DUVASU

An Overview of University Post-graduate Research

(2001-2013)

U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan
Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU)
Mathura- 281 001 (U.P.) INDIA

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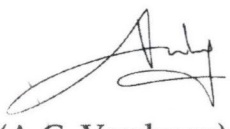
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FOREWORD

Like other Agricultural and Veterinary Universities of country DUVASU has a common and prime mandate of continuous improvement and imparting the teaching, research and extension in Veterinary & Animal Sciences, Fisheries and Livestock Product Technology and thus contributing its share in building the economy of the nation. The constituent college of Veterinary & Animal Sciences of this university is rated as the premier veterinary institution of the country and is the first Veterinary College of Post independent India to have started with full fledged B.V.Sc. & A.H. degree programme and then subsequently the post graduate M.V.Sc. and Ph. D. degree programmes in various disciplines of Veterinary and Animal Sciences. Post graduate Researches conducted in various departments are the training to the candidates in research methods and solve the immediate problems which the state/nation is facing. It is not only an essential requirement for the award of post graduate degree but also is a true reflection of the quality of academic standards of the university. Several landmark researches have been conducted in the laboratories of the college and excellent research findings have been compiled in various theses. These findings are although preserved in the libraries and achieves in bound forms, their dissemination and applicability is sometimes limited due to non availability in original forms or in diskettes. The compilation of these findings into "Theses Abstracts" form is a "ready at hand" service to the researchers who want to trace the developmental status with concepts and design to final interpretation and outcome of researches in various disciplines at various times. The University has thought up a plan to publish this "Overview of University Post-graduate Research" since the inception of this university in 2001 for the benefit of the scientific community. The editorial committee has done a yoeman's job in compiling this difficult task. I congratulate them for having ventured in this direction and bring out this useful publication. It will give a glimpse of Researches which have been done in the past. This will also help to avoid duplication of research work which are being/ will be conducted in the various laboratories of the country and thereby will save the valuable resources which can be more profitably utilized for conducting new researches and thus paving way for national prosperity. I hope this publication will serve as a very useful reference tool to the Scientists and Teachers engaged in various disciplines of Veterinary and Animal Sciences and other allied disciplines. I congratulate to all those who have contributed to this publication.



(A.C. Varshney)
Vice Chancellor

PREFACE

University laboratories serve as an unending fountain head for flow of research findings. Researches are the outcome of the continuous hard work put in for long time by the solitary Scientist or the combination with one or many others or by the team of workers from different discipline to knock out to find solution for a single burning problem. These findings are published in various reports and journals. The "Theses" which is submitted for award of the degree at Master's or Doctoral level incorporates the whole hearted band whole time hard work put in by the research scholar under the guidance of his supervisor who is his mentor. It is the devotion of scholar and benevolence of advisor which truly reflected in the thesis and hence reveals the quality of mind and heart of the two. Not unoftenly the researchers who qualify their Masters' or Doctoral programmes themselves by chose the field practice and have no connections whatsoever with the academics. Generally the Masters' or Doctoral theses of the researchers remain unreported in wide publication catch dust in the libraries. "An Overview of University Post-graduate Research " compilation is an effort to coup up with this loss. The abstracts compiled in this volume date back from the start of the DUVASU in 2001 and all abstracts of M.V.Sc. and Ph.D. Theses so far compiled have been presented in this volume. Any reader can have a glimpse of type and trend of the academic researches which were being conducted during these years. The volume will serve as a ready reference to the various research scientists in different disciplines of Veterinary & Animal Science and help them in choosing the problems, methodology and the guidelines for conduct of researches. This will also save duplication of research project allotment at various degree levels in various universities.

We hope the volume will serve as a very important tool to the scientists and researchers in giving shape to future line of action in research and trend. This will also save the loss of valuable resources and funds which are likely to drained due to simply duplication of researches.

The publication division and editors feel grateful to the Hon'ble Vice Chancellor for kindly giving his a "Go ahead" signal to the publication of this compilation.

All the colleagues and friends from various departments have very generously cooperated in collection and compilation of the information. The Incharge, Printing and Publication division took extra pains to bring out this priceless publication in short time.

– Editors

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Animal Genetics and Breeding

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M.V.Sc.

1. Molecular Characterization of Casein Polymorphism in Indian Goat Breeds

Prakash Kumar and S.N. Shukla

Study on milk protein polymorphism will provide the information for selection of the goats, which can be utilized for better milk yield, protein yields as well as cheese production. The genetic variants of aS_1– Cn, aS_2– Cn, β– Cn, K– Cn, β– LG and a-LA genotypes were identified by sodium dodecyl Sulphate polyacrylamide gel electrophoreses (SDS-PAGE) in milk samples of Jamunapari, Barbari, Marwari, Sirohi and Jakhrana goats. The aS_1– Cn allele was confirmed by PCR typing in all five Indian goat breeds. The genotype frequency of aS_1– Cn were 0.96, 0.91, 0.93, 0.81 and 0.90 in five Indian goat breeds mentioned above. The Sirohi and Marwari breeds were significantly ($P<0.05$) different from Jamunapari and Barbari breeds in protein content. The findings of present study suggested that the milk protein variants in Indian goat breeds may be used as selection criteria to increase total casein, total calcium and total protein as well as milk yield.

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2. Genetic study on the Reproductive Traits of Hariana Cows

Ashish Kumar Srivastava and K.C. Sharma

The performance records of 211 Hariana cows, progeny of 27 sires, born during the year 1966 to 1985 and maintained at DDD Farm, Mathura and Livestock Farm, Madhurikund (Mathura) were utilized in this study. The least squares means of age at first oestrus, age at first conception and age at first calving were 1091.43±45.85, 1107.56±57.88 and 1394.02±57.54 days, respectively. The heritability estimates of age at first oestrus, age at first conception and age at first calving were obtained as 0.720±0.284, 0.785±0.289 and 0.768±0.288, respectively. The least squares means of post-partum oestrus interval, service period and first calving interval were 185.43±11.18, 240.53±27.01 and 671.71±209.04 days, respectively. The least squares mean of breeding efficiency of Hariana cows was estimated to be 72.57±5.27 percent. Economics of keeping Hariana cows up to the completion of their first lactation and up to the age of subsequent second calving was also calculated. It was concluded that suitable measures should be taken to reduce the age at first oestrus, age at first conception and age at first calving to the level of first group heifers (756, 795 and 1200 days, respectively) by paying more attention on breeding and management practices.

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3. Assessing Genetic Diversity among Some Livestock Species through DNA Markers

Sanjeev Kumar Agrawal and R.C. Sharma

Genetic diversity among the five livestock species i.e. Goat, Sheep, Cattle, Buffalo and Mithun was estimated using two type of DNA markers namely- randomly amplified polymorphic DNA (RAPD) marker and micro satellite associate sequence amplification (MASA) markers. The high molecular weight genomic DNA was successfully isolated from all the five livestock species. These primers detected good amount of polymorphism. The number of total band amplified with different primers ranged from 7 to 10 in goat, 7 to 9 in sheep, 6 to 7 in cattle, 8 to 10 in buffalo and 7 to 10 in Mithun. The genetic distance, pooled over the primers ranged from 0.318 to 1.041. Goat and sheep showed the minimum genetic distance and maximum genetic distance was observed in Mithun and goat. It was concluded that the livestock species have low

to moderate genetic similarity with each other and at this level the sheep and goat are more genetically similar to each other in comparison to other livestock pairs. Mithun showed maximum genetic similarity with buffalo and least genetic similar with goat.

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4. Genetic study of production efficiency and reproduction efficiency traits in Sahiwal cattle maintained in northern Indian plane

Deepak Bhatia and V.K. Arora

The study was undertaken on the data of purebred Sahiwal cows comprising performance records of 873 Sahiwal cows, progeny of 48 sires, maintained at State Livestock cum Agriculture Farm, Chakganjaria, Lucknow (U.P.) and distributed over a period of 42 years (1957 -1998). In total eight production efficiency and reproduction efficiency traits viz. milk yield per day of first lactation, milk yield per day of first calving interval, milk production efficiency per kg body weight, milk production efficiency per day per kg body weight, milk production efficiency per kg metabolic body weight, milk yield per day of age at second calving and breeding efficiency by both Tomar and Wilcox method were studied. The estimate of heritability for various traits, genetic and phenotypic correlations among different traits was also calculated. It was concluded that the strong positive genetic correlation between milk yield per day of age at second calving with milk yield per day of first calving interval (0.97±0.20), milk production efficiency per day per kg body weight at calving (0.89±0.20), milk production efficiency per kg metabolic body weight (0.89±0.20) and milk production efficiency per day per kg metabolic body weight (0.87±0.20) indicates that milk yield per day of age at second calving trait can very well replace the traits mentioned above for evaluation of field animals for better economic evaluation and selection. The study also revealed that heavier cows have lower breeding efficiency.

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5. Serum Lysozyme a Marker for Enhancing Immunocompetence in Goat

Prakash Chand Sharma and S.N. Shukla

An attempt has been made in the present study to generate the information on innate immunity. Lysozyme brings about bacteriostatic as well as indirect bactericidal activity against pathogens and contributes considerable to keep the quality of stored milk. A total of 290 sera samples comprising, 172 Barbari goats, 66 Jamunapari goats and 52 Muzaffarnagari sheep were analyzed for serum lysozyme activity. Serum lysozyme activity in Barbari, Jamunapari goat and Muzaffarnagari sheep was 0.30±0.30, 1.45±1.00 and 0.16±0.113µg/ml and varied from 0.48 to 1.607µg/ml, 0.139 to 3.236µg/ml, 0.034 to 0.566 µg/ml respectively. Serum lysozyme activity varied from breed to breed and species to species. Mean lysozyme activity of Jamunapari goat was higher than Barbari goat. Muzaffarnagari sheep was showing very low lysozyme activity. The observed genetic variation (high and low serum lysozyme level) could be useful for improvement of resistance against bacterial disease by marker-assisted selection.

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6. Genetic and Phenotypic Studies on the Persistency of Milk Production in Murrah Buffalo

Kailash Chandra and R.C. Sharma

The h^2 estimates for 30, 60, 90, 120, 150 and 180 days cumulative milk yield were statistically significant. Significant h^2 estimate for peak yield 300 days milk yield and lactation period have been also reported. Study revealed that peak yield had a significant and positive genetic correlation with 300day milk yield, lactation period and persistency of milk predictor. It was

found that 150 days cumulative milk yield is the best predictor of first lactation milk yield. The study was undertaken to assess the genetic parameters of various economic traits of Murrah buffaloes kept at DDD Farm, Mathura, State Livestock-Cum-Agriculture Farm, Madhurikund and Babugarh Farm (Meerut). Statistical analysis was carried out by utilizing least square and maximum likelihood (LSMSLMW) method. Least squares mean of all six economic characters undertaken in this study have been reported significant variations due to farm, season, age and weight at first calving were noted in all the traits.

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7. Leptin Gene Polymorphism in Indian Goat Breeds

Shivendra Kumar Singh and S.N. Shukla

The investigation was undertaken to analyze the Restriction Fragment Length Polymorphism in leptin gene in Jamunapari and Barbari goat breeds and to evaluate the leptin gene in relation to growth performance. Leptin gene sequences were obtained in Indian goats for the first time and two regions of leptin gene were amplified and analyzed also. The sequences of exon-2 ranges from 153 bP to 265 bP, while sequences of intronic region showing mean about 388 bP while only one sequence showed 283 bP of leptin gene.

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8. Role of Male Germ plasm in Conservation of Bhadawari Buffalo

Amitosh Kumar and K.C. Sharma

The study was undertaken with the objective of conservation of Bhadawari buffalo through male germ plasm. In all 120 collections were taken from 10 Bhadawari bulls during the months of October 2004 to March 2005. The overall averages for ejaculate volume, initial pH, mass motility, individual motility and sperm density were found as 1.68±0.07 ml, 6.54±0.02, 2.67±0.09, 48.23±0.61 percent and 0.95±0.02 million/mm^3, respectively. Percent live spermatozoa, percent total abnormal live spermatozoa, percent head abnormalities of spermatozoa, percent tail abnormalities of spermatozoa and cold shock resistant spermatozoa averaged 68.32±1.00, 14.25±0.53, 3.29±0.34, 10.96±0.31 and 27.27±0.27 percent respectively. On the basis of most important semen characteristics i.e. mass motility, sperm density and percent live spermatozoa Bhadawari bulls were graded. The preservability of semen was also studied at 4-5°C and at -196°C. Tris-egg yolk extender was found significantly better than modified EYC extender for maintaining higher percentage of motile spermatozoa at different storage periods. Haematological attributes recorded for all Bhadawari bulls were in normal rage indicating that they were healthy and disease free. It was concluded that vigorous selection of bulls for semen quality and the use of good dilutor will assist the conservation of Bhadawari breed of buffalo.

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9. Genetic Evaluation of Sahiwal Cattle using Animal Model

Govind Narayan Tiwari and H.N. Singh

The present investigation was carried out on data of 947 purebred Sahiwal cows, progeny of 109 sires, maintained at State Livestock Cum Agriculture Farm, Chakganjaria, Lucknow (U.P.). The data was distributed over 52 years with 13 periods and each period of 4 years. The statistical analysis was done by animal model (DFREML version 3.0 dos and Least Square and maximum likelihood method) PC-2 version computer software. The overall mean of age at first calving, calving interval, total lactation milk yield, 300 days lactation milk yield, dry period, lactation length and average daily milk yield were estimated as 1238.18±221.37 days, 465.32±96.81 days, 1741.72±574.03 kg., 1596.59±479.87 kg., 153.45±76.02 days, 312.08±74.64 days and 5.62±1.49 kg, respectively. The corresponding coefficient of variation were 13.59%, 19.81%,

32.94%, 27.91%, 47.85%, 23.89% and 23.38%, respectively. There was highly significant effect of period of calving on age at first calving, lactation length, total lactation milk yield and 300 days lactation milk yield and a significant effect of season of calving was seen on age at first calving, dry period, lactation length, total lactation milk yield, 300 days lactation milk yield and average daily milk yield. Heritability of reproduction traits and production traits ranged from 0.120±0.03 to 0.269±0.084 and 0.181±0.041 to 0.23±0.046, respectively. There was positive genetic correlation between age at first calving, calving interval, dry period, 300 days lactation milk yield, average daily milk yield and total lactation milk yield and negative genetic correlation with lactation length. The DFREML breeding values helped in selection of best sires on the basis of age at first calving, total lactation milk yield, 300 days milk yield and average daily milk yield indicating that selection on basis of various traits is the best method over selection on basis of single traits.

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10. Genetic Study on Growth characters in Hariana Cattle

Ved Prakash Rai and R.C Sharma

The performance record of 319 Hariana heifers, progeny of 33 sires, born during 1966 to 1972, maintained at Livestock Farm at Babugarh (Ghaziabad) and at Madhurikund Farm (Mathura) was taken for the present study. The least square means of birth weight, body weight at 3, 6, 9, 12, 18, 24 and 30 months, first lactation peak yield and first lactation 300 days milk yield were 23.63±0.35 kg, 55.67±0.77 kg, 87.38±1.22 kg., 117.84±1.71 kg., 147.96±2.02 kg, 192.20±2.64 kg, 239.23±2.85 kg, 278.45±3.26 kg, 5.02±1.00 kg, and 1025.75±5.74 kg., respectively. Farm effect was significant ($P<0.01$) for body wt. at birth 3, 6, 9, 12, 18, 24 and 30 months age and for first lactation peak yield. The year effect were significant for body wt. at 3, 6, 12, 18, 24 month age, first lactation peak yield and first lactation 300 days milk yield. The effect of season of birth/ calving was found non-significant on all traits under study. Heritability estimates of body wt. at birth, 3 month age were low and for body wt. at 6 and 9 month age were significant and moderately high (0.438±0.025). The h^2 estimates for first lactation peak yield and first lactation 300 days milk yield were 0.574±0.30 and 0.436±0.02, respectively. The genetic correlation was positive and highly significant for body wt. at 12, 18, 24 and 30 months of age, first lactation peak yield and first lactation 300 days milk yield. It can be concluded that Hariana heifers must be selected for higher production on basis of 12 months body wt. and for attaining high body wt. at 12 months age utmost care with good husbandry practices should be done.

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11. Studies on MHC Haplotyping in Guinea fowl and its association with general immunocompetence

Atul Gupta and H.N. Singh

In the present investigation, a total of 76 guinea fowl (GF) birds of Lavender variety and 15 birds each of white leghorn (WL), Aseel (AS) and Red Cornish (RC) were used for studying the polymorphism in MHC genes, B1 domain of BLB2 gene using SSCP and PCR-RFLP techniques and genotyping of guinea fowl population for B-haplotyping. After genomic DNA extraction, PCR was carried out with LEI0258 locus specific primers. Monomorphic pattern was observed in GF for micro satellite marker study with 247 alleles on LEI258 locus, however, polymorphism was observed in chicken breed with 5 alleles (205-608bp), 11 alleles (234-590bp) and 7 alleles (193-295bp) in WL, AS and RC, respectively. Only one B-haplotype i.e. B18 was observed in GF while, WL had B13, 13.2, 15.2, 17.22, 73 and BW11 B-haplotypes; AS had 1, 1.2, 2, 5, 6, 8, 11.1, 15, 15.2, 18, 19.1, 22, 29, 62, 73 and 74 B-haplotypes and RC had 2, 5, 11, 11.1, 13, 13.2, 15, 15.1, 17, 27, 29, 61 and BW11 B-haplotypes. There was presence of 6 SSCP patterns for BLB2gene in guinea fowl suggesting the possibilities of more than one B-haplotypes in GF. The GF population was

grouped on basis of total HA titre against SRBC at 10th weeks of age were low HA antibody titre (LHA), medium HA antibody titre (MHA) and high HA antibody titre (HHA) groups with means HA titre of 3.36±0.11, 5.85±0.11 and 8.53±0.19, respectively. PCR-RFLP of B-domain of BLB2 gene using Hae III restriction enzyme revealed Allele A and allele B with AA and AB genotypes. The frequency of AA and AB genotypes by Hae III RE in GF was 0.4 and 0.6, respectively in low titre group; 0.6 and 0.4, respectively in medium titre group and 0.5 and 0.5, respectively in high titre group. While the frequency of AA and AB genotype by Taq I restriction enzyme were 0.6 and 0.4 in low titre group, 0.5 and 0.5 in both medium and high titre group, respectively.

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12. Immunocompetence profiling and DNA Polymorphism in disease resistance genes in Aseel and Kadaknath native chickens

Parmatma Singh and H.N. Singh

In the present investigation, 242 Aseel chicks and 179 Kadaknath chicks breed of native chicken were evaluated for immunocompetence traits viz., Antibody response to SRBC, CMI response to PHA-P, serum lysozyme activity and serum IgG level and DNA polymorphism at ChB6, caspase-1, IAP-I and ZOV3 genes along with association among immunocompetence traits. Humeral immune response to SRBC was measured through HA test. CMI response to PHA-P was done by measuring the difference between the thickness of the foot web before and after the injection of 0.1% PHA-P. Lysozyme concentrate was estimated by lysoplate assay using *Micrococcus lysodieckticus* as substrate. Serum IgG level was measured by single radial immunodiffusion assay with rabbit antchicken as antisera. The data on immunocompetence traits were analyzed by least square analysis of variance taking age group and sex as fixed effects in the model. The HA titre was 9.22±0.20 (2-21) and 7.49±0.25 (2-15) in Aseel and Kadaknath, respectively. Serum lysozyme level was 2.13±0.3 ig/ml (1.47 to 3.35 ig/ml) and 2.02±0.5 ig/ml (1.42 to 3.77 ig/ml); average serum Ig concentrate was 10.61±0.25 mg/ml and 10.07±0.20 mg/ml; average CMI response to PHA-P was 0.156±0.013 mm (10^{-2}) and 0.207±0.013 mm (10^{-2}) in Aseel and Kadaknath, respectively. Heritability estimates for CMI response was medium, whereas for lysozyme and IgG was very low. PCR-RFLP technique using PvuII, Hsp92II, BgeI and SnaBI restriction enzyme for ChB6, caspase-I, IAP-I and ZOV3 genes, respectively was used on twelve birds from each breed with six from high HA titre group and from low HA titre group. The Pvu II RE digestion of 215bp amplicons of ChB6 genes generated AA (147 bp) genotype, BB (215 bp) genotype and AB (147 and 215 bp) genotype in Aseel with a frequency of 0.25, 0.25, 0.5, respectively. However, AA and BB genotypes were observed in Kadaknath with equal frequencies of 0.5. The Hsp92II RE digestion of 1070 bp amplicons of caspase-I generated AB (109, 122, 227, 244, 312 and 421 bp) and BB (109, 122, 227, 244 and 312 bp) genotype with a frequency of 0.75 and 0.25, respectively while only AB genotype with 1.0 frequency was observed in Kadaknath. BgeI RE digestion of 394 bp amplicons of IAP-I gene generated AA (254 and 40 bp) and AB (254, 140 and 394 bp) genotype with frequency of 0.14 and 0.86, respectively in Aseel and 0.38 and 0.62, respectively in Kadaknath. The SnaBI RE digestion of 320 bp amplicons of ZOV3 gene revealed AB (270 and 320 bp) and BB (320 bp) genotypes with frequency of 0.5 and 0.5, respectively in Aseel and 0.6 and 0.4, respectively in Kadaknath. Aseel showed higher humeral response to sheep erythrocytes and higher serum lysozyme level than Kadaknath thus showing higher immunocompetence. Varied levels of humeral immune response in Aseel can be exploited for development of higher immunotolerant birds through selective breeding.

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13. Genetic Studies on Some Economic Traits of Sahiwal Cattle

Amita Sharma and K.C. Sharma

The data for present study were collected from the government livestock farm, Chak Ganjaria, Lucknow, Uttar Pradesh, comprising performance record of 450 daughters from 1983 to 2006 sired by 40 bulls and having minimum 5 daughters under each sire. The least squares means of age at first calving, first service period, first calving interval, first lactation total milk yield and estimated as 1069.533±7.639 (days), 274.109±8.438 (days), 566.111±8.550 (days), 6.266±0.055 (Kg.), 1411.194±17.756 (Kg.), 378.136±4.596 (days), 1868.798±32.954 (Kg.) and 3.122±0.047 (Kg.) The heritability estimates were worked out by paternal half sib correlated method. The heritability estimates for age at first calving, first service period, first calving interval, first lactation peak yield, first lactation 300 days milk yield, first lactation period, first lactation total milk yield and milk yield per day of first inter-calving period were found as 0.140±0.011, 0.077±0.016, 0.079±0.018, 0.365±0.148, 0.439±0.159, 0.402±0.154, 0.765±0.201 and 0.578±0.178 respectively. It may be concluded that for the selection of genetically superior Sahiwal cows, age at first calving, first lactation peak yield and first lactation 300 days milk yield should be incorporated in a selection index.

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14. Role of Semen additive in improving the keeping quality of buffalo on the morphology of spermatozoa

Sumati Kumar and K.C. Sharma

The present investigation was carried out for the evaluation of semen of Murrah and Bhadawari bulls, on the basis of important physical characteristics. In all, 66 ejaculate of two Murrah bulls and 61 ejaculates of two Bhadawari bulls were examined to study physical attributes of semen. Average ejaculate value was 2.89±0.08 ml. and 3.35±0.12 ml. for Murrah and Bhadawari bulls respectively. The present experiment was undertaken to compare the efficiency of extender viz- milk. D_2 and tris to know the extent of preservability of buffalos semen at refrigeration temperature. The Murrah semen with tris extender up to 48 hours of storage was significantly superior to the Bhadawari and Murrah semen preserved in milk or D_2 extender. The overall fertility rate was 32.77 percent. It was interesting to note that storage age of semen from first day of use to third day had no significant impairment in fertility with any of the treatment. Tris-egg yolk extender with cysteine hydrochloride is recommended for preservation of buffalo semen successfully at 4°C-5°C up to storage age of 48 hours.

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15. Evaluation of Sahiwal Sires on the basis of early production performance of their Daughter's

Sumit Kumar and K.C. Sharma

The present investigation was carried out for genetic evaluation of Sahiwal sires by best linear unbiased prediction (BLUP) sire evaluation methods. The data were collected regarding first lactation performance of 485 Sahiwal cows for the period 1983 to 2006 maintained at Government Livestock farm, Chak Ganjaria, Lucknow. The heritability estimates for 90, 120, 150, 180 days part milk yield, peak yield, days to reach peak yield and 300 days milk yield were 0.129±0.042, 0.145±0.045, 0.237±0.063, 0.303±0.075, 0.384±0.089, 0.365±0.086 and 0.322±0.078 respectively. All these estimates were medium in magnitude and significant. The estimated breeding values for peak yield ranged from 0.126 to 0.107 Kg. and for 90, 120, 150 and 180 days milk yield ranged from – 36.860 to 29.934, - 36.096 to 32.218, - 50.096 to 28.667, and - 72.048 to 34.771 respectively and – 28.527 to 25.368 for 300 days milk yield. On the basis of results it may

be concluded that performance of daughters in their first lactation on the basis of yield and 120 days (4 months) milk yield may be used to select sires with good reliability rather than to wait 300 days or total lactation milk yield.

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16. Genetic studies on reproduction traits in Gangatiri cattle breed

Ramsahay Yadav and Deepak Sharma

The present investigation was undertaken on 202 Gangatiri cows progeny of 11 sires calved during 1993 to 2008 maintained at Government Livestock farm, Arazilines, Varanasi for the genetic study of various reproduction traits. Average least square mean and heritability estimates of age at puberty, age at first conception, age at first calving, gestation length, service period, calving interval and breeding efficiency were 1359.38±20.51 days and 0.266±0.203; 1365.96±20.97 days and 0.324±0.218; 1649.42±21.01 days and 0.315±0.216; 283.46±0.75 days and 0.023±0.135; 187.30±16.03 days and 0.398±0.236; 471.06±16.08 days and 0.408±0.238; and 82.50±1.72 per cent and 0.283±0.208, respectively. The significant effect of sire was seen on all the traits except gestation length and significant effect of period of calving was seen on age at puberty, age at first conception, and age at first calving. The effect of season of calving on all the traits was found to be non-significant. The linear regression of age of puberty and age of first conception on age of first calving (in days) was found to be highly significant whereas linear regression of service period, calving interval and breeding efficiency on first lactation total milk yield were found to be highly significant. There was negative genetic correlation of breeding efficiency and gestation length with all other traits non-significant with higher standard error. The genetic and phenotypic correlations between age at puberty, age at first conception and age at first calving were estimated as high positive and significant indicating that similar genes were involved for the expression of these traits. The genetic correlations of service period with calving interval were high and positive. The phenotypic correlations between milk production and breeding efficiency traits were found to be highly negative and significant and indicated that breeding efficiency traits may be effective in selection programme for genetic improvement. The phenotypic correlations of calving interval with breeding efficiency and first lactation total milk yield; service period with calving interval, breeding efficiency and first lactation total milk yield; age at first conception with age at first calving; age at puberty with age at first conception and age at first calving were highly significant. The environmental correlations between all the traits were found to be significant except age at puberty with gestation length, gestation length with breeding efficiency and first lactation total milk yield. It indicated that very much favorable to augment the efficiency of these traits in similar environment.

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17. Genetic studies on production traits of Gangatiri cattle

Umakant Jaiswal and K.K Chauhan

The present investigation was done for production performance traits of Gangatiri cows maintained at GLF, Arazilines, Varanasi. The data of 202 Gangatiri cows' progeny of 11 sires distributed over a period of 16 years from 1993 to 2008 were utilized in this study. The overall means of FLPY, FLMY, FLL and FDP were estimated as 4.91 kg, 1032.57 kg, 256.74 days and 201.51 days, respectively. The average of body measurements viz. hearth girth, paunch girth, body length and height at withers were analyzed as 152.66, 181.48, 110.33 and 124.40 cm, respectively. The coefficients of variation for all the traits, except body measurements were high indicating the possibility of selection for each trait. The period of calving had highly significant effect ($P<0.01$) on FLPY and season of calving had non-significant effect for all traits. The linear regression of FLMY on age at first calving (days) was found to be significant ($P<0.05$), except

FLPY, FLL and first dry period. Sire had significant effect on most of the economic traits. The heritability estimates for FLMY, FLPY, FLL and FDP were estimated as 0.639±0.29, 0.127±0.17, 0.352±0.23, and 0.701±0.30, respectively. The coefficients of correlations between body measurements with production traits have a positive and significant association. Phenotypic correlations between heart girth, paunch girth and height at withers with milk yield, peak yield and lactation length were observed highly significant (P<0.01), while body length with peak yield had significance at 5% level. The association of body measurements with production may facilitate solution of animals for milk producing ability. These results concluded that the improvement of Gangatiri cattle production traits, the breeding strategies followed to bring genetic improvement needs accurate selection and progeny testing bulls, for improving productivity along with improvement in managemental practices in herd.

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18. Comparison of various methods for efficient sire evaluation and genetic study of different economic traits in Sahiwal cattle

Peaush Kumar Singh and V.K. Singh

Under present study, data were collected regarding first lactation performance of 506 Sahiwal cows for the period 1986 to 2009 maintained at State Livestock cum Agriculture Farm, Chak-Ganjaria, Lucknow. The breeding values of different sires for various first lactation traits were estimated by LSQM, SRLS and BLUP methods. The least square means for MY 90, MY 120, MY 150, MY 180, MY 300 days and Lactation Period were 518.504±19.366, 687.717±25.956, 840.796±31.820, 988.978±37.575, 1389.916±59.528 kg and 358.378±16.617 days respectively in Sahiwal cattle. The effect of period on MY 90 and MY 120 days was found significant (P<0.05) and on MY 150, MY 180 and MY 300 days was found non-significant. The effect of season on MY 90, MY 120, MY 150, MY 180 and MY 300 days was found significant (P<0.01). Effect of AFC groups on MY 90, MY 120, MY 150, MY 180, MY 300 days was found non-significant. The magnitude of heritability of production traits depends upon the variability in sires for additive genetic component. So these traits can be improved by applying selection in these animals. The genetic and phenotypic correlations between cumulative part yields and first lactation yield were positive and highly significant. According to regression equation analysis, the actual value of 300 days milk yield is very close to the predicted values on the basis of 90 days milk yield others. It was concluded that performance of daughters in their first lactation on the basis of 90 days milk yield may be used to select sires with good reliability rather than to wait for 300 days or total lactation milk yield. Among reproduction traits Calving interval is a trait which reveals the management and environment condition of the farm animal. Larger calving interval is counter-checked and could be controlled by reducing service period and dry period. The effort should be made to reduce the calving interval up to standard (12 months) prescribed for Sahiwal cattle. Estimates of BLUP method were emerged as most reliable among three methods because it has more precise values. Ranking of sire was done on the basis of their breeding values estimated through BLUP method of sire evaluation. Ranking of sire is useful in case of better sire for future generation.

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19. The Study of PIT-1 Gene Polymorphism in Sahiwal cattle by using PCR-RFLP

Ankur Chauhan and Madhu Tiwari

In the present study, identification of PIT-1 gene polymorphism and its association with milk production traits was undertaken in 77 Sahiwal cattle maintained at NDRI, Karnal by using PCR-RFLP technique. Amplification of DNA sample revealed 600 bp product and restriction digestion with *Hinf I* showed three types of genotypes, namely, AA (600 bp), AB (600, 343 & 257 bp) and BB (343 & 257 bp) genotypes. The frequency of BB genotypes was highest (64.98%) in all screened samples, followed by AB genotype (31.16%). However, the AA genotype was the least frequent (3.89%). The allelic frequency of PIT-1 A & B alleles were 19.48% and 80.51%, respectively. Association studies of PIT-1 gene with milk production traits showed that AFC, GP, DP & LP had non-significant variation among all the three genotypes over all four lactation. However, a significant difference ($P \leq 0.05$) was found among the three genotypes for total milk yield and milk yield at 300 days with AA genotype showing higher value than AB and BB genotypes in the first lactation. From present investigation, the *Hinf I*/PCR-RFLP revealed polymorphic pattern of PIT-1 gene in Sahiwal cattle and association studies showed significant effect of an allele on total milk yield and milk yield at 300 days.

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Ph.D.

1. Sire evaluation for early and lifetime economic traits and development of selection criteria in Sahiwal cattle

V.K. Singh and H.N. Singh

The study was conducted on the data of Sahiwal cattle maintained at Livestock cum agriculture farm, Chakganjaria, Lucknow (U.P.). The performance records of 882 Sahiwal cattle were analyzed to estimate genetic variance and covariance for various economic traits viz. age at first service, age at first calving, first calving interval, milk yield, first lactation period and dry period. The study was conducted to estimate relationship between early and lifetime economic traits. Breeding value of sires was calculated by using BLUP method. Suitable selection criteria were developed through constructing selection indices for improvement in milk yield of Sahiwal cattle. Optimizations of early lifetime traits for maximum lifetime production were also estimated.

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2. Studies on genetic resistance of small ruminants to natural injection with gastrointestinal nematode

K.K. Chauhan and S.N. Shukla

The present investigation on genetic and non-genetic control of resistance and susceptibility pattern of *H. contortus* in goats in semi arid tropics was carried out on total 855 progenies of 46 sires of Barbari and Jamunapari goats maintained at CIRG, Makhdoom. Statistical analysis was done as logarithmic transformation of Faecal egg count (FEC) data by Log_e (FEC+100); Arisin transformation of hematological (PCV%) data and least square analysis of transformed data using mixed model and maximum likelihood computer program. Barbari breed was more susceptible to *H. contortus* infection than Jamunapari at all ages. Sex had an effect on LFEC & PCV trends in both breeds and also LFEC had no significant ($p>0.01$) difference between single and multiple birth at all ages in Barbari breed. However, Jamunapari breed had significant ($p<0.05$) difference in LFEC between single and multiple born kids at 12 months of age. Season I (March-April) born kids had lower LFEC and higher PCV than kids born in season II (Sept.-Oct.) in both breeds at 3, 6 & 9 months age. Age had significant effect on LFEC & PCV in both breeds. The effect of age of kids on immunity indicated that LFEC in Barbari breeds was higher than Jamunapari breed at all ages. The h^2 estimates for LFEC at 3, 6 and 9 month of age were 0.22± 0.11, 0.29±0.12, 0.37±0.14, respectively in Barbari and 0.16±0.14, 0.16±0.14, 0.38±0.20 respectively in Jamunapari goats and that of PCV was 0.29±0.12, 0.44±0.15 and 0.44±0.15, respectively in Barbari and 0.48±0.23, 0.31±0.18 and 0.07±0.11, respectively in Jamunapari goats. The h^2 estimates of body weight were high at 3 months of age and subsequently decreased at 6 & 9 months of age in both breeds. There was highly positive phenotypic correlation between LFEC & PCV in both breeds at all ages. Sires had significant effect on LFEC & PCV indicating that the traits are influenced genetically. The selection and mating between RxR, RxS & SxS parents showed resistance level in ratio of 12:6:2, 13:13:9 and 2:5:10, respectively in Barbari goats. The regression of LFEC on live weight and live weight gain was not significant at all ages in Barbari goat and on live weight in Jamunapari breeds. Season had significant effect on LFEC at late pregnancy stage. Jamunapari does had higher worm load as compared to Barbari does at all physiological stage. LFEC showed an increasing trend from mating to early lactation stage and decreasing trend from late lactation to dry period stages in both breeds in both breeding season. Resistant sires had low LFEC and higher PCV at all ages in both breeds. During physiological stages Jamunapari goats showed lower immunity than Barbari goats due to higher milk production.

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3. Genetic architecture of DRB3 gene in crossbred cattle

A.K. Ghosh and S.N. Shukla

The bovine lymphocyte antigen (BoLA)-DRB3 gene encodes cell surface glycoprotein that initiates immune response by presenting processed antigenic peptides to $CD4^+$ T helper cells. The present investigation was proposed to undertake with the objectives to elucidate allelic variants of DRB3 gene, to clone and sequence DRB3 alleles and to characterize various DRB3 alleles of crossbred cattle. DNA was isolated from blood samples of randomly selected 200 crossbred (Jersey×HF×Sahiwal) cattle maintained at IDF, GBPUAT, Pantnagar, Uttarakhand. DNA fragments were amplified using one pair of primers (Forward-5'CACATTTCCTGGAGTATTCT3' and reverse-5'ACCCCCGTAGTTGTGTCT3') spanning over the exon 2 of DRB3 gene. SSCP patterns of the amplified fragments were studied by PAGE. Six new alleles as A, B, C, D, E and F having frequencies 0.2675, 0.245, 0.12, 0.2525, 0.0825 and 0.0325, respectively and twelve genotypes as AB, AD, AE, AF, BB, BC, BD, BE, CD, DD, DE and EF having frequencies 0.23, 0.215, 0.06, 0.03, 0.04, 0.11, 0.04, 0.03, 0.13, 0.04, 0.04 and 0.035, respectively were identified. Genotypes had significant effect on mastitis. Animals with AB, AD, AF, BC and CD genotypes were found more prone to mastitis highlighting the presence of A or C or D allele in the genotype affecting more to mastitis and therefore could be considered for marker assisted selection (MAS). Association of BoLA-DRB3 gene with economic traits indicated that genotypes have significant effect on age at first calving, daily milk yield, and peak yield, but no significant association was observed in first lactation length and first lactation milk yield.DRB3 gene was cloned and positive clones were subjected for sequencing. Comparing variability of different amino acid sequences it was observed that the functional mutation in allele E was maximum followed by B, F, A, C and D allele. From percent similarity study and phylogenetic relationship it was found that maximum similarity was between B and F alleles followed by D, A, C and E allele. Using Swiss Model Workspace the modeling of identified hypothetical three dimensional protein structures encoded by six different alleles was done. The antigen binding sites of the proteins coded by each allele were successfully identified. The higher consensus percentile rank indicated the better antigen binding site. As per the physico-chemical characteristics of the proteins were concerned proteins coded by B and F alleles were unstable.

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M.V.Sc.

1. Effect of calf starter and soy powder on the performance and rumen microflora of pre-weaning calves

Lokendra Kumar and Aditya Kumar

The study was conducted for investigating the effect of calf starter and soy powder on the growth, nutrient intake, feed conversion ratio, rumen fermentation pattern, glucose and volatile fatty acids level in blood plasma in cow calves up to 3 month of age. Three groups of about 15 days old calves were taken, each having four calves, one as control i.e. reared under farm condition and other two as experimental, one of which were fed *adlib* calf starter up to 3 months and other group was given calf starter as in IInd group plus 100 gm. soy powder after boiling in water in morning and evening with free supply of drinking water. Fortnightly growth, feed consumption daily as well as fortnightly and feed conversion ratio were recorded in each group. Besides this the rumen liquor sample at fortnightly interval (30, 45, 60 and 75 days of feeding) was also collected from each calves by stomach tube for study the development of micro flora in rumen and effect of feed on the growth of micro flora population. A comparative evaluation of total body weight gain and feed conversion ratio of all the three groups viz. control, treatment Ist and treatment IInd were 12.27, 21.42, 20.57 kg and feed conversion ratios were 2.15, 1.74, and 2.32, respectively. This variation in growth and feed conversion ratio of calves gives an indication that calf starter and soy powder have some effect on the tissue of the body that causes the better growth of the calves in experimental groups than control one. As regard the nutrient intake by the calves in terms of DM, CP, CF, EE, NFE, ash, calcium and phosphorus in control and treatment groups were 93.11, 6.24, 24.89, 1.55, 50.61, 8.99, 0.47 and 0.25 kg in control, 148.09, 22.4, 28.43, 3.36, 84.84, 13.37, 2.26 and 1.12 kg in treatment I and 184.65, 41.01, 25.86, 13.65, 89.10, 13.76, 2.14 and 1.33 kg in treatment II respectively. The rumen pH, total fungal and bacterial, protozoal count population in calf starter and soy powder have no impact. The value of volatile fatty acids and ammonia nitrogen shows that these were higher in treatment I and II group which may be due to the higher amount of soluble carbohydrate and good quality protein in calf starter and soy powder. The blood picture in terms of glucose and volatile fatty acids in control and treatment groups were 47.50, 60.00, 55.00 meq/lt of volatile fatty acids and 108.50, 114.11, 109.00 mg/dl of glucose respectively. This shows that the calf starter and soy powder have some effect on fermentation pattern of the rumen and increases the volatile fatty acids and glucose level. From this short term study with small numbers of calves, it was concluded that feeding of calf starter is essential to the calves at early period for obtaining better growth and early maturity.

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2. Use of different level of shilajit as growth promoter in broiler

Dharmendra Vyas and Aditya Kumar

The study was conducted for investigating the effect of shilajit as a growth promoter on the production performance, nutrient utilization and carcass quality characteristics in the broiler chicks. In this experiment five group of 4th day old chicks were taken, each having thirty chicks one group as a control and four as experimental which were fed shilajit at the level of 40, 60, 80 and 100g per 100 kg of feed. Experimental diet provide from 4th day to 7th week along with *ad libitum* water supply. The feed consumption and body weight of the birds were recorded at weekly interval. Two metabolic trials were conducted at the end of 4th week and 7th week of age respectively to find out the digestibility of organic nutrient and retention of calcium and phosphorous. A comparative evaluation of all the five group viz. control 40, 60, 80 and 100g of shilajit show that average body weight gain and efficiency of feed conversion ratio were 39.47g,

2.36 in control, 44.68g, 2.29 in 40g shilajit, 43.43g, 2.25 in 60g shilajit, 44.76g, 2.22 in 80g shilajit, 44.99g, 2.18 in 100g shilajit group respectively. This variation in production performance of broiler, gives as indication that shilajit have some effect on the tissue of the body that causes the better growth and utilization of the nutrient. The digestibility of nutrients and retention of nitrogen, calcium and phosphorous of both the trials were non-significantly difference among the groups. The carcass quality characteristics and dressing yield were non-significantly different in all the five groups. This can be concluded that addition of 40 to 100g shilajit per 100 kg of ration would be fed to broilers then their growth rate would be more than the normal. While the digestibility of nutrients and carcass quality would not be affected too much. For the final conclusion long term study with more number of birds with addition of different level of shilajit would be needed.

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3. Effect of multi-enzyme levels on the performance of commercial broilers

Sudhir Kumar Rathore and Aditya Kumar

The present study was undertaken to find out the effect of multienzyme supplementation on the performance of broiler chickens, viz. growth, feed intake, feed gain ratio and metabolizability of nutrients. The effect of experimental diet on carcass parameters was also investigated. One hundred fifty, day old commercial broiler chicks were randomly distributed to five dietary treatment groups consisting of thirty birds in each group. Similar, managemental conditions were maintained for all the birds during the period of seven weeks. Diet (T_1) was computed as per BIS (1992) to meet out metabolizable energy, crude protein and limiting amino acid (lysine and methonine) requirement of birds to serve as control. Enzyme supplemented diets i.e. T_2 (T_1+multienzyme @ 25 gm/100kg feed), T_3 (T_1+ multienzyme @ 50gm/100 kg feed), T4 (T_1+ multienzyme @ 75gm/100 kg feed), T_5 (T_1 + multienzyme @ 100 gm/kg feed) were formulated by adding commercial multienzyme. The average body weight gain and average feed intake per bird were recorded weekly and feed gain ratios were calculated for all treatments for different growth periods. Two metabolic trials were conducted first at the age of 4^{th} week and 2^{nd} at the age of 7^{th} week for the evaluation of digestibilities of dry matter, ether extract, crude fiber, nitrogen free extract and retention of nitrogen, calcium and phosphorus. Four birds per treatment were sacrificed at 7^{th} week of age to study carcass parameters. At the 3^{rd} day (beginning of experiment), the average live weight of chicks was 73.66 gm. Total body weight gain in overall growth period was 2227.66 gm in T_3, 2222.96 gm in T_2, 2164.98 IN T_4, 2152.98 gm in T_5 and 2020.27 gm in control. Enzyme supplementation increased body weight gain in all four groups in contrast to control group. However, the differences were non-significant among all the groups. The feed intake was statically similar during entire experimental period in all the groups. Enzyme supplementation diet showed non-significant increase in feed consumption than control. Improvement in FCR was statistically similar in T_2, T_3, and T_4 while improvement in FCR of T_5 groups was in between with these three groups (T_2, T_3, and T_4) and control (T_1). The over all, FCR of T_1, T_2, T_3, T_4, T_5 were 2.38, 2.22, 2.19, 2.25, 2.29 respectively. Dry matter metabolizability was significantly higher in enzyme supplemented groups during overall growth period. Enzyme supplementation resulted in significant improvement in nitrogen, calcium and phosphorus retention than control. Effects of treatment on carcass characteristics were not significantly different among the groups.

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4. Performance of guinea pigs on different rations

Vivek Gupta and Aditya Kumar

Present investigation was carried out to study the effect of feeding of different types of cereal grains (viz. gram, maize, oat, barley) on overall performance of guinea pig particularly on their growth rate, feed conversion ratio, nutrient utilization and blood glucose level. The present study was under taken to formulate the balance and economic diet for guinea pig by replacement of costly feed ingredients with cheap and easily available grains. Thirty five healthy guinea pigs (*Cavia porcellus*) of twenty one days were procured and these animals were adopted on a normal diet for three days. After adaptation, the individual guinea pig were weighted and then randomly divided into 5 groups of 7 animals in each having mean body weight of 153.43 g and fed five different type of complete ration. The rations contain similar ingredient (viz. wheat bran, berseem meal, soybean meal, fish meal, mineral mixture and vitamin-C) except grains, in which the gram was replaced by maize, oat, barley and a group was kept without addition of grain, also. The total experiment period lasted for 10 weeks duration, at each weak end total feed offered; live body weight gain and feed left over of each group were recorded. One digestion trial of three days duration at 8th week of experiment was also conducted with four guinea pigs in each group, to find out the digestibility of various nutrients and balance of Ca and P. During the entire experiment period G. pigs were offered feed and water *ad libitum* and besides the ration weighted amount of green berseem was also provided. To evaluate the best ration for guinea pig growth rate, feed gain ratio, nutrient utilization and blood glucose level were recorded. Result showed that the average dry matter intake (DMI) and body weight gain was higher in T-2 group, whereas lowest in T-5 group, while the other groups values were similar as compare to control. The feed gain ratio for group T-5 was significantly higher, while the other groups have similar feed gain ratio as compare to control. Dry matter digestibility coefficient of five experimental rations from T-1 to T-5 was 76.22, 78.75, 78.29 and 70.81, respectively. The digestibility of nutrients of T-5 ration is significantly lower as compare to control ration. The blood glucose, retention of calcium and phosphorus were not varying significantly between different groups. It was concluded that gram can easily be replaced from guinea pig diet with cheap grains like maize, oat and barley without affecting growth, feed gain ratio and digestibility of nutrients.

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5. Effect of different levels of crude fibre on the growth of weaned rabbits

Madan Murari Patel and Aditya Kumar

In this experiment investigations were carried out to study the effect of feeding of different levels of crude fibres (viz. 8%, 12%, 16%, 20%) on overall performance of rabbits particularly on their growth rate, feed conversion ratio, nutrient utilization and blood glucose level and total volatile fatty acid to evaluate a standard crude fibre level ration for feeding to rabbit in laboratory and rabbit rearing centers. Twenty healthy rabbits (*Oryctolagus cuniculus*) of 4-5 week old were procured and were adopted on a normal diet for three days. After adaptation, the individual rabbit were weighted and then randomly divided into 4 groups of 5 animals in each having an average 421.50 g weight. They were fed four different rations having crude fibre viz. 8%, 12%, 16% and 20%. All the rations contain similar ingredients and green berseem. The total experiment period lasted for 9 weeks duration, at each weak end total feed offered; live body weight gain and feed left over of each group were recorded. One digestion trial of three days duration at last week of experiment was also conducted with four rabbits in each group, to find out the digestibility of various organic nutrients and balance of Ca and P. During experimental

period rabbits were offered feed and water *ad libitum* and weighted amount of green berseem was also provided as per level of CF. To evaluate the best CF ration for rabbit growth rate, feed gain ratio, nutrient utilization and blood glucose level and total volatile fatty acid of all groups were recorded. Result showed that the average feed consumption was 37.74, 37.09, 43.15, and 49.11 g per rabbit per day in group T-1 to T-4, respectively. The highest average feed consumption (on dry matter basis) was observed in T-4 group, whereas lowest in T-2 group. The overall weekly average body weight gain was 74.44, 98.44, 84.67, and 88.89 g. The body weight gain is highest in T-2 group, whereas the lowest weekly body weight gain is observed in T-1 group. When compare all the groups the T-2 group has significantly differed from the T-1 group and higher than the T-3 & T-4 groups. The weekly average feed gain ratio in T-1, T-2, T-3 and T-4 group was 3.61, 2.64, 3.73 and 3.97, respectively. The feed gain ratio of T-2 is significantly lower than the other groups due to optimum level of crude fibre in the diet. Digestibilities of nutrients in T-2 group were significantly higher as compare to other groups. The blood glucose level, retention of calcium and phosphorus in rabbit were not significant and shows an indication of positive growth in rabbits. From this experiment it is concluded that 12% level of CF in T-2 ration was good for optimum growth, FCR and digestibility of nutrients etc than the 8%, 16% and 20% level of CF in the rabbit diet.

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6. Screening of medicinal plants as feed additive using *in vitro* gas production test

Jyoti Sachan and Ravindra Kumar

Plants rich in secondary metabolites (saponins, tannins, essential oils, etc.) have antimicrobial activity which can be exploited for selective inhibition of a particular group of microbes in the rumen. We have screened a large number of plant extracts for their potential to change the digestibility and gas production in *In vitro* gas production test using buffalo rumen liquor. Out of 10 plant extracts/spices tested using *In- vitro* gas production test, 4 increased the gas production and digestibility potential by *In vitro* gas production test with buffalo rumen liquor. pH of rumen liquor was not affected by incorporation of herbal feed/spices tested as compared to control. Gas production (ml/gDM) was significantly higher on *Syzygium aromaticum* (130.87) and Ferula asafetida (138.33) as compared to control (119.49). The *In-vitro* true digestibility was significantly ($p<0.05$) higher by addition of *Cuminum cyminum Linn* (seed) and *Trigonella foenun-graecum* (Seed). The *In vitro* organic matter digestibility was significantly ($P<0.05$) for addition of *Trigonella foenun-graecum* (Seed) higher by compared to control. Addition of *Ferula asafetida* and *Aloe barbadanis* (Plant extract) tends to improve DMD and OMD in dose dependent manner. *Cucurma longa* and *Piper nigrum* had no effect on the various Parameters studied. *Acacia Concinna* extracts suppressed IVDMD and IVOMD of feed, various levels of the extract should be tested to find out a suitable. From present study this can be concluded that *Cuminum cyminum Linn., Trigonella foenun-graecum, Ferula asafoetida* and *Aloe barbadanis* has potential to be used as feed additive to improve the digestibility of feed.

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7. Manipulation of dietary cation-anion difference to reduce nutrient deficiency in peri-partuirent cows

Bokan Abhay Mohanrao and Vinod Kumar

The advanced pregnant animal's diets can be manipulated simply by adding relatively more anions or cations which affect blood buffering capacity and acidity. In present study, twelve Haryana cows in advanced pregnancy divided into 3 groups i.e. G1, G2 and G3 (n=4)

experimental animals received diets containing +11, +21 and +31 mEq per 100 g DM DCAD. Requirement of the animals were fulfilled by feeding basal ration containing concentrate mixture, maize fodder and wheat straw with supplementation of 83, 50, and 109g/d mineral mixture per cow in G1, G2 and G3, respectively. All experimental animals were maintained from -30 days pre-partum to +7days postpartum experimental period. Daily Feed intake and fortnightly body weights were not significantly different (P>0.05) in three groups. Feeding of +11 mEq/100 g of DM DCAD diets improved blood calcium levels in periparturient cows in their last month of pregnancy without affecting dry matter intake and balance of other minerals. Feeding of +21 mEq/100 g of DM DCAD diets provide sufficient buffer and mineral balance for pregnant animals and improve milk yield post calving. Feeding of diet higher than +31 mEq/100 g of DM DCAD raised blood pH more than 7.3 and leads to metabolic alkalosis. Feeding of manipulated DCAD did not adversely affect the reproductive health of cows in all three respective groups. It can be concluded that feeding of +11 mEq/100 g of DM DCAD diet during advanced pregnancy maintain blood Ca homeostasis from bone and however feeding of +21 mEq/100 g of DM DCAD diet can provide normal mineral balance and improve milk production in periparturient cows.

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8. Effect of supplementing rumen-protected lysine and methionine on growth performance and nutrient utilization of growing Hariana cattle

Jai Kumar Singh and Debashis Roy

Present study was conducted to see the effect of supplementing rumen protected methionine (RPM) and rumen protected lysine (RPL) on growth performance, blood biochemical and nutrient utilization of growing Hariana heifers. Eighteen growing Hariana heifers were randomly distributed into three groups i.e C, T1 and T2 on body weight basis. Chemical compositions of all the dietary components were found to be in normal range. Animals in T1 and T2 group were supplemented with 1 g RPM, 5 g RPL and 2 g RPM, 10 g RPL along with basal diet. The average body weights (kg) and metabolic body weight (kg $W^{0.75}$) of heifers were not significantly different (P>0.05) within groups at all the fortnights. Animals of T1 and T2 groups showed significant increase in fortnightly body weight gain and ADG in 4th and 5th fortnight, respectively as compared to control. Though DMI (Kg) did not differ significantly within groups, DMI% decreased (P<0.05) significantly in both the treatment groups at 1st, 4th, 5th and 6th fortnight.FCR was found lower (P<0.05) for T1 group as compared to control at 3rd and 4th fortnights whereas, both T1 and T2 groups were lower (P<0.05) in FCR in comparison to control at 5th fortnight.Body condition score (BCS) of animals were found similar in all the groups throughout the experimental period. The overall average BCS ranged from 2.17 to 3.80. The total protein ranged from 7.22 to 7.78 (g/dl) in control, 6.59 to 7.68 (g/dl) in T1 and 6.31 to 7.10 (g/dl) in T2 group, respectively. The plasma albumin concentration of experimental animals varied from 3.42 to 3.43 (g/dl) in control, 3.06 to 3.46 (g/dl) in T1 and 3.10 to 3.39 (g/dl) in T2 groups. Initially Immunoglobulin concentration was found to be 29.60, 29.60 and 29.58 mg/dl in control, T1 and T2 groups, respectively. Final values at the end of third month of the trial were 29.67, 29.69 and 29.72 mg/dl respectively. Though, AST activity of T2 (78.03 IU/l) was significantly higher (P<0.05) than control (61.12 IU/l) at the end of second month of trial, ALT and AST activities were statistically similar. Though BUN concentration of treatment groups was lower than control group during whole trial period but the difference was not statistically significant. Average plasma creatinine concentration varied from 0.80 to 1.47 mg/dl in control, 0.38 to 0.75 mg/dl in T1 and 0.41 to 0.57 mg/dl in T2 groups, respectively. At the end of trial, the creatinine concentration was found lower (P<0.05) in both treatment groups as compared to control groups. Digestibility coefficients of all the nutrients i.e DM, OM, CP, EE, CF, NFE, NDF and ADF were found similar

in treatment and control groups. Though digestibility coefficient of CP were higher in both the treatment groups than control, the difference was not statistically significant. Similarly digestible nutrient intake was also found similar in control and treatment group. In conclusion, supplementing basal diet with rumen protected lysine and methionine was found beneficial in terms of weight gain and feed efficiency without affecting protein metabolism, liver function and nutrient digestibility.

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9. Effect of strategic mineral mixture supplementation on nutrient utilization and blood mineral profile of heifers

Vivek Prasad Gupta and Vinod Kumar

The present study was to access the mineral status of different feed stuffs, animals and strategic mineral mixture supplementation on nutrient utilization, growth performance and blood biochemical in heifers. Commonly available feeds and fodders used for feeding of animals were assessed for mineral content. Dry roughage like wheat straw is found deficient in Ca (3.33%), P (52.00%), Cu (54.75%) and Zn (60%) than critical level. However green fodder like maize and sorghum is deficient in Na and Mn. Percent deficiency of Na in maize and sorghum was 16.67 and 73.33% respectively. Level of Mn deficiency in maize and sorghum were 42.83 and 73.83%, respectively. Leguminous green like berseem was found to be deficient in Na and deficiency level was 84.67% than critical level. Regarding cereal grain (barley, oat and wheat grain) were deficient in Ca, Na, Mn and Cu and level of deficiency were ranges between 67.33 to 84.00%, 46.67 to 83.33%, 3.25 to 20.00 and 7.50 to 11.25%, respectively. In present findings, cattle and buffalo of different stage of maturity and stage of production were assessed for their mineral status and plasma levels of different minerals were above the recommended critical level except Cu. In animal trail, eighteen heifers were randomly blocked into three (G1, G2 and G3) groups having six animals in each on body weight basis and fed for 60 days. The requirements of experimental animals were met by feeding concentrate mixture, green fodder, wheat straw (NRC, 2001). Experimental heifers either received a basal diet devoid of supplemental mineral mixture (G1) or supplemented with type 1 (G2) and type 2 (G3) mineral mixture. Effect of feeding type 1 and type 2 mineral mixtures on nutrient intake was recorded daily. However effect on body weight change and blood biochemical was recorded at 0, 15, 30, 45 and 60 days of mineral mixture supplementation. Supplementation of type 1 and type 2 mineral mixtures did not have any effect ($P>0.05$) on dry matter intake (DMI) and body weight change. In present findings supplementation of type 1 and type 2 mineral mixtures have significant effect ($p<0.05$) on Ca and Na absorption (gram/day). However, supplementation of mineral mixture did not affect intake, absorption and faecal excretion of P and Mn. Feeding of type 1 and type 2 mineral mixtures in heifer have significant effect ($P<0.05$) on intake, absorption and out go of Cu and Zn among three respective groups. Plasma Ca, Cu, Zn, Fe and Mn level was significantly affected ($P>0.05$) by supplementation of mineral mixture and plasma level was found higher in group G2 and G3. Conversely, plasma level of Na and P was not affected by feeding type 1 and type 2 mineral mixtures. In present findings, supplementation of strategic area specific mineral mixture did not have any effect on liver function, immunity and antioxidant status of heifers fed on type 1 and type 2 mineral mixtures. In conclusion, feed stuffs and animal's blood were deficient in vital minerals and supplementation of type 1 and type 2 mineral mixtures in heifers help to improve their plasma mineral status without altering nutrient utilization, growth performance and blood biochemical.

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Ph.D.

1. Chromium picolinate supplementation to layers and broilers for designer eggs and designer broiler meat production

Jyoti Palod and Aditya Kumar

The present investigation was carried out to study the effect of chromium picolinate supplementation on the production performance, carcass traits, carcass composition, certain serum biochemical and health status related parameters in commercial broilers and production performance, egg quality traits, egg composition, certain serum biochemical and health status related parameters in commercial layers. In the first phase one hundred and fifty, day old broiler chicks were procured and kept on deep litter system. For first 2 days, chicks were kept on standard starter ration and plain water. On third day, one hundred and twenty eight broiler chicks were selected on the basis of uniform average body weights and were divided into 4 treatment groups i.e. control, water containing organic chromium @ 200ppb, 400 ppb and 600 ppb (each treatment with two replicates), having sixteen birds each for a period of 6 weeks. Production parameters were recorded on weekly basis. A metabolic trial of 4 days duration was conducted at the end to study the effect of organic chromium supplementation on retention of nutrients. At the end of trial 6 broilers from each replicate group were slaughtered to measure different carcass traits and carcass composition. Representative blood samples were also collected at the time of slaughter to determine certain serum biochemical parameters. In second phase experiment was conducted with layers for 30 weeks. During this feeding trial, growth and production parameters were fortnightly studied. Egg quality and egg composition parameters were studied for 23 -26 weeks (Phase I), 27-30 weeks (Phase II) and 23 - 30 weeks (overall period) while nutrient retention, lipid profile of yolk and serum biochemical parameters were studied at the end of trial i.e. at the end of 30th week. Based on the results of Phase I, another experiment was conducted with the same experimental design except difference in dose rate. The dose rate was 0, 800, 1600 and 2400 ppb levels of chromium in treatment groups 1 to 4, respectively. In this phase blood was collected at the end of experiment for biochemical as well as health status related studies. From this study it is concluded that the production performance of broilers in the present investigation was best in 600 and 800 ppb levels of chromium supplemented groups in Experiments I and II respectively. However, between 600 and 800 ppb level groups, later dose level group performed better. For designer meat production most of the parameters showed best results at 600 and 2400 ppb level groups in Experiments I and II, respectively. However, broilers provided 1600 ppb organic chromium showed statistically similar response comparable to 2400 ppb level. When all the levels of chromium supplementation were considered 600 ppb level of chromium supplementation was best for designer meat production in the present investigation. Growth performance of layers during starter and grower period and production performance of laying hens was best at 600 and 2400 ppb levels of chromium supplementation in Experiment I and II, respectively. Considering the 600 and 2400 levels of chromium supplementation in comparison to their respective control, 600 ppb level was found to be better as compared to 2400 ppb level. The important parameters considered for production of designer egg production (cholesterol, HDL-cholesterol, LDL-cholesterol and chromium content) revealed 600 ppb level to be best in Experiment I and 2400 ppb chromium level in Experiment II. All these parameters were statistically similar for 1600 and 2400 ppb levels of chromium. Most of the parameters considered for designer eggs were found to be better at 1600 ppb level as compared to other levels of organic chromium supplementation. Therefore, it can be finally concluded from the results of present investigation that organic chromium supplementation through water may be advised at 800 ppb level to improve production performance of broilers, 600 ppb level for designer meat production, growth performance of starter and growers and production

performance of laying hens and 1600 ppb level for designer egg production which is the choice of health conscious consumers these days.

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2. Evaluation of different varieties of maize for their composition, *in vitro* rumen fermentation and production performance in lactating cows

Shalini Vaswani and Ravindra Kumar

The present study was conducted to know the chemical composition, *in vitro* rumen fermentation pattern and performance of different maize varieties. Seeds of Sorghum (Purva) along with four normal maize varieties (HTHM 5101, DHM 117, HM 5 and Shaktiman/ 900M Gold) and three QPM varieties (HQPM 5, HQPM 7, HQPM 9/ Vivek) of maize procured from CIMMYT Centre, New Delhi were grown in different plots of ILFC, DUVASU, Mathura under same environmental and agronomic conditions. Fodder from these varieties was harvested at 45-50 days (pre-cob) and 80-90 days (post-cob) stage after sowing. Maize grains were also collected at post cob stage. The nutrient composition of Sorghum, different varieties of maize fodder at pre-cob and post-cob stage and maize grain was found in the normal range. The variety DHM 117 at pre-cob stage exhibited higher values for CP (10.62%), EE (2.70%) and NFE (65.49%) whereas, the content of CF (16.16%) and TA (5.03%) was found lower in comparison to other varieties. The CP content decreased and the DM, CF, NDF, ADF, Cellulose and ADL content of different varieties increased at post- cob stage in comparison to pre-cob stage of fodder. The CP (14.06%), EE (8.97%), CF (3.37%) and TA (4.20%) of grains of DHM 117 variety was found higher in comparison to other maize grain varieties. The Ca (%), Fe (ppm) and Mn (ppm) was reported to be higher in the pre-cob and post-cob maize fodder than the grains of different varieties of maize. The Mn (ppm) content of HQPM 9 variety was found higher at both pre-cob (102.66) and post-cob (72.54) stage than other maize varieties (33.58 -55.56) and (25.92-52.02) at pre and post-cob stage respectively. *In vitro* rumen fermentation parameters of different varieties of maize fodder singly and in combination wheat straw+ maize fodder (50:50) and wheat straw + fodder+ concentrate (40:40:20) in different feeding systems were analyzed using *in vitro* gas production test with buffalo rumen liquor. The IVDMD (%) and IVOMD (%) was significantly ($P<0.05$) higher in HQPM 7 and HQPM 9 varieties. The ammonia nitrogen (12.16 mg/dl) and PF (5.06) was significantly ($P<0.05$) higher in DHM 117 variety. The microbial protein synthesis (mg) and the pH did not differ significantly ($P>0.05$) among different varieties. The IVDMD (%) was significantly ($P<0.05$) higher for HQPM 5 (53.79) and HQPM 7 (53.30) varieties when the different varieties were incubated along with wheat straw in 50:50 ratio. The pH and ammonia nitrogen did not differ significantly. The partitioning factor (PF) was significantly higher ($P<0.05$) for variety Shaktiman (7.18) and lower for DHM 117 (4.28) variety. The IVDMD (%) and IVOMD (%) was significantly ($P<0.05$) higher for HQPM 5, HQPM 7 and HQPM 9 varieties when the varieties were incubated with wheat straw and concentrate in 40:40:20 ratio. The pH, microbial protein synthesis (mg) and partitioning factor (PF) did not differ significantly among different varieties. Thus, under different feeding systems, the QPM varieties i.e. HQPM 5, HQPM 7 and HQPM 9 have shown better digestibility and other rumen fermentation parameters in comparison to other varieties. *In vitro* net methane (1.50 ml), methane per gm dry matter (7.47ml/g), and methane per gm digestible dry matter (14.13 ml/g DM) was significantly ($P<0.05$) lower for DHM 117 variety as compared to other varieties with goat rumen liquor. Similarly net methane (3.27 ml), methane per gm dry matter (16.27 ml/g) and methane per gm digestible dry matter (16.86 ml/g DM) was also lower for maize grain of DHM 117 variety with goat rumen liquor. The milk yield (kg/d), 4% FCM milk yield (kg/d) and milk composition (%) (Fat, protein, lactose, total solid and SNF) of Sahiwal cows fed with different varieties of maize fodder was

statistically similar at pre and post-cob stage. Nutrient intake and digestibility of different groups of cows fed with different varieties of maize fodder were studied at pre-cob and post-cob stages of fodder. During pre-cob stage, the DMI (kg), green fodder intake (kg) and total roughage intake (kg) was also significantly ($P<0.05$) higher for DHM 117 variety. The DCP (g/d) and TDN (kg) was significantly higher for DHM 117 variety. Digestibility (%) of DM, OM and CP was significantly ($P<0.05$) higher for DHM 117 variety. At post cob stage, DMI (kg), green fodder intake (kg) was significantly ($P<0.05$) higher for DHM 117, HQPM 9 and HTHM 5101 variety. The DCP (g/d) was significantly ($P<0.05$) higher for variety HTHM 5101. The TDN (kg) showed non-significant difference but, the TDN (kg/100kg BW) and TDN (g/ $kgW^{0.75}$) was significantly ($P<0.05$) higher for DHM 117 variety. Digestibility (%) of DM and OM was significantly ($P<0.05$) higher for DHM 117, HM 5 and HTHM 5101 variety. From present study, it can be concluded that nutrient composition varies with different varieties grown in similar environmental and agronomic conditions. The methane production was least for grain and fodder of DHM 117 variety of maize. Milk yield and composition was not found to be significantly affected by different varieties of maize fodder while digestibility and intake of nutrients was found to be affected.

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Livestock Products Technology

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M.V.Sc.

1. Effect of chicken meat on quality characteristics and storage stability of noodles prepared from different flours

Akhilesh Kumar Verma and Vikash Pathak

Snacks like noodles are fast foods relished by all segments of society due to variety, convenience and capability to satisfy the short term hunger. The consumption of snack foods is increasing day by day due to rapid urbanisation, increase in per capita income and socio-economical changes and finally the changing life styles of people. The snack food market is one of the largest markets in the world but most of the snacks available in the market are cereal based and lack in essential nutrients. So various cereal flours were replaced with different levels of minced chicken meat to enhance the nutritional value for the assessment of suitability and compatibility of chicken meat noodles. Emulsions prepared from various flours replaced with different meat levels were subjected to measure pH and emulsion stability. The results obtained for these parameters revealed decreased pH as well as emulsion stability with the increase in meat level in all the formulations. This might be due to acidic nature and lower binding ability of meat. The physico-chemical analysis of the fresh chicken meat and control noodles revealed increasing trend of moisture in all of the products while ash, protein, fat, water absorption index, water solubility index increased in whole wheat flour, rice flour and refined wheat flour based chicken meat noodles and decreased in soybean chicken meat noodles with increase in the level of meat. However, other parameters such as crude fibres and cooking loss showed overall decrease in the values with increase in meat levels whereas, volume and weight increase parameters decreased on increase of meat levels in all of the products except soybean flour based noodles. The selected products i.e. P1, P2, P3 & P4 were stored for 30 days at 30±2°C under aerobic packaging and subjected for quality analysis on 0, 10, 20 & 30th day of storage. The data obtained for storage study overall revealed increasing trend in water activity, moisture, free fatty acid, TBA, water absorption index, crispiness, TPC and yeast and mould whereas decreasing pattern in values of protein, crude fibre, fat, ash, water solubility index, hardness, work of shear and all sensory attributes over the storage period of 30 days. All the sensory attributes and some physico-chemical parameters decreased non-significantly ($P>0.05$) and were very well within the acceptable limit. TPC and yeast and mould counts increased in all the groups throughout the storage period but within the safe limit. Overall sensory parameters revealed the maximum scores for P2 (refined wheat flour based chicken meat noodles containing 40% minced chicken meat) followed by P3>P4>P1. The cost of production of this product was also under the medium range (Rs 38.72 per 80 gm chicken meat noodles including cost of 8 gm of taste maker) as compared to other selected chicken meat noodles i.e. P1, P3 & P4 (43.52, 50.72 & 34.72 per 80 g chicken meat noodles including cost of 8g of taste maker for P1, P3 & P4 respectively).

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2. Studies on development on quality characteristics of self stable chicken sticks

Gaurav Kumar and Vikash Pathak

Chicken meat sticks three different flours (gram flour, rice flour and refined wheat flour) were taken separately in four different batches i.e. G1, G2, G3 and G4; R1, R2, R3 and R4; and W1, W2, W3 and W4 respectively replaced with different levels of minced chicken meat (0, 50, 60 and 70%) for each flour. The freshly prepared sticks were subjected to physico-chemical, sensory and proximate analysis for final selection of meat level in each flour. Values for pH of emulsion and sticks both decreased high significantly ($P<0.01$) with increase level of minced chicken meat in all three flours in linear way while emulsion stability of dough showed an increasing trend in gram

flour based meat sticks and decreasing trend in rice flour and refined wheat flour based meat sticks in highly significant ($P<0.01$) manner with increased level of meat incorporation. A highly significant decrease ($P<0.01$) was noticed in cooking yield for all treatments with increased in minced chicken meat due to higher moisture content in meat (70-75%) as compared to flours used in study. Moisture and ash was significantly ($P<0.01$) higher in all treatments replaced with increased levels of minced chicken meat in chicken meat sticks as compared to control of respective flour. Protein and fat decreased high significantly ($P<0.01$) in gram flour based chicken sticks with increase in chicken meat level but increased high significantly ($P<0.0$) in meat sticks prepared by replacement of rice and refined wheat flour with different levels of chicken meat. The overall mean scores sensory parameters like appearance and color, texture, flavor, mouth coating, meat flavor intensity and overall acceptability were observed highest in G4, R3 and W4 for gram flour, rice flour and refined wheat flour based chicken meat sticks among all treatments from each flour. Saltiness had no significant ($P>0.05$) difference between treatments for any flour. Meat sticks prepared with different flours, G4 containing 70%, R3 containing 60% and W4 with 70% of minced chicken meat replaced with gram flour, rice flour and refined wheat flour respectively, were finally selected to study quality characteristics of shelf stable chicken sticks on 0, 10, 20 & 30th day of storage. TBA, FFA and water activity decreased high significantly ($P<0.01$) with the advancement of storage period and was highest in G4. Moisture, protein, fat and ash contents were highest in G4. Moisture increased high significantly ($P<0.01$) in all treatments throughout the storage period. The values of hardness and work were observed highest in R3>W4>G4 but crispiness values for G4>R3>W4. There was no significant ($P>0.05$) difference in mean TPC, yeast and mould count in between treatments on 0, 10, 20 and 30th day during storage of chicken sticks. *Staphylococus*, Coliforms and *Salmonella* were not detected during whole storage period in any treatments due to high processing temperature and hygienic handling and packaging of product. The scores for all sensory parameters including overall acceptability were highest in G4 and decreased significantly ($P<0.05$) in all treatments except saltiness.

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3. Technology development and quality assessment of chicken meat biscuits

Raj Kumar Jaiswal and Vikas Pathak

Keeping in view of demand of biscuits and intervention of nutrition in them, present investigation was carried out to study the technology development and quality assessment of chicken meat biscuits by replacement of refined wheat flour with different levels (0%, 40%, 50% and 60%) of chicken meat powder prepared by mincing and dehydration of chicken meat which were abbreviated as A, B, C and D respectively. Various physico-chemical properties, proximate estimation, microbiology, texture analysis and sensory evaluation were carried out on 0, 10th, 20th and 30th days of storage at ambient temperature in aerobic and vacuum packaging, where as emulsion stability of raw dough and cooking yield of freshly prepared chicken biscuits were evaluated on 0 day only. There was significant difference ($P<0.05$) found for emulsion stability of dough between treatments, but no significant difference was found between A, B and C. Cooking yield decreased significantly ($P<0.05$) with increase in meat incorporation and found to be highest in A, followed by B>C>D. A linear decrease was found significantly ($P<0.05$) in pH with replacement of refined wheat flour with increasing level of chicken meat powder, due to acidic nature of meat in both packaging conditions. TBA and FFA values for chicken meat biscuits were found to be significantly ($P<0.05$) increased with increased level of meat incorporation due to higher fat in meat as compared to refined wheat flour. Fat, moisture and ash content increased significantly ($P<0.05$), but protein content increased highly significantly ($P<0.01$) with increase in chicken meat powder level. Work of shearing and shear force values

decreased in linear way A>B>C>D due to higher moisture level in meat powder as compared to refined wheat flour. No significant (P>0.05) difference was observed in between the treatments for TPC and yeast and mould count in all four days of storage but increased high significantly (P<0.01) in aerobic and significantly (P<0.05) in vacuum packaging over storage period. The vacuum packaged chicken meat biscuits showed almost negligible microorganism growth due to absence of air. The total absence of *Staphylococus, Coliform* count and *Salmonella* count was reported during whole storage period in both packaging conditions. The color and appearance and texture scores decreased with increase in meat powder incorporation, but all the variants were well accepted by all sensory panelists. Meat flavor, meat flavor intensity, mouth coating and salting scores increased with increased chicken meat powder level. The semi trained sensory panelists accepted all formulation of meat biscuits, but the highest overall acceptability scores were awarded to C among all the treatments. On the basis of result obtained, chicken meat biscuits containing 50% of chicken meat powder (C) can be selected as the best treatment in both packaging conditions.

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4. Studies on quality evaluation of some important edible byproducts of Barbari goats (*Capra hircus*)

Pramila Umaraw and Vikas Pathak

The production of edible byproducts is concomitant with meat production. The present utilization of edible organs is much lower than its potential. Edible byproducts of Barbari kids constitute about 3% of the live weight of an animal of which liver contributed maximum (1.47%). This could increase the saleable cost of animal by 6.94%. Physicochemical, proximate, mineral, fatty acid profile, texture, colour, and microbiological analysis was conducted taking *Longissimus dorsi* muscle as reference. Physicochemical properties revealed a higher pH values in all organs as compared to reference muscle. Cooking loss varied significantly (p<0.05) among the organs. Maximum cooking loss was observed in kidneys (34.67%). Proximate analysis of each organ was conducted to find out the nutritive value. The moisture, protein, fat, ash, carbohydrate and energy values differed significantly. Except liver all organs evinced higher moisture values than muscle. Protein content of offals was significantly (p<0.05) lower than that of muscle. Liver and heart exhibited high protein content in comparison to other organs (19.66% and 16.08%). Fat content was found to be highest in brain followed by heart (8.49%). Ash content was significantly (p<0.05) highest in spleen (3.30%) whereas carbohydrate value was highest for liver (1.76%) and brain (1.85%). Percent energy value was significantly (p<0.05) different among all organs studied. Liver had the highest energy value (133.8%). Mineral profile analysis revealed significant difference between muscle and organs and even differed significantly among them. Kidney had highest sodium content (202.39mg/100g), potassium content was highest in testicles (362.61mg/100g). Copper, iron and zinc were found to be highest in liver (6.97mg/100g), spleen (31.1661mg/100g) and muscle (4.1561mg/100g). Fatty acid contents displayed significant difference among organs and muscles. Each organ had its characteristic fatty acid content. Saturated fatty acid content differed significantly and spleen evinced the highest value (54.95%) although monounsaturated fatty acid content was highest in muscle (40.36%). Polyunsaturated fatty acids were maximum in liver (22.54%). PUFA/SFA ratio of liver (0.49) was similar to the recommended level. Spleen, brain and testicles showed favorable n6/n3 ratio. All edible byproducts exhibited characteristic textural and color parameters. Liver required the maximum shear force and work of shear (121.48N and 32.19 kg-sec). The total viable count (TVC), Coliform count showed slight differences for all organs studied. The *Staphylococcus* counts were low with little differences among organs.

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5. Development and quality assessment of meat momos

Tanuja and Vikas Pathak

The present study was conducted for the development and quality assessment of health oriented meat Momos. The meat level and cooking time were optimized on the basis of physic-chemical parameters, colour and textural profile and sensory attributes. The 50% meat level with 30 minutes cooking time was selected with significant ($P<0.05$) difference in cooking yield, moisture content, protein content, and non-significant difference in color and textural profile. The optimized product was improved with the incorporation of three different percent of corn and potato starch (4%, 6% and 8% separately) in the dough during the preparation of chicken momos. The chicken momos containing 6% corn starch in dough was selected as the best treatment on the basis of physicochemical, color and textural parameters and sensory attributes. There was significant ($P<0.05$) difference observed in cooking yield, moisture content, protein content, fat content, carbohydrate and energy values. The mean L^*, and a^* values were differ significantly ($P<0.05$) and highest for 6% corn starch but b^* value differ non-significantly and increased with potato starch. The textural parameters i.e. hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness values increased slightly in a non-significant manner with increased level of corn as well as potato starch. Potato starch showed more hardness, gumminess and chewiness values than corn starch. The mean appearance, flavour, texture, mouth coating and overall acceptability values were significantly ($P<0.05$) higher for momos with 6% corn starch. Further study was conducted by replacing the chicken meat with four different variants of fish meat i.e. 25%, 50%, 75% and 100% for enhancing the nutritional value of meat momos. There was no significant difference was found in mean pH value, cooking yield, weight gain and water activity but significant difference ($P<0.05$) was observed in mean moisture content, fat and ash content with increased level of fish. The mean L^*, a^* and b^* values differ in non-significant manner in which lightness and yellowness slightly increased with fish meat but redness decreased. The hardness, springiness, gumminess and chewiness values decreased but adhesiveness and cohesiveness values increased non-significantly with fish meat incorporation. The mean flavour, texture, meat flavor intensity, mouth coating and overall acceptability values were highest for F3 (75%) in comparison to other treatments. Meat Momos prepared with the incorporation of 75% fish meat were finally selected to study the quality characteristics at 0, 3, 6, 9 and 12 day of storage with control and 100% fish meat level at refrigeration temperature (4±1oC). The mean pH value was non-significantly higher for F4>F3>S2. The mean values for TBA and FFA increased in highly significant ($P<0.01$) manner with the advancement of storage period and found to be highest in F4. The Total plate count, Psychrophillic count and Yeast and mould count were significantly ($P<0.01$) higher but within the safety limits upto 9th day of storage. *Coliforms* and *Salmonella* were not detected during whole storage period in any treatment due to steam cooking and hygienic handling during processing and storage. The scores for all sensory parameters including overall acceptability were significantly ($P<0.05$) higher for F3 as compared to F4 and S2.

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Poultry Science

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M.V.Sc.

1. Quality and shelf-life of spent hen meat cubes

Subhash Chandra and P.K. Shukla

A study was undertaken to evaluate the quality and shelf-life of spent hen meat cubes. Spent hen minced meat cubes were evaluated for physic-chemical properties, microbiological quality and organoleptic (sensory) attributes at regular intervals from 0 to 9 days of refrigeration storage (4±1°C). There were four products (1) Control, (2) Tetra sodium pyrophosphate (TSPP) (3) Tocopherol and (4) TSPP with tocopherol. Chicken cubes of very good acceptability and nutritive value could be prepared by the incorporation of TSPP or TSPP with tocopherol. Chicken cubes packed in polyethylene bags for a period of 9 days in refrigeration (4±1°C) condition, remains acceptable for consumption. The efficacy of different food additives used in the preparation of spent hen meat cubes in the present investigation was best for TSSP with tocopherol followed by TSPP and tocopherol. There was an increase in microbial count with increasing storage intervals. However, the various microbial counts were well below the threshold value of log 7.0 in these products. Thus, the products were microbiological safe up to 9 days of refrigeration storage. Incorporation of TSPP or TSPP with tocopherol significantly ($P<0.01$) improved the quality and ultimately extended shelf-life of product. The overall acceptability of product (cubes) at 9th day of storage remained good. However, the overall acceptability of control and tocopherol containing samples was significantly low as compared to TSPP or TSPP with tocopherol containing samples. Hence, TSPP along with tocopherol may be added to improve the quality and shelf-life of spent hen meat cubes.

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2. Effect of dietary antistressors on the performance of commercial broiler in tropical climate

Neeraj Garg and P.K. Shukla

An experiment was undertaken to study the comparative performance of commercial broiler fed either the standard broiler ration or the commercial broiler fed ration supplemented with various antistressors. Ninety one week old straight run commercial broiler chicks were procured and divided into six dietary treatment groups comprising of three replicates and five chicks in each replicate. The first group was kept as control diet not supplemented with any kind of antistressors. The other four treatment groups were supplemented with ascorbic acid (200 ppm), iodine (4 ppm), ammonium (1%) and soda bicarb (0.5%). The last group was supplemented with all the antistressors in the concentration as described above. The total experimental period lasted for six weeks, starting from second week to seventh week of age. The average mean ambient temperature during the experimentation was recorded as 31.52±0.47 °C and mean relative humidity as 68.69±0.92%. The cumulative feed consumption up to 7 week of the birds was not significantly ($P\leq0.05$) affected by the supplementation of antistressors in the diet. However, the control group and the birds given ascorbic acid (200 ppm) showed a numerical decrease in over all feed consumption. The overall total body weight gain of the commercial broiler was not significantly ($P \leq 0.05$) affected by the supplementation of various antistressors. The overall feed gain ratio was not significantly ($P \leq 0.05$) affected by the supplementation of various antistressors in the diet. However, the birds kept on the diet having all the antistressors showed improvement in feed conversion ratio as compared to the control. The balance of crude protein, calcium and phosphorous were recorded to be positive for all the treatment groups during both hr metabolic trials i.e.. at 4 weeks and 6 weeks of age. However, the retentions of these nutrients were not significantly ($P \leq0.05$) affected by the supplementation of dietary antistressors. The carcass quality characteristics were not significantly ($P \leq 0.05$)

affected by the various dietary antistressors. Mortality during the entire period of experimentation ranged between 0 to 7.77%, which was considered to be normal and was not attributed to any of the experimental treatments. In the present investigation, the production parameters were not found to be significantly affected by supplementation of various dietary antistressors. However, a numerical improvement regarding these parameters was evident. The temperature variations and R.H. registered in this study, therefore, seemed to be insufficient to induce heat stress in the commercial broiler or probably these birds were well adapted to the tropical climate.

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3. Estimation of genetic diversity in different poultry species by using different DNA based markers

Sandeep Sudhakar Joshi and P.K. Shukla

A study was undertaken to estimate genetic diversity among five poultry species i.e. chicken, duck, guinea fowl, quail and turkey using two types of DNA markers namely randomly amplified polymorphic DNA (RAPD) markers and microsatellite associated sequence amplification (MASA) markers. Genomic DNAs were isolated from blood (nucleated RBCs) of 10 birds (5 males + 5 females) in each of the five poultry species with a yield of 200-300 μg per 50μl blood. The genetic similarity between the species, pooled over the primers ranged from 0.169 to 0.329, which suggested a very low degree of genetic similarity among the poultry species. Among these, the minimum genetic similarity (0.169) was observed between duck and turkey. In general, the duck showed lower genetic similarity with other species also. Chicken and guinea fowl showed maximum genetics similarity at the low level of genetic correlation. The genetic distances, based on band sharing (DS) among the five poultry species pooled over primers were 1.112 to 1.778. While the minimum genetic distances were observed between guinea fowl and chicken (1.112), the turkey and duck were most distant (1.778). The between genetic similarity estimates, based on both type of the markers varied not only in magnitude, but also for trend among the poultry species. The genetic similarity estimates based on MASA markers (0.286 to 0.679) were higher than those based on RAPD markers (0.169 to 0.329). Using RAPD markers, minimum genetic similarity was observed between duck and turkey (0.169), followed by turkey and quail (0.202), duck and chicken (0.224), chicken and turkey (0.227), duck and quail (0.230), guinea fowl and turkey (0.233), guinea fowl and quail (0.271), duck and guinea fowl (0.274), chicken and quail(0.282), and chicken and guinea fowl (0.329). However, the trend for genetic similarity among the poultry species is different, when the estimates are based on MASA markers. Using MASA markers, the minimum genetic similarity was estimated between duck and guinea fowl (0.286) followed by duck and turkey (0.444), duck and quail (0.453) quail and turkey (0.458), duck and chicken (0.480), chicken and guinea fowl (0.500), chicken and turkey (0.533), guinea fowl and quail (0.542), guinea fowl and turkey (0.588) and chicken and quail (0.679). Thus, it may be concluded that RAPD as well as MASA markers are very effective in detecting the genetic polymorphism among the poultry species and establishing genetic relatedness among them. Further, duck seemed to have minimum genetic similarity with other species.

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4. Performance of commercial broiler fed low levels of different pesticides and performance enhancer

Rishesh Kumar Misra and P.K. Shukla

A study was carried out to determine the effect of two pesticides Fenvalerate (FEN) and Methyl parathion (MPT) with or without a performance enhancer 'Zigbir' on the

production performance, carcass quality characteristics, haematological and biochemical in commercial broilers. One hundred one week old broiler chicks were selected on the basis of uniform average body weight and were divided into ten dietary treatment groups of two replicates each and five birds in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet (control), basal diet + 500 ppm Zigbir, basal diet + 75 ppm FEN, basal diet + 75 ppm FEN+500 ppm Zigbir, basal diet + 100 ppm FEN, basal diet + 100 ppm FEN+ 500 ppm Zigbir, basal diet + 25 ppm MPT, basal diet + 25 ppm MPT+ 500 ppm Zigbir, basal diet + 50 ppm MPT, basal diet + 50 ppm MPT+ 500 ppm Zigbir. Numerical decreases in the feed intake were recorded in the groups fed with 25 ppm MPA. The overall improvements in the feed consumption following supplementation of Zigbir with these pesticides were also recorded. There was numerical decrease in overall body weight gain in the pesticide supplemented groups. However, a slight increase in overall body weight gain was recorded following supplementation of Zigbir (500 ppm) with the pesticides. Neither the processing shrinkage nor the dressed yield (cut-up yield or giblet yield) of the birds were significantly ($p<0.05$) affected by the dietary levels of MPA (25 or 50 ppm) and Fen (75 or 100 ppm) with or without supplementation of Zigbir. The hemoglobin concentration and the PCV of the birds were significantly ($p<0.01$) reduced by the dietary levels of FEN (750or 100 ppm) or MPA (25 or 50 ppm). However a slight alleviation in the corresponding values of these attributes were observed in the birds, in the respective Zigbir supplemented groups. The various enzyme profile in the serum (SGOT, SGPT and alkaline phosphates) showed a significant ($p<0.01$) increase in their mean values in the group fed with FEN (75 or 100 ppm) and MPA (25 or 50 ppm). However the supplementation of Zigbir at a level of 500 ppm with these pesticides showed a slight beneficial effect in reducing the mean values of the enzymes. Thus, it may be concluded that Methyl parathion was more toxic than Fenvalerate in corresponding concentration. Further, Zigbir proved to have a beneficial effect in alleviating these adverse effects due to these pesticides.

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5. Influence of dietary probiotic and performance enhancer on the performance of commercial broilers

Raj Kumar Srivastava and P.K. Shukla

A study was undertaken to determine the influence of dietary probiotic and performance enhancer on the performance of commercial broilers. Ninety one week old broiler chicks were divided into five dietary treatment groups, each consisting of three replicates and six chicks in each replicate. The birds were subjected to dietary treatments *viz.* control diet, control diet + saccharomyces cervisiae (yea-Sacc^{1026}) 1gm/10kg of feed, control diet + yea-Sacc^{1026} 2 gm/10 kg of feed, control diet + control + yea-Sacc^{1026} 1gm/10kg of feed + Zigbir 2.5gm/10kg of feed and control + yea-Sacc^{1026} 1gm/10kg of feed +Zigbir 5gm/10kg of feed, respectively. The total experimental period lasted for six weeks, starting from second week to seven week of age. The cumulative feed consumption up to 7 week of age of the birds was not significant ($p<0.05$) affected by the supplementation of Yea-Sacc^{1026} and Zigbir in the diet. However, a numerical increase in feed consumption have been recorded in the groups feed with Yea-Sacc^{1026} ^{2gm}/10kg of feed and Zigbir 5gm/10kg of feed. Body weight of the broiler chicks at weekly intervals during the entire experimental period from 2 to 7 weeks of age were not significant ($P<0.05$) affected by the Yea-Sacc^{1026} and Zigbir. However, a numerical increase in the overall body weight gain was observed in the groups supplemented with Yea-Sacc^{1026} and Zigbir. The overall feed ratio was not significant ($P<0.05$) affected by the supplementation of Yea-Sacc^{1026} and Zigbir in the diet. However, a numerical improvement in the overall feed gain ratio have been observed, in the birds feed with dietary Yea-Sacc^{1026} either at 1gm or 2gm/10kg of feed and Yea-Sacc^{1026} 1gm with 2.5 Zigbir per 10kg of feed or 2gm Yea-Sacc^{1026} with 5gm Zigbir per 10kg or feed. The carcass

quality characteristics (percentage shrinkage due to fasting, blood loss, evisceration loss, dressed yield, cut-up yield and giblet yield) were not significant ($P<0.05$) affected by the Yea-Sacc[1026] and Zigbir. Mortality during the experimental period was recorded between 0 to 2.77 percent which is considered to be normal broiler house mortality and was not attributed to any of the experimental treatments. The present investigations indicated that the overall performance of the broilers did not reveal significant impact of supplemental Yea-Sacc[1026] with or without Zigbir because probably the diet was adequate in all the nutrients and also the birds were not in stress condition.

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6. Effect of dietary supplementation of lactobacillus sporogenes and Zigbir on the performance of broiler

Anand Prakash Singh and P.K. Shukla

A study was undertaken to assess the comparative efficacy of Lactobacillus sporogenes (Uni-Sankyo limited, Ratnagiri, Maharashtra) and Zigbir (Natural Remedies) on performance of commercial broiler. Ninety one week old straight run broiler chicks were divided into five groups of three replicates each and six chicks in each replicate. The birds were subjected to dietary treatments *viz*. Control (basal diet), basal diet + 0.02% lactobacillus sporogenes (probiotic), basal diet + 0.04% probiotic, basal diet + 0.02% probiotic with 0.025% Zigbir, basal diet + 0.04% probiotic with 0.05% Zigbir respectively. The total experimental period lasted for six weeks, starting from second week to seven weeks of age. The cumulative feed consumption was not significantly ($p<0.05$) affected by the supplementation of probiotic and Zigbir in the diet up to 7th weeks of age of the commercial broilers. However, numerical increases in feed consumption have been recorded in the groups fed with probiotic (0.04% or 0.02%) with Zigbir (0.025% or 0.05%). The body weight gain of the broiler chicks' interval during the entire experimental period form second Zigbir. However, a numerical increase in overall body weight gain have been observed except treatment group -2 (supplemented with 0.02% probiotic), from that of control. There were no significant differences observed among the different treatment groups pertaining to carcass quality characteristics and cut-up-parts. Mortality during the experimental period was recorded between 0 to 6.67 percent, which is considered to be normal broiler house mortality and was not attributed to any of the experimental treatments. Thus, it may be concluded that dietary probiotic and the performance enhancer failed to produce the significant desired effects, probably because the diets were adequate in all the nutrients and the birds were kept practically in stress free condition.

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7. Biocholine as a replacer of choline chloride in commercial broilers

Vinod Kumar and P.K. Shukla

A study was undertaken to determine the effect of biocholine on the performance of commercial broilers. One hundred and five one week old straight run broiler chicks were divided into five dietary treatment groups, each consisting of three replicates and seven chicks in each replicate. The birds were subjected to dietary treatments *viz*. Basal diet + choline chloride @ 1400ppm (control), basal diet + biocholine (BC) @ 300, basal diet + BC @ 400ppm, basal diet + BC @ 500ppm, basal diet + BC @ 600ppm. The total experimental period lasted for six weeks starting from 2nd to 7th week of age. The cumulative feed consumption up to 7 week of age did not reveal any significant ($p<0.05$) impact of BC. However, a significant decrease ($p<0.005$) was observed in 2nd week in the group fed BC @ 500 ppm. The overall feed consumption in the birds fed with BC at different levels showed a numerical decrease in feed consumption from that of control diet up to 7th week of age. The overall cumulative body weight gain of broiler did not reveal any

significant ($p<0.05$) impact of supplementing BC at different levels. The overall cumulative feed gain ratio of the broiler did not reveal significant ($p<0.05$) difference between the different treatment diets. However, a numerical improvement in the overall feed gain ratio have been observed in the birds fed with the dietary BC @ 300 ppm. There were no significant differences observed among the different treatment groups pertaining to carcass quality characteristics and cut-up-parts. There was no significant difference recorded in the SGPT, SGOT and alkaline phosphatase level among the different treatment groups at 7th week of age. No gross and microscopic pathological lesions have been observed in the different visceral organs like liver, spleen, bursa, kidney, lung and heart in the birds fed with different levels of biocholine. Thus, it may be concluded that biocholine may be used as a replacer of choline chloride in commercial broilers.

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8. Studies of different pesticides and biocholine on performance of commercial broiler

Sanjeev Kumar and P.K. Shukla

An experiment was carried out to determine the effect of two different pesticides-Fenvalerate (FEN) and Methyl parathion (MPA) with or without biocholine (BC) on the performance of commercial broilers. One hundred and fifty one week old straight run broiler chicks were divided into ten dietary treatment groups, each consisting of three replicates and five chicks in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet + choline chloride @ 1400ppm (control), basal diet + BC @ 500 ppm, basal diet + FEN @ 150 ppm, basal diet + FEN 150 ppm + BC @ 500 ppm, basal diet + FEN 200 ppm, basal diet + FEN 200 ppm + BC @ 500 ppm, basal diet + MPA 75 ppm, basal diet + MPA 75 ppm + BC @ 500 ppm, basal diet + MPA 100 ppm and basal diet + MPA 100ppm+ BC @ 500ppm. The overall feed consumption of the birds fed with FEN 200 ppm was significantly reduced compared to control. Similarly, the overall feed consumption of the birds fed with MPA 100ppm was significantly reduced compared to that of control. The overall cumulative body weight gain of the broilers was significantly ($p<0.01$) reduced in group fed with FEN 200 ppm alone or with BC and MPA 75 ppm with BC or MPA 100 ppm. The hemoglobin concentration and the PCV of the birds were significantly ($p<0.01$) reduced by the dietary levels of FEN (150 or 200 ppm) or MPA (75 or 100 ppm). However, a slight alleviation in the corresponding values of these attributes was observed in the birds, in the respective BC supplemented groups. The various enzyme profile in the serum (SGOT, SGPT and alkaline phosphates) showed a significant ($p<0.01$) increase in their mean values in the groups fed with FEN (150 or 200 ppm) and MPA (75 or 100 ppm). However, supplementation of BC at a level of 500 ppm with these pesticides showed a significant beneficial effect in reducing the mean values of these enzymes. Thus, it may be concluded that Fenvalerate even at the level of 150 ppm adversely affected the performance of the commercial broilers. However, Methyl parathion produced the adverse effect on the performance of the birds even at lower levels (75 ppm or 100 ppm) and was more toxic than Fenvalerate in corresponding concentration. BC proved to have a little beneficial effect in alleviating these adverse effects due to these pesticides.

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9. Genetic polymorphism between wild and domestic chicken using DNA markers

Amit Tomar and P.K. Shukla

A study was undertaken to estimate the DNA polymorphism among red jungle fowl (RJF) and modern day descendants namely, White Leghorn (WL), Rhodes Island Red (RIR), Red Cornish(RC), White Plymouth Rock (WR), Aseel (AS) and Kadaknath (KN). Three different types

of DNA markers i.e. Randomly Amplified Polymorphic DNA (RAPD), Microsatellite/ Minisatellite Associated Sequence Amplification (MASA) and Microsatellite Markers were used to detect the polymorphism. A total of 20 random primers were used for RADP analysis. While minimum genetic similarity (BS) was observed between RJF & WL, followed by RJF & RIR, maximum BS was observed between WL & RIR followed by that between AS & KN. In general, RJF showed minimum similarity with all the breeds. However, it showed comparatively more BS with native chicken breeds as compared to the exotic breeds. While maximum genetic distance (DS) was observed between RJF & WL, followed by RJF & RIR, minimum DS was observed between WL & RIR, followed by that between AS & KN. In general, RJF showed maximum DS with all breeds. However, it showed comparatively lesser DS with native chicken breeds as compared to the exotic breeds. For MASA analysis, three oligo-nucleotide primers were used in the study. While minimum BS was observed between RJF & RC followed by RJF & RIR, maximum BS was observed between both native breeds i.e. AS & KN followed by RC & WR. While the minimum DS was observed between native breeds AS & K followed by RC and WR, the maximum DS was observed between RJF and RC, followed RJF & WL. A total of 8 microsatellite markers were used for microsatellite Marker Analysis. The between breed BS estimates pooled over different microsatellite markers was minimum between RJF & WL followed that between RJF & RC, the maximum BS between RJF & RC followed by RC & WL. In general, RJF showed lower BS with all the other three breeds compared to other combinations and among three breeds. RJF showed maximum BS with AS. While the maximum DS estimated was between RJF & WL, the minimum DS was between AS & RC followed by RC & WL. In general, RJF showed higher DS with all the other 3 breeds compared to other combinations and among 3 breeds. It showed minimum genetic distance with AS. Thus, it may be concluded that irrespective of type of markers used, Red Jungle fowl showed lower genetic similarity with all the domestic chicken breeds in comparison to the levels of genetic similarity among the domestic chicken breeds.

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10. Effect of herbal preparations on performance of broilers

Ashish Ashok and P.K. Shukla

The present investigation was carried out to study the effect of Herbiotic FS and Livoliv-250 on the performance of broilers. Eighty four one week old straight run broiler chicks were divided into four dietary treatment groups, each consisting of three replicates and seven chicks in each replicate. The birds were subjected to dietary treatments viz. Basal diet- Control, Basal diet+ Livoliv-250 @250 ppm, basal diet + Herbiotic-FS @ 250 ppm, basal diet+ Livoliv-250@ 250ppm+ Herbiotic-FS @ 250 ppm. The total experiment period lasted for six weeks starting from 2nd to 7th weeks of age. The cumulative feed consumption did not reveal any significant ($p<0.05$) impact of Herbiotic-FS and Livoliv-250. However, a significant decrease ($p<0.05$) was observed in 5th and 6th weeks in the diet fed with Herbiotic-FS and Livoliv-250. The overall feed consumption in the bird fed with Herbiotic-FS and Livoliv-250 showed a numerical decrease in feed consumption from that of control diet up to 7 weeks of age. The overall cumulative body weight gain of the broilers did not reveal significant ($p<0.05$) difference by supplementation of Livoliv-250 or Herbiotic-FS (250 ppm) as compared to that of control. Further, a numerical improvement in the overall feed gain ratio was observed in the birds fed with the dietary Livoliv(250 ppm), Herbiotic-FS (250 ppm) and Herbiotic-FS (250 ppm) + Livoliv (250 ppm) at 2nd, 3rd and 4th week age respectively as compared to that of control. The balance of protein, calcium and phosphorous were recorded positive during the metabolic trials at 4th and 6th week of age. However, these were not significantly affected by either Herbiotic-FS or Livoliv-250. There were no significant differences observed among the different treatment groups pertaining to carcass quality

characteristics and cut-up-parts. There was no significant difference recorded in the SGPT, SGOT and alkaline phosphatase level among the different treatment groups at 7th week of age. Further, no significant differences were observed in the haematological parameters, serum albumin, globulin and albumin: globulin ratio at 7 weeks of age. No gross abnormalities were observed in the different visceral organs like liver, spleen, bursa, kidneys, lungs and heart in the birds fed with Livoliv (250 ppm) or Herbiotic-FS (250 ppm). From the present investigation it is evident that Herbiotic-FS and Livoliv-250 had no significant impact on the different performance traits of the broilers, probably due to the reasons that the diets were adequate in all the nutrients and there was no stress factor on the birds during the experimentation.

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11. Effect of dietary probiotics on the performance of commercial broilers

Vijay Kumar Jaiswal and P.K. Shukla

The present investigation was carried out to study the effect of dietary supplementation of probiotic on the performance of commercial broilers. Eighty four one week old straight run broiler chicks were divided into four dietary treatment groups, each consisting of three replicates and seven chicks in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet (Control), basal diet+ L.sporogenes and S. cerevisiae (Improval) @ 1000 ppm, basal diet + L.sporogenes (Sporolac) @ 100 ppm, basal diet + S. cerevisiae (Yea-Sacc) @ 500 ppm. The total experimental period lasted for six weeks starting from 2 to 7 weeks of age. The birds fed with probiotic at different levels showed a significant increase ($p<0.05$) in feed consumption from that of control diet upto 7 weeks of age. However, a overall cumulative body weight gain of the broilers revealed no significant ($p<0.05$) impact due to dietary supplementation of probiotics (*L. sporogens & S. cerevisae*) or their combination up to 7 weeks of age. The overall cumulative feed gain ratio of the broilers did not reveal significant ($p<0.05$) difference between the different treatment diets. The balances of protein, calcium and phosphorous were recorded positive during metabolic trials at 4th and 6th week of age. However, these were not significantly affected by addition of probiotic in the basal diet. There were no significant differences observed among the different treatment groups pertaining to carcass quality characteristics and cut-up-parts. There was no significant difference recorded in the SGPT, SGOT and alkaline phosphatase level among the different treatment groups at 7th week of age. Further, no significant differences were observed in the haematological parameters, serum albumin, globulin and albumin: globulin ratio at 7 weeks of age. No gross abnormalities were observed in the different visceral organs like liver, spleen, bursa, kidneys, lungs and heart in the birds fed with probiotic in the basal diet. The present investigation indicated that the overall performance of the broilers did not reveal significant impact of supplementation of probiotic because probably the diet was adequate in all the nutrients and also the birds were not in stress condition.

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12. Preparation of some meat convenient products by value addition

Ajay Tripathi and P.K. Shukla

The present study was undertaken to evaluate the effect of incorporation of two proteins viz., soy flour and black gram flour at different levels in restricted chicken nuggets. Various attempts were made to standardize the products from the recipe and finally one product was taken as standard which was 10% added water level, 15 minute mixing time and 15 minute cooking time. Restructuring of the product was done by replacing lean meat with soy flour (10%) and Bengal gram flour (10%). Restructured products were evaluated for physic-chemical properties and microbiological quality. There were three products; (1) control, (2) soy product

and (3) Bengal gram product. There was a marginal increase in cooking yield and water holding capacity with increase in the level of soy protein and Bengal gram protein. There was a significant increase ($p<0.01$) in pH with incorporation of soy protein and Bengal gram protein. Moisture content of control, soy and Bengal gram treated nuggets did not differ significantly. The moisture of the product packed in the polythene packing was significantly ($p<0.01$) higher than the aluminum foil packing. The protein content of control, soy and Bengal gram treated nuggets differed significantly. There was no significant effect on ether extract on treatment, storage as well as in packing. Thio-barbituric acid content increased significantly ($p<0.01$) with incorporation of soy protein but not because of Bengal gram protein. The total plate count was recorded more in polythene packed product. The effect of storage was highly significantly ($p<0.01$) as the total plate count was increased rapidly during storage. Psychrophillic count for polythene packing was significantly ($p<0.01$) higher to that of aluminum foil packaging. Restructuring had a significant ($p<0.01$) effect on general appearance of the products. The effect on flavor was highly significant with incorporation of soy protein and Bengal gram protein. Overall mean values for polythene packaging are significantly ($p<0.01$) higher to that of aluminum foil packaging. Texture values for polythene packaging were significantly ($p<0.01$) different from that of aluminum foil packaging. For polythene packaging overall juiciness values were significantly ($p<0.01$) different to that of aluminum foil packaging. The effect on flavor was significantly ($p<0.01$) different to that of aluminum foil packaging. Hence, it may be concluded that restructuring of poultry meat by soy flour and black gram flour may enhance the value of meat and provide better acceptability, nutrition and value addition from low cost raw material. Besides use, the value of chicken meat can be enhanced by using restructured meat technology.

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13. Effect of herbal CRD powder on growth and immunocompetence performance of commercial broiler

Prashant Satya and P.K. Shukla

The present investigation was carried out to study the effect of dietary supplementation of herbal CRD powder on the performance of commercial broilers. Seventy five one week old straight run broiler chicks were divided into five dietary treatment groups, each consisting of three replicates and five chicks in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet (control), basal diet + Herbal CRD powder @ 500ppm, basal diet + Herbal CRD powder @ 1000ppm, basal diet + Herbal CRD powder @ 2000ppm, basal diet + Herbal CRD powder @ 5000ppm. The total experimental period lasted for six weeks starting from 2 to 7 week of age. The cumulative feed consumption did not reveal any significant ($p<0.05$) impact of herbal CRD powder. The overall cumulative feed ratio gain of broiler did not reveal any significant ($p<0.05$) difference between the treatment diets. The balance of protein, calcium and phosphorous were recorded positive during both the metabolic trials. However, these were not significantly affected by addition of herbal CRD powder in the basal diet. There were no significant differences observed among the different treatment groups pertaining to carcass quality characteristics and cut-up-parts. There was no significant difference recorded in the SGPT, SGOT and alkaline phosphatase level among the different treatment groups at 7th week of age. Further, no significant differences were observed in the haematological parameters, serum albumin, globulin and albumin: globulin ratio at 7 weeks of age. No gross and microscopic pathological lesions have been observed in the different visceral organs like liver, spleen, bursa, kidney, lung and heart in the birds fed with different levels of herbal CRD powder. The total HA tier, 2-Mercaptoethanol resistant (MER) and 2- Mercaptoethanol sensitive (MES) antibody were estimated on day 0, 5, 10 and 15 day post injection (dpi). There was no significant difference in HA titer at different stages among the treatment groups. In addition no significant difference

was observed in cell mediated immune response as a measure of foot web index among the various treatment groups. The present investigation indicated that the overall performance of the broiler did not reveal significant impact of supplementation of herbal CRD powder because probably the diet was adequate in all the nutrients and also the birds were not in stress condition.

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14. Effect of supplemental ascorbic acid and herbal vitamin C replacer (Phyto 'C') on performance of broilers exposed to immobilization stress

Ajeet Kumar Gupta and P.K. Shukla

The present investigation was carried out to study the effect of Synthetic Vitamin C and Phyto 'C' on the production performance, biochemical and pathological changes in the commercial broilers, exposed to immobilization stress. One hundred and ten, one week old straight run broiler chicks were divided into eleven dietary treatment groups, each consisting of two replicates and five chicks in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet+ Control, Basal diet + Stress, Basal diet + Synthetic Vitamin C @ 100 ppm + Stress, Basal diet + Synthetic Vitamin C @ 200 ppm + Stress, Basal diet + Synthetic Vitamin C @ 300 ppm + Stress, Basal diet + Phyto 'C' A @ 100 ppm + Stress, Basal diet + Phyto 'C' A @ 200 ppm + Stress, Basal diet + Phyto 'C' A @ 300 ppm + Stress, Basal diet + Phyto 'C' B @ 100 ppm + Stress, Basal diet + Phyto 'C' B @ 200 ppm + Stress, Basal diet + Phyto 'C' B @ 300 ppm + Stress. Immobilization stress for 1 hr/ day by tying their legs was given at age of three weeks onwards up to seventh week of age in all experimental groups except treatment 1. The total experimental period lasted for six weeks starting from 2 to 7 week of age. A numerical improvement in the overall feed gain ratio have been observed in the birds fed with the dietary Phyto'C' A (200 ppm), Phyto 'C' 12 B (100 ppm) and Phyto 'C' B (200ppm) at 7^{th} week of age respectively as compared to that of control. There was a numerical increase in the values of Alkaline Phosphatase in the group fed with Phyto 'C' as compared to that of control, which could probably be due to the increased liver activity. An alleviation in the total serum protein in the diet containing Synthetic Vitamin C (100, 200 & 300 ppm), Phyto 'C' A (100 ppm), Phyto 'C' B (100, 200 & 300 ppm) has been recorded as compared to that of control, a slight increase in the serum albumin and globulin was observed in the diet containing Synthetic Vitamin C or Phyto 'C' to that of control. At 7^{th} week of age, a slight increase in the plasma level of Ascorbic acid was observed in the diet containing Synthetic Vitamin C (200 & 300 ppm), Phyto 'C' A (100 ppm) and Phyto 'C' B (200 ppm) to that of control. It was concluded that vitamin C (ascorbic acid) supplementation to commercial broiler chick ration @ 200 ppm was beneficial during stress period.

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15. Random genetic difference between Red Jungle Fowl and domesticated chicken

Amit Kumar and P.K. Shukla

The present study was undertaken with the objective to detect genetic polymorphism between Red Jungle Fowl and domestic chicken and to find RJF specific RAPD marker (s). A total of ten (3 males and 7 females) Red jungle fowl (RJF) and 10 (5 males and 5 females) White Leghorn representing Domestic fowl (DF) were used. Out of 30 random primers capable of generating distinct and repeatable RAPD profile, only 5 primers (17%) could detect polymorphism between the RJF and DF. The five polymorphic random primers i.e. P_9, P_{10}, P_{19}, P_{20} and P_{26} were used for further analysis using individual DNA samples from RJF and DF. From these 5 random primers, a total of 27 loci were amplified and 8 of these (about 29%) were found to be polymorphic

between the RJF and DF. These primers varied in generating the extent of polymorphism and proportion of polymorphic loci ranged from 16.66% (1 out of 6 loci) by P_9 to 33.33% (2 out of 6 loci) by P_{10}. The band sharing estimates for within group genetic similarity in RJF and DF groups ranged from 0.89 to 0.96 and from 0.91 to 0.98, respectively. Similarly, the within group genetic similarity estimates, based on band frequency in RJF and DF ranged from 0.68 to 0.95 and from 0.80 to 0.94, respectively. The between group genetic similarity estimates from band sharing (BS) and band frequency (BF) between RJF and DF, BS estimates ranged from 0.89 to 1.01, while the BF estimates ranged from 0.78 to 0.99. Genetic distance estimates based on band sharing ranged from 0.043 to 0.213, while the genetic distance estimates based on band frequency ranged from 0.010 to 0.248. Using pooled DNA samples, 5 out of 30 random primers were identified as polymorphic between RJF and DF and were used further to detect the polymorphism between the RJF and DF using individual's genomic DNA from RJF birds and DF birds. A total of 8 loci, out of 27 amplified loci were found to be polymorphic. The primer P9 detected only 1 polymorphic loci, which showed comparatively low frequency in both the breeds. The primer P_{10} detected 2 polymorphic bands and both the bands showed higher, but varying frequencies in RJF and DF. The primer P_{19} detected 2 polymorphic loci. Out of these, 1430 loci showed very high frequency in RJF (0.9) while in DF; it had a frequency of 0.1 only. The primer P_{26} detected 1 polymorphic loci, whose frequency was higher in RJF (0.8) as compared to DF (0.2). Hence, out of these 8 polymorphic loci, no locus was specific to RJF or DF.

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16. Effect of herbal supplements on performance of commercial broilers

Raj Narayan Namdev and P.K. Shukla

The present investigation was carried out to study the effect of dietary of herbal supplements on the production performance of commercial broilers. One hundred and fifty one week old straight run broiler chicks were divided into five dietary treatment groups, each consisting of three replicates and ten chicks in each replicate. The birds were subjected to dietary treatments *viz.* Basal diet (control), basal diet + Turmeric @0.5%, basal diet + Arjuna @0.5%, basal diet + Amla @1.0%, basal diet + Turmeric @0.5% + Arjuna @0.5% + Amla @1.0%. The cumulative feed consumption did not reveal any significant ($p<0.05$) impact of herbal supplements. The overall cumulative body weight gain of the broiler at 2nd, 3rd and 5th weeks of age revealed significant ($p<0.05$) impact due to dietary herbal supplements. However, the overall body weight gain showed non-significant numerical increase from that of control diet at up to 7th week fed with herbal supplements. The balance of protein, calcium and phosphorous were recorded positive during both the metabolic trials. However, these were not significant affect by addition of herbal supplements in the basal diet. There were no significant differences observed among the different treatment groups pertaining to carcass quality characteristics and cut-up-parts. There was no significant difference recorded in the SGPT, SGOT and alkaline phosphatase level among the different treatment groups at 7th week of age. Further, no significant differences were observed in the haematological parameters, serum albumin, globulin and albumin: globulin ratio at 7 weeks of age. No gross and microscopic pathological lesions have been observed in the different visceral organs like liver, spleen, bursa, kidney, lung and heart in the birds fed with different levels of herbs. The present investigation indicated that the overall performance of the broiler did not reveal significant impact of herbal supplements because probably the diets were adequate in all the nutrients and also the birds were not in stress condition.

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17. Effect of probiotic and herbal supplement on the performance of commercial broilers

Mahendra Tiwari and H.N. Singh

One hundred and eighty one week old straight run broiler chicks were divided into three dietary treatment groups, each consisting of three replicates and twenty chicks in each replicate. The birds of the first group were fed on a basal diet supplemented with probiotic Biovet YC @ 50g/quintal of the basal diet. The second group birds were fed on a basal diet supplemented with herbal mixture (1% Amla pulp powder, 0.5% turmeric powder, 0.5% Neem leaf powder). The third group was fed a control diet/ basal diet. The experimental diets were fed to these groups from second to six weeks of age. Birds in the probiotic supplemented group had significantly higher ($P<0.05$) body weight compared to the control group throughout the experiment. The herbal supplemented group had also significantly higher ($P<0.05$) body weight compared to the control group throughout the experiment. Further, the probiotic supplemented group had significantly higher ($P<0.01$) body weight compared to the herbal supplemented group at 3rd (505.61 vs. 486.12) and 4th week (773.15 vs. 750.76) of age. There were no significant differences in the FCR of commercial broilers among the various treatment groups during 1-4 week, 4-6 week and 1-6 week period. However, FCR was apparently better in the herbal supplemented group compared to the probiotic and control group during 4-6 week (2.50 vs. 2.65 & 2.71) and 1-6 week period (2.38 vs. 2.42 & 2.47). HA titer was significantly higher ($P<0.01$) in the herbal (8.33) supplemented group compared to the probiotic (6.16) and control group (4.33) at 6 weeks of age. Further the HA titer was significantly higher ($P<0.01$) in the probiotic fed group compared to the control group at 6 weeks of age. Foot web index was significantly higher ($P<0.01$) in the herbal supplemented group (0.816) compared to probiotic (0.65) and control group (0.54). Further, the foot web index was significantly higher ($P<0.01$) in the probiotic fed group compared to the control group at 6 weeks of age. Hence, it may be concluded that feeding of herbal supplement (0.5% turmeric powder, 0.5% Neem leaf powder and 1% Amla pulp powder) may elicit growth and immune-competence traits of broilers.

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18. Effect of antibiotic and herbal supplement on the performance of commercial broilers

Mrityunjay Goswami and H.N. Singh

One hundred and eighty one week old straight run broiler chicks were divided into three dietary treatment groups, each consisting of six replicates and ten chicks in each replicate. The birds of the first group were fed on a basal diet supplemented with antibiotic Stafac 20@ 50g/quintal of the basal diet. The second group birds were fed on a basal diet supplemented with herbal mixture (0.5% Amla pulp powder, 0.25% turmeric powder, 0.25% Neem leaf powder). The third group was fed a control diet/ basal diet. The experimental diets were fed to these groups from second to six weeks of age. There was no significant difference in the weekly body weight of broilers among the treatment groups except at 4th week where the body weight of the control group was significantly higher ($P<0.05$) compared to the group in which there was herbal supplementation. There was no significant difference among various treatment groups in the FCR of commercial broilers during 1-4 week, 4-6 week and 1-6 week period However, at 6th week of age, FCR was numerically better in the antibiotic and herbal supplemented group compared to the control group (2.04 & 2.04 Vs 2.32). In addition, FCR in the herbal supplemented group (1.83) was numerically better during 4-6 week compared to the control group (1.91) and antibiotic supplemented group (1.91). Log 2 value of HA titer against 1% SRBC was significantly higher ($P<0.01$) in the antibiotic (7.33) and herbal supplemented group (6.66) compared to the

control group (5.5) at 6 weeks of age. However, no significant difference was noted between the antibiotic and the herbal supplemented group. The *in vivo* T-cell mediated immune response to phytohaemagglutinin (PHA-P) measured as foot web index was significantly higher ($P<0.01$) in the antibiotic group (0.64) compared to the herbal (0.31) and the control group (0.29). In addition, foot web index was apparently higher in the herbal supplemented group than the control group. The present investigation indicated that the feeding of herbal supplements did not have significant impact on the overall performance of the broilers because probably the diets were adequate in all the nutrients and also the birds were not in stress condition. However, the results obtained in the present study indicate that the addition of herbs to the diets of broilers may enhance immunity. Hence, it may be concluded that feeding of herbal supplement (0.25% turmeric powder, 0.25% Neem leaf powder and 0.5% Amla pulp powder) may elicit the immune-competence traits of broilers.

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19. Identification of sexes in poultry using DNA based methods

Manoj Kumar Singh and H.N. Singh

The present study was undertaken to develop a simple and accurate system of DNA based sexing method in poultry. A resource population of guinea fowl, chicken, turkey and quail which is maintained at Experimental farm at CARI, Izatnagar was used. From each population two healthy males and two healthy females were randomly selected and were utilized for further experiment. Initially, the genomic DNA from both the sexes in chicken and guinea fowl was used as template for sex specific PCR assay. In chicken, two bands were observed in female, while only one band was observed in male. While the common band was of 360 bp, the female specific band was 376 bp. However, in guinea fowl, only one band was i.e. 360 bp was observed in both the sexes. While in guinea fowl and turkey, the band was of the same size (i.e. 360 bp), in quail, it was comparatively bigger in size i.e. 380 bp. In chicken, a 370 bp fragment was amplified in female bird, while no amplification was observed in male. Similarly in guinea fowl, a 370 bp fragment was amplified in female bird, while no amplification was observed in male. In all the species including guinea fowl, a 590 bp fragment was amplified in both the sexes. In view of developing a single tube PCR assay, attempts were made to multiplex the PCR for EE0.6 sequence PCR and 16S r RNA PCR. The whole blood and the lysate were used as template in place of genomic DNA. No amplification was observed. It was found that all the sets of primers are not effective in differentiating the sexes even in more closely related poultry species. Further, the W chromosome specific primers were effective in sex differentiation in chicken, but not in guinea fowl. The CHD gene specific primers were also effective in differentiating the sexes only in chicken, but failed in the other closely related poultry species i.e. guinea fowl, quail and turkey. Also, the CHD gene specific primers showed the size difference in CHD W and CHD-Z homologous sequences even between closely related poultry species. The EE0.6 specific primers were very effective in sex differentiation in guinea fowl, but also in other closely related poultry species such as chicken, quail and turkey. A multiplex PCR assay using EE0.6 specific primers and 16S r RNA primers is very effective in sex differentiating in guinea fowl as well as in other closely related poultry species such as chicken, quail and turkey. Attempts to simplify the multiplex PCR assay failed with the use of whole blood or lysate after proteinase K digestion as template in place of genomic DNA as it did not result in PCR amplification.

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20. A study on comparative efficacy of antibiotic and Tulsi leaf powder on the performance of commercial broilers

Shailendra Kumar Singh and H.N. Singh

A study was proposed to determine the effect of growth promoters like probiotics vis-à-vis Tulsi leaf powder (TLP) at graded levels on the performance of commercial broilers. Two hundred and twenty five one week old straight run broiler chicks were divided into five dietary treatment groups, each consisting of three replicates and fifteen chicks in each replicate. The birds of the first group were fed on a basal diet supplemented with antibiotic Stafac 20 @ 50g/quintal of the basal diet. The second group birds were fed on a basal diet supplemented with 0.2% TLP. The third group was fed the control diet along with 0.5% TLP a control diet/ basal diet. The fourth group was fed the control diet along with 1.0% TLP. The fifth group was fed the control/ basal ration (22.5% CP & 2830 K cal/kg ME). The experimental diets were fed to the various treatment groups from second to six weeks of age. Body weight was significantly higher ($P<0.05$) in the control group and 1% TLP group compared to the other groups at 2nd week of age. However, 4th wk onwards body weight was apparently higher ($P<0.05$) in the 0.5% TLP group compared to the other groups and at 6th week of age, body weight of 0.5% TLP feed group was significantly higher ($P<0.05$) compared to the other groups. The control group had significantly better ($P<0.05$) FCR compared to the other groups at 2nd week of age. FCR was significantly better ($P<0.05$) in the 0.5% Tulsi supplemented group compared to the other groups during 1-6 week period. Log 2 value of HA titer was apparently higher in the 0.5% TLP compared to the other groups. The antibiotic fed group had apparently higher HA titer compared to the control group. Foot web index was apparently higher ($P<0.01$) in the 0.2% TLP and 0.5% TLP compared to the antibiotic and the control group. Microbial load in fecal sample was lowest in antibiotic group (150×10^4) followed by 1.0% TLP group (170×10^4), 0.5% TLP (175×10^4), control (180×10^4) and 0.2% TLP group (200×10^4). The present investigation indicated that the feeding of 0.5% TLP supplement had a significant impact on the overall performance of the broilers. The results obtained in the present study indicate that the addition of 0.5% TLP to the diets of broilers may enhance immunity. Further, it was noted that 0.5% TLP supplementation improved the overall FCR of commercial broilers. Hence, it may be concluded that feeding of 0.5% TLP may elicit growth performance and immuno-competent traits of commercial broilers.

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21. Effect of probiotic and herbal supplement on the performance of turkey poults

Jitendra Singh Rajput and H.N. Singh

Ninety one week old straight run turkey poults were divided into three groups, each consisting of three replicates and ten poults in each replicate. The birds of the first group were fed on a basal diet supplemented with probiotic Biovet YC @ 50g/quintal of the basal diet. The second group birds were fed on a basal diet supplemented with herbal mixture (1% Amla pulp powder, 0.5% turmeric powder, 0.5% Neem leaf powder). The third group was fed a control diet/ basal diet (28% CP & 2800 Kcal/kg). There was no significant difference in the average weekly body weight of turkey poults among different treatment groups during the experimental period. There were no significant differences in the FCR of commercial broilers among the various treatment groups during 1-4 week, 4-8 week and 1-8 week period. Log 2 value of HA titer was apparently better in the herbal (9.00) supplemented group compared to the probiotic (8.00) and control group (7.83) at 8 weeks of age. There was no significant difference in foot web index of different treatment groups during throughout the experiment. Results indicated that foot web index was numerically better in the herbal supplemented group (0.58) compared to

probiotic (0.55) and control group (0.49). Microbial load in fecal sample was lowest in herbal group ($72x10^5$) followed by probiotic group ($88x10^5$) and control group ($104x10^5$). The present investigation indicated that the feeding of herbal supplements did not have significant impact on the overall performance of the turkey poults. This may be due to the fact that the diets were adequate in all the nutrients and also the birds were not in stress condition. However, the results obtained in the present study indicate that the addition of herbs to the diets of turkey poults may enhance immunity. Hence, it may be concluded that feeding of herbal supplement (0.5% turmeric powder, 0.5% Neem leaf powder and 1% Amla pulp powder) may elicit the immuno-competent traits of turkey poults.

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22. A qualitative study on spent quail meat (bone-in) pickle and their storage stability

Hari Om Singh and H.N. Singh

In the present study, attempts were made to determine the proximate composition of spent quail meant and to access the suitability of acetic acid (A), lactic acid and vinegar for preparation of spent quail meat (bone-in) pickle. The recipe was standardized for optimum cooking time and minimum oil requirements. The standardized products after cooling were packed in two types of rigid packages (glass and PET) and stored at room temperature for further study. These were subjected to storage studies on 0, 30, 60 and 90th day of storage. The meat samples of spent quails analyzed for its pH, percent moisture, percent crude protein, percent ether fat and percent total ash were 5.42, 72.72, 19.35, 3.90 and 1.20 respectively. The standardized recipe of spent quail meat (bone-in) pickle contained 12% oil, 40% cook-out on meat weight basis, 2.5% each of acetic acid/ lactic acid/ vinegar in cook-out, 4% salt, 20% green curry stuff, 4% dry spice mixture and 1000g spent quail (bone-in) meat. It was found that combination of acetic acid in the pickle was found most suitable. In the present study almost negligible changes in the pH values of all these products were observed during 90 days storage period. The low pH values in the product were found suitable for safety and longer shelf life. TBA values were found increased with the increase in storage period and on an average it increased 3 folds than fresh product during 90 days storage. Among the package glass jars was evidenced with higher TBA value than PET jars. Moisture content of the products was found decreased with increase in storage period but fat, protein and ash contents were found increased with increasing storage period. Microbial counts were increased with increase in storage period. Almost in all products, treatment of the product was significantly affected by the storage period but no significant effect of package was observed during storage period. In general, microbial counts in all the pickle products were well below threshold value of log 7.0/g. Sensory scores of standardized products for almost all the attributes slightly decreased except sourness with increases in storage period. Among the package no significant variation in the sensory scores of various pickle products were found. However, products stored in PET jars were scored slightly higher sensory scores in almost all attributes. It may be concluded that all the combination of acids used in present study were very well accepted by the sensory panelists but combination with acetic acid was found to be best. The standardized recipe for all these products were found organoleptically better, microbiologically safer and shelf stable at room temperature for 90 days and PET jars was proved durable and convenient for pickle storage.

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Ph.D.

1. A complete ex-vivo embryo culture system for transgenesis and reconstitution of chicken

Jitendra Kumar Chauhan and P.K. Shukla

A total of 535 fertile eggs of crosses between naked neck colored broiler males with RIR layer females, aged between 35 to 40 weeks, obtained through artificial mating were set in different experiments using ex-vivo embryo culture (EC) technique. Simultaneously, 30 eggs from same strain were also set in the incubator as control. A total of 255 chicks hatched from 535 embryo transfers. The results of mortality and abnormal chicks produced in embryo culture and normal incubation revealed that percentage of abnormal chicks produced in embryo culture system were 0.78% and 0.0% in control. The overall post hatch survival rate was about 47.66% in EC and 86.66% in control. The survival rate was reduced to 18.33 and 49.33% respectively and total numbers of chick were produced 55 and 148 respectively by single stage technique and double window ex-vivo embryo culture system. Regarding comparison of survival rate of embryos in ex-vivo embryo culture system and control eggs showed approximately 63.63% of embryos survived to day 4 of incubation but then some losses occurred as a result of transfer to the next culture system. The survival rate of embryos in culture was up to 9.09% at day 17. There was a peak of embryo loss in last but 5 days prior to hatch. The survival rate was nil on hatching, but in the control eggs, approximately 90.90% chicks were hatched on 22nd day of hatching. Analysis for effect of pre-incubation storage of hatching eggs showed that the hatchability of stored eggs through 0-8 days resulted in reduction from 92 to 87.33% but in case of ex-vivo culture system hatchability was reduced drastically from 48 to 0%. The hatchability after 8 days of storage eggs pre-incubation, there was no growth on embryo but in case of normal; incubation hatchability was reduced by 5.34%. Our findings indicate that, if broiler eggs are stored at 15°C with 45% RH (as practiced commercially), the blastoderm is not likely to reach hypoblast stage unless incubated for up to 5 hours at 36°C. Nevertheless as long as incubation commences within 2 weeks, embryo development is not compromised. In conclusion, it can be said that during pre incubation storage, of chicken blastoderm develops in a temperature-dependents manner towards a hypoblast stage and beyond. But, irrespective of egg storage temperature, embryo survival is reduced in eggs stored longer than 2 weeks, in case of normal incubation. But in case of ex-vivo embryo culture system, hatchability declines drastically in pre-incubation storage eggs. For eggs which were more than 7 days of storage, there will be no development occurring at all.

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Veterinary Anatomy

SNo.	Titles	Name of Scholars	Name of Major Guide	Years	Page No.
		M.V.Sc.			
1.	Morphological, histological and histochemical studies on the metanephros, ureter and urinary bladder of goat (*Capra hircus*) in prenatal period	Abdur Rezzaque Choudhury	Dr. Chandra Pal	2001	53
2.	Gross, Histological and Certain Histochemical Observations on the Prenatal Thymus of Goat (*Capra hircus*)	Dr. Madhav Prasad	Dr. Ajay Prakash/ Dr. Archana Pathak	2011	54
3.	Gross, Histological and Certain Histochemical Observations on the Prenatal Liver of Goat (*Capra hircus*)	Gajendra Singh	M.M. Farooqui/ Dr. Ajay Prakash	2011	54
4.	Gross, Histological and Histochemical studies on Pelvic urethra and Accessory sex glands of Prenatal Goat (*Capra hircus*)	Abhinov Verma	Dr. Archana Pathak/ Dr. M.M. Farooqui	2012	55
		Ph.D.			
1.	Morphological, histological and histochemical studies on male genital system of goat (*Capra hircus*) in prenatal period	Dr. M.M. Farooqui	Dr. Chandra Pal	2004	56
2.	Correlative Anatomy of testis and Accessory sex glands of Gaddi Goat (*Capra hircus*)	Dr. Archana Pathak	Dr. R.S. Katiyar	2006	58
3.	Gross Histological and Histochemical studies on the urinary system of goat (*Capra hircus*)	Ashwani Kumar	Dr. Raja Ram.	2006	59
4.	Gross morphological, histological and histochemical and ultrastructural studies of the intestine of guinea fowl	Dr. Sriprakash	Dr. R.S. Katiyar	2011	61

1. Morphological, histological and histochemical studies on the metanephros, ureter and urinary bladder of goat *(Capra hircus)* in prenatal period

Abdur Rezzaque Choudhury and Chandra Pal

Macroscopic and microscopic studies were conducted on the metanephros, ureter and urinary bladder of 41 goat embryos/foeti in different stages of gestation. Grouped into 10-30 days), II (31-60 days), III (61-90 days), IV (91-120 days) and V (121-150 days) having 10 foeti in each group except group I (0 30 days) which contained only one embryo. The metanephrogenic mass was observed at 1.5 cm CRL stage (28 days) caudolateral to the mesonephros. Metanephros in the group II (30-60 days) were extended from 2nd to 5th lumbar transverse processes. Both the metanephroi were irregularly ovoid and redish brown in colour. The cortex and medulla could not be demarcated upto 50 days of gestation. At 84 days of gestation, the developing metanephroi were placed under 3rd to 5th lumbar transverse processes. Renal pyramids were observed at 69 days of gestation which became more distinct at 88 days. Renal columns became distinct on 115 days of gestation. At 135 days, renal crest, renal column and interlobar arteries were well distinct. The biometric studies revealed that the right metanephros was heavier, longer, wider and thicker than the left one in all the groups. The cortex and medulla were well demarcated at 58 days of gestation. The growth rate of cortex and medulla was higher in group III (61 -90 days) as compared to the other groups. The renal corpuscles were observed in the process of differentiation up-to the group V (121-150 days) just beneth the capsule. The diameter of renal corpuscle decreased as the age of the foetus advanced. At 58 days of gestation the differentiating mesenchymal cells, located just beneath the capsule, were arranged in chain like fashion and subsequently formed the cluster, the forerunners of nephron. The dimensions of the juxtaglomerular cells increased from group II to V. The distal convoluted tubules were less convoluted at 46 days of gestation than the proximal convoluted tubules. There were lined by simple cuboidal epithelium with less eosinophilic cytoplasm. The different dimensions of proximal and distal convoluted tubules decreased from group II to V. macula densa cells observed in the group II onwards and their dimensions increased as the age advanced.Differentiating thick descending limb of Henle's loop was observed in the medulla at 58 days of gestation, lined by simple cuboidal to pyramidal epithelium. Arched collecting tubules at 58 days of gestation, were lined by simple cuboidal to low columnar epithelium. Straight collecting tubules had cuboidal to low cuboidal epithelium. The diameter of this segment significantly increased from group II to V. The papillary ducts, located in the medulla, contained tall columnar epithelium with nuclei situated more towards the lumen in group II. Renal pelvis was lined by 3 to 8 layered transitional epithelium from group II to V group. The ureter arose from m the hilus and turned backward and entered the urinary bladder forming trigone vesicae with the pelvic urethra. Statistical analysis showed that the length of the right ureter was higher than the left one and diameter of the middle part was least from the proximal and distal parts of ureter in all the groups. At 48 days of gestation, the ureter was lined by transitional epithelium which contained 2-3 cell layers and changed to 4-5 layers of cells. The urinary bladder presented well defined three parts viz. neck, body and vertex at 48 days of gestation. Stastical analysis revealed that the length of the neck of the neck of urinary bladder was higher in female than male in all the groups. Lamina propria and submucosa could not be demarcated throughout gestation period.

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2. Gross, Histological and Certain Histochemical Observations on the Prenatal Thymus of Goat (*Capra Hircus*)

Madhav Prasad and Ajay Prakash

Gross, histological and histochemical studies were conducted on the thymus of goat foetuses/embryos ranging from 16.87 to 151.32 days of gestation. The material was divided in to early, mid and late prenatal groups. In early prenatal period only histological studies were conducted. Grayish to pale coloured cervical and thoracic parts of thymus were generally superficially lobulated. The cervical part extended from thoracic inlet upto the larynx and its right portion was connected with the thoracic part. Irregularly quadrilateral thoracic part lied on left side in cranial mediastinum thoracic opposite the first rib to third intercostal space. Biometric parameters of late prenatal period were significantly higher than the mid prenatal period goat thymus. At 16.87 days of gestation the thymic primordium consisted of eosinophilic and basophilic cell types where as in 24.37 days of old embryo the primordium had epithelial reticular cells, lymphoblasts, fibroblasts and mesenchyme cells. At 52.51 days of gestation the lobulation of thymic parenchyma was ill developed and poorly organized. The capsule, trabeculae and septae were more pronounced in the thymus of late prenatal period than the mid prenatal goat. The parenchyma of the lobules was not divisible in to cortex and medulla upto 73.13 days of gestation. Beyond 52.51 days of gestation the parenchyma contained epithelial palisades which were poorly visible in the cortex and absent in the medulla and at 140.69 and 151.32 days of gestation. Hassall's corpuscles were absent upto 73.13 days of gestation. The developed were either unilamilar or multilamilar type. The thymocytes and epithelial reticular cells were positive for PAS and glycogen. Their nuclei had positive reactions for DNA. These were negative for acid mucopolysaccharides, alkaline phosphatase and acid phosphatase.

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3. Gross, Histological and Certain Histochemical Observations on the Prenatal Liver of Goat (*Capra Hircus*)

Gajendra Singh and M.M. Farooqui

Morphological, histological and histochemical observations were made on 18 goat embryos/foeti of either sex ranging from 17 days to full term. The experimental units were divided in to three groups viz. Group1 (1-30 days); Group 2 (31-90 days) and Group3 (91- till term), having 6 embryos/foeti in each group. A pea sized roughly quadrilateral bilobed brownish liver was observed at 1.4 cm CRL (27days) of gestation. At this stage, the liver comprised of parietal and visceral surfaces and its borders were not well demarcated. The liver was covering almost whole of the abdominal cavity at 47 days of gestation the liver comprised of two distinct equal lobes. Caudate lobe was distinct between right lobe and portal fissure of the liver. The faciform and lesser omentum ligaments were well distinct at 92 days of gestation. At 126th day of gestation, the liver occupied about two third part of abdominal cavity and its long axis was extended from the 7th rib to 13th rib. The right lobe was larger than the left lobe. At 140 days of gestation visceral surface was mostly covered by abomasum. Till the full term the liver was not completely oriented on the right side of the abdominal cavity as in adult stage. The biometrical studies revealed that there was increase in weight, length, width and thickness of liver as the age of the foetus increased. There was a positive correlation between foetal weight, CRL, age and liver length, width, thickness and weight. Microscopically the liver was enclosed by thin capsule containing mesenchymal cells and had thin reticular fibrils. In group 2 the capsule became fibrous and had fine collagen, reticular fibrils and mesenchymal cells and was moderate positive for PAS and AMPs. In group 3 the capsule became highly fibrous and was mild AMPs, PAS and Alkaline phosphatase positive. At 0.9 cm CRL (17 days) of gestation, the primordium of the liver

appeared as an irregular solid strands of hepatocytes separated by irregular and broad blood spaces. The haemopoetic cells formed haemopoetic islands which showed intense PAS reaction. Large multilobular basophilic nucleated and lighter eosinophilic cytoplasmic megakaryocytes cells showing moderate PAS reaction were seen throughout the study. Thin reticular fibrils were first noticed around the hepatocytes on 17th day of gestation. The fine collagen fibres were first noticed at 47 days of gestation in the capsule which form the bundles in the capsular connective tissue and blood vessel walls from the 92 days and ownwards.Very thin elastic fibres were also observed in the elastic lamina of blood vessels at 94 days of gestation and they became more developed in further stages of group 3. At 49 days of gestation the radial arrangement of hepatocytes and bi nucleated to tetra nucleated cells were observed at many places. Developing central veins and blood vessels of different shapes and sizes were observed at 47 days and haemopoetic islands from 17 days ownwards. The haemopoetic islands showed intense reaction for AMPs. The hepatic cells were mild to moderate positive for Best's carmine, PAS,AMPs and their nuclei showed mild to moderate Feulgen reactions. Differentiating triangular to star shaped Kuppfer cells were appeared on 49 days. Lobule formation was not so distinct at 49 days. The portal triad first observed at 49 days of gestational stage which became distinct at 69 days of gestation. Beyond this stage further organization of parenchyma was continued due to differentiation of connective tissue.Haemopoetic activity was more toward the periphery of lobule than the centre. The cytoplasm of hepatocytes and hepatblasts Showed blue iron particles, argentaffin granules, mild alkaline phosphatise activity and distribution of lipid droplets. At full term stage the hepatocytes were compactly arranged and their cytological characters were identical to adult one. The number of megakaryocytes reduced near full term however, the number of RBCs was more and they were present in the lumen of blood vessels and in sinusoids. The micrometrical studies revealed that there was a decreasing trend in diameter of hepatocytes from group 1 to group 2 and then increasing trend from group 2 to group 3. A continuous increasing trend in diameter of nuclei of hepatocytes, thickness of hepatic plate, diameter of central vein and capsular thickness was recorded. A continuous decreasing trend in diameters of blood spaces, distance between two central veins and number of hepatocytes per unit area was recorded was observed.An increasing trend in diameter of haemopoetic foci from group 1 to group 2 and then decreasing trend from group 2 to group 3 was evident.

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4. Gross, Histological and Histochemical studies on Pelvic urethra and Accessory sex glands of Prenatal Goat (*Capra Hircus*)

Abhinov Verma and Archana Pathak

Gross, histological and histochemical studies were conducted on the pelvic urethra and accessory sex glands in prenatal goats, divided into five group viz; Group I (0-30 days), Group II (31-60 days), Group III (61-90 days), Group IV (91-120 days) and Group V (121-150 days) of gestation- with 6 animals in each group. At 28 days of gestation within the cluster of mesenchymal cells, a cleft was formed, which form the future urogenital sinus. Grossly, the pelvic urethra was formed as tube like structure at 49 days of gestation. At 55 days of gestation the primordia of vesicular and bulbourethral glands appeared in the cranial and caudal part of pelvic urethra, respectively. The primordia of prostate gland also appeared at 55 days of gestation in the wall of developing urethra. Biometrical parameter of pelvic urethra increased with increased in age and weight of foetus throughout the gestation period. The various layers of pelvic urethra were formed distinctly at 57 days of gestation. The lumen of urethra was lined by 4-5 layers of polyhedral shape cells at 59 days of gestation. At 71 days of gestation, it was lined by transitional epithelium. The primordia of vesicular glands appeared as lateral outpocketing of mesonephric duct at 55 days of gestation. The duct formation was started with the aggregation of

large mesenchymal cells at 59 days of gestation. At 70 days of gestation, the luminated duct were also present with solid ducts. These were lined by simple to stratified squamous epitheliun in group III, but in group IV these were lined by 2-3 layers of cuboidal epithelium. Folding o mucosa was seen in the later part of last trimester of gestation. In group V the ducts were linec by stratified cuboidal to columnar epithelium. At 55 days of gestation, the primordia of future pars disseminata of the prostate gland was observed as cluster of cells within the wall o developing urethra. On 57 days of gestation, solid prostatic ducts were present in the lamina propria submucosa of pelvic urethra. At 71 days of gestation, the luminization process was seen in some of the solid ducts. Prostatic ducts were confined to the dorsal aspect of pelvic urethra in its cranial part but as we move caudally they were present in the dorsolateral, lateral and ventral part of the same. The solid secretory end pieces were found at 93 days of gestation, at the terminal part of the duct, concentrated at the periphery of lamina propria submucosa. The primordium of bulbourethral gland was seen as compact mass consisted of polyhedral cells, at 55 days of gestation. One compact mass was consisted of central lumen surrounded by 2-3 layers of concentrically arranged mesenchymal cells. The other compact mass was solid consisted of cluster of polyhedral cells. The duct formation started at 57 days of gestation. At 59 days of gestation, luminated as well as nonluminated ducts were present. These were lined by stratified cuboidal to columnar epithelium in group III. But in group V, they were lined by simple to stratified cuboidal epithelium. The solid secretory end pieces (mucous and serous) were recorded at 140 days of gestation.

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Ph.D.

1. Morphological, histological and histochemical studies on male genital system of goat (*Capra hircus*) in prenatal period

M.M. Farooqui and Chandra Pal

The morphological, histological and histochemical studies were conducted on the male genital system of 70 goat embryos or foeti in different stages of gestation. The embryos/foeti were grouped into I (0-30 days), II (31-60 days), III (61-90 days), IV (91-120 days) and V (121 day to till term) having 14 embryos/foeti in each group. Biometric studies were made on various parts male genital organs. In addition to routine histological observations, histochemical studies were conducted for polysaccharides, acid mucopolysaccharides, alkaline and acid phosphatases, lipids and DNA. The genital ridge was observed at 1.2 cm CRL (23rd day) stage on the ventromedial aspect of mesonephros as a linear extension, extended from second thoracic segment to the last sacral vertebra. At 42nd day, the genital ridge developed into a cylindrical structure, the testes. The left testis was heavier, thicker and longer than the right one. Microscopically at 1.2 cm CRL stage, the genital ridge contained an accumulation of mesenchymal cells, immature RBCs, differentiating fibroblasts and primordial germ cells. At 44th day of gestation, capsule was composed of 2-5 layers of mesenchymal cells, fibroblasts, large cells and capillaries just below the germinal epithelium. The sex cords contained two types of cells at 44th day of gestation viz. large and small cells. At 56th day of gestation, differentiating Sertoli cells were also observed in the sex cords. The large cells, spherical in shape were placed peripherally up to 68th day of gestation. Some of the cells started moving towards the future lumen site. The hypertrophic and degenerating changes were observed from 65th day of gestation onwards. Small cells were located close to the basement membrane of sex cords. On 125th day of gestation these cells were situated slightly away from each other. At 56th day of gestation, some of the small cells differentiated into a roughly pyramidal shaped with indistinct cell boundaries known as sertoli cells. At 44th day of gestation, few of the mesenchymal cells located in between the sex cords, started developing into Leydig cells. At 56th day of gestation, these cells were present in groups of 2-3 cells along with differentiating fibroblasts. At this stage, on the basis of staining characters, size of the nuclei and position of their nucleoli, four different forms of differentiating leydig cells was recognized viz; Type-I, Type-II, Type-III, Type-IV. The anlagen of the mediastinum testis was identified on 44th day of gestation as the area devoid of sex cords. It was placed at cranio dorsal end of testis and shifted centrally after 65th day of gestation. On 42nd day of gestation, a peritoneal fold detached from the caudal end of gonad and extended up to the abdominal floor, referred as gubernaculum (inguinal ligament), whichbecame harder, longer and cord like in 5th group. On 23rd day of gestation, on the ventromedial surface of mesonephros a sharp convex ridge was observed over which two ducts ran obliquely downwards and backward referred as Mullerian (lateral one) and mesonephric/Wolffian (medial one) ducts. At 60th day the cauda epididymis appeared as elongated structure. The left epididymis was longer, wider and heavier than the right one. Microscopically, the mesonephric duct (epididymis) was located just beneath the tunica albuginea of the epididymis lined by high simple cuboidal to columnar epithelium at 25th day of gestation. At 88th day of gestation, the duct became more tortuous. The demarcation between the epididymis and the vas deferens was observed at 60th day of gestation due to formation of cauda epididymis. Up to 88th day of gestation the vas deferens was entirely in the abdominal region. From 89 to 96th day of gestation, it was present in the inguinal canal along with other spermatic cord structures. After 98th day, the vas deferens had extra abdominal and abdominal parts. The non-ampulated part was straight in the region of testis. Microscopically at 66th day of gestation vas deferens was lined by simple columnar epithelium with a very small lumen surrounded by a closely packed, concentrically arranged mesenchymal cells, which

showed slight tendency of pseudostratification at 88th day of gestation. The anlagen of the seminal vesicle was first observed on 55th day of gestation ahich became tube like on 68th day. A groove was observed between the two glands. The gland showed lobulation on its dorsolateral aspect of cranial end at 84th day of gestation. The gland became roughly rounded after 88th day of gestation. It was placed on the junction of neck of the bladder and the initial part of pelvic urethra. Microscopically the lateral out pocketing of mesonephric duct occurred on 56th day of gestation surrounded by mesenchymal cells. In third month of gestation, the gland was composed of dense mesenchymal tissue and different section of luminised ducts. In III and IV groups, the ducts were lined by simple columnar epithelium. At 125th day, the large duct was lined by high pseudostratified columnar epithelium. The prostate gland was represented by pars disseminata that extended through out the length of the pelvic urethra. The pars disseminata appeared whitish in colour while urethral muscle was red about 98th day onwards, of gestation. Microscopically, the primordia of the prostrate gland was appeared on 56th day of gestation as an aggregation of solid cells just beneath the transitional epithelium of urethra and were located in the underlying lamina propria and submucosa. The pars disseminata was covered by a layer of circularly arranged developing smooth muscle fibre and urethral muscle. Solid tubules got canalized on 66th day of gestation. Up to 3rd month of gestation the pars disseminata was confined to dorsolateral aspect of the urethra. The glandular tissue was distributed all around the urethral lumen after 108 day of gestation. Most of the secretory end pieces were solid, however, canalization began, on 118th day onwards. Simple columnar or pyramidal cells lined these end pieces. The paired pea shaped primordia of bulbourethral glands appeared on 55th day of gestation on either sides of dorsolateral aspect of pelvic urethra near the ischeal arch. The glands became rounded in the early third month of gestation and covered by bulboglandularis muscle. Microscopically the primordia of the bulbourethral gland was observed on 53rd day of gestation composed of mesenchymal mass and solid clusters of cells surrounded by indistinct muscle bundles. It developed as an outgrowth from the urethral epithelium into mesenchymal mass. The canalization of solid mass of the cells took place on 56th day of gestation. The septae containing mesenchymal cells, fibroblasts and capillaries invaginated in between the tubules. At 108th day of gestation the gland was divided into distinct lobules by means of fibro muscular septae. The secretory end pieces were solid up to 115th days of gestation. On 116th day of gestation spherical, irregular or pyramidal cells lined the secretory end pieces. There was no separate pelvic urethra up to 47th day of gestation. The colliculus seminalis and urethral crest were indistinct upto 2nd month of gestaion. The penile urethra was lodged in the ventral surface of the penis in the urethral grooves. At 53rd day and onwards the transitional epithelium lined the pelvic urethra. The penile urethra was represented by urethral plate at 25th day of gestation. At 48th day of gestation it contained solid mass of cells in terminal part of penis while its initial part showed degeneration of centrally placed cells. At 56th day, the lumen was lined by transitional epithelium. In the glans region it was highly folded near term. At 23rd day of gestation, the external genitalia appeared in the form of cone shaped projection, the genital tubercle, which was located ventral to the cloacal opening. In later stages it gets elongated. The urethral plates and the groove on ventral aspect of tubercle were observed at 16 mm stage. The genital tubercle began to migrate towards the umbilicus at 42 days of gestation. The primordia of galea glandis appeared on dorsolateral aspect of the glans at 48th day of gestation. The development of anogenital raphae started on 48th day of gestation. At 72 day of gestation, the glans reached close to the umbilicus. The sigmoid flexure was very much evident with its both curves on 98th day onwards. In cross sections, the body of the penis had varying shapes in different regions.

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2. Correlative Anatomy of testis and Accessory sex glands of Gaddi Goat (*Capra hircus)*

Archana Pathak and R.S. Katiyar

A study was conducted on the testes of 30 male Gaddi goats, divided into 3 groups viz; prepubertal (0 day to 18 months), pubertal (<18 months to >5 yrs) and post- pubertal (>5 yrs) of age. The testis was covered by fibroserous tunica albuginia having outer fibrous layer and inner vascular layer with smooth muscle fibers at birth. The tunica albuginia continuously grew in thickness from prepubertal to postpubertal animals. The septula testis arising from the tunica albuginia, divided the parenchyma in lobules and converged at mediastinum testis. Few differentiated and undifferentiated leydig cells were present in the intertubular spaces, which enormously increased in pubertal animals and replaced by fibroblasts in the postpubertal animals. The parenchyma was comprised of solid sex cords in new born kids, converted into luminated tubules after 6 months of age and had clear cut tubuli contorti and tubuli recti leading to the mediastinum testis. In "0" day old kids, sex cords were filled with undifferentiated small cells located at peripheray along the basement membrane and large primordial germ cells centrally. By the six months of age, latter started showing sign of degeneration and thus luminization of tubules started. Spermatogenesis started in the seminiferous tubules of 12-18 months goat. In pubertal animals all the stages of spermatogenic cells and Sertoli cells were identified in the seminiferous tubules. The population of gonia cells and primary + secondary spermatocytes were 35% and 30% respectively. But in postpubertal animals gonia cells were reduced to <30% and Primary +secondary spermatocytes <20%. Many degenerating seminiferous tubules showed giant cells. The stroma and parenchyma ratio which was 35:65 at birth became 30:60 at 6 months, 25:75 at 12 months and 15:85 in pubertal and 20:80 in postpubertal animals. The seminiferous tubules grew wider in diameter continuously and consistently from birth to puberty. In late prepubertal and pubertal animals, eight types of different cell associations were encountered. Epididymal tubule diameter increased from caput to cauda in all age group of animals. The growth was fastest in the prepubertal animals in all segments. The tubules were lined by simple columnar epithelium in the caput region, pseudostratified in corpus and cauda regions. In late prepubertal and pubertal animals all segments were lined by pseudostratified columnar ciliated epithelium of different heights and comprised of 4 types of cell. At birth the lining epithelium of vas deferens was simple columnar, which changed to pseudostratified columnar ciliated epithelium in pubertal animlas. The mucosal glands in the ampulla were few in new born kids, became numerous and widely spread in the pubertal and post-pubertal animals. Vesicular gland was small white cord like, became "?" shaped at 6 months and "S" shaped at 12 months, located at the level of junction of ampulla of vas deferens. The proste gland comprised of corpus prostate and pars disseminata the former lay as a band like structure close to the junction of vesicular gland. The bulbourethral glands were paired glands located on either sides of urethra close to ischial arch. With the advancement in age, the stroma and parenchyma ratio reduced in all the three accessory sex glands viz; vesiucular gland, prostate gland and bulbourethral gland. The result revealed that the testes, epididymis, ampulla of vas deferens, vesicular gland, prostate gland and bulbourethral gland grew simultaneously during the postnatal life of animal. There was an all around growth in their biometrical parameters and the parenchyma. The function of secretory acini increased maximum in pubertal animals. In postpubertal animals the degenerative processes were set in testis which was indicated by the growth of giant cells in the seminiferous tubules, degeneration of spermatogenic cells and seminiferous tubules and increment of fibroblasts and macrophages in the intertubular area. The epididymal tubules start showing signs of incarceration, contracture and replacement by proliferating cells especially the fibroblasts. The accessory sex glands exhibited signs of

exhaustion, degeneration and replacement by fibrous elements along with reduction in their normal process of secretion.

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3. Gross Histological and Histochemical studies on the urinary system of goat (*Capra hircus*)

Ashwani Kumar and Raja Ram

The gross histological and histochemical studies were conducted on 35 apparently healthy goats (*Capra hircus*) of either sex from nondescript breed, divided into three groups viz; prepubertal, pubertal and postpubertal. The age of the animals was ascertained on the basis of dentition. The right and left kidneys were fixed in position by renal fascia and partly or completely covered in perirenal fat. The colour of the kidneys varied from brownish red to dark bluish red. The right kidney extended from the last rib to the second lumbar vertebra and related medially to the posterior vena cava. Left kidney extended from the third to fifth lumbar vertebra. Both kidneys were bean shaped and smooth. The left kidney was generally larger in size and weight. The ureter began at the renal pelvis and terminated at the bladder. Study revealed that the right ureter was longer than the left one. The parenchyma of the kidney was divided into an outer cortex and inner medulla. It was surrounded by a connective tissue capsule distinctly divided into an outer fibrous layer and inner muscular layer formed by smooth muscle. The cortical stroma was divided into cortical labyrinth and medullary rays. The cortical labyrinth consisted of renal corpuscles, PCT, DCT, arched collecting tubules, interstitial connective tissue, blood vessels and capillaries. The renal corpuscles lay in the cortical labyrinth and consisted of a tuft of capillaries surrounded by Bowman's capsule. The diameter of renal corpuscles was maximum in the juxtamedullary region. In the glomerulus the diameter of afferent arteriole was slightly larger than the efferent arteriole. The diameter of glomeruli significantly increased in the postpubertal animals. The PCT formed the major portion of the cortical labyrinth. These are lined by single layer of low columnar or pyramidal cells with rounded nuclei. The DCT was generally present near the vascular pole of the glomerulus. It was lined by simple cuboidal epithelium. The diameter of these tubules increased with increase in age. The arched collecting tubule which joins the nephron with the straight collecting tubules of medullary rays was lined by a single layer of cuboidal or columnar epithelium. Its diameter also goes on increasing with increased in age. The medullary rays comprised of straight portion of proximal tubules, thick segment of Henle's loop and straight collecting tubules. The straight portion of proximal tubule was comprised of pyramidal cells with dark stained eosinophillic cytoplasm. Loop of Henle was lined with low cuboidal cells and the straight collecting tubule was line with cuboidal or columnar epithelium. Numerous openings of papillary duct opened on the tip of the renal papilla forming area cribrosa. The mucosa of ureter formed a flap before opening into the urinary bladder. The mucosa of the urinary bladder was folded or appeared smooth depending on the degree of distention of organ. The mucosa in the pelvic urethra was longitudinally folded. Its lining epithelium epithelium varied from flattened to tranditional epithelium. The lumen of penile urethra was folded in its beginning near the ischial arch and became more folded in its distal part. The epithelium of penile urethra wsa transitional but may be changed into stratified squamous at external urethral orifice.

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4. Gross morphological, histological and histochemical and ultrastructural studies of the intestine of guinea fowl

Sriprakash and R.S. Katiyar

The present study was aimed at promotion and advancement of the knowledge with respect to the Gross morphological, histological, histochemical, and ultrastructural studies of the intestine in guinea fowl from day 1 through 180. The changes in the intestinal morphology, histological, histochemical and Ultrastructure with the advancement of age were studied. There was significant increase in the weight and length of all the segments of intestine with age. Maximum increase in the length and weight was observed during first 30 day of the post hatch life. The wall thickness of the intestine and diameter were also increased with age. Histologically, the wall of the intestine consisted of tunica mucosa, tunica muscularis, tunica submucosa and tunica serosa. Mucosa of entire intestine showed villi of variable shape and size. The surface epithelium consisted of columnar shape chief cells, the goblet cells, rounded oval shaped globular leukocyte, and intraepithelial lymphocyte. The goblet cells were neatly shaped goblet, the globular leukocyte were observed in the basal half of the intestinal epithelium. The lamina propria was composed of loose connective tissue which had collagen and reticular fibers which increased with the advancement of age. The cellular components observed in the lamina propria were mainly large and small lymphocytes together along with a few eosinophilic leucocyte. Meissner's plexus were present in the submucosa layer. Histochemically, the intestinal goblet cells and brush border of villi showed strong PAS reaction. Pronounced activity of Alkaline phosphatase. Acid phosphatase and lipase were observed in villus epithelium of 15 to 180 day old guinea fowl. Ultrastructurally, the structural variations among similar type of cells were not observed in the different part of intestine. The chief cells having basally located oval nuclei and prominent nucleolus. Globular leucocytes and lymphocyte were sandwiched between the epithelial cells throughout the intestine in all age groups. Mast cells were located in the lamina propria just beneath the epithelium. The results obtained indicate that the changes observed in different age group may contribute significantly in satisfying the functional requirements of guinea fowl during development.

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Veterinary Biochemistry

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2.	Standardization of biological and immunological assays of pregnant mare serum godanotropin (PMSG) in immature rats	Dr. Uzma	Dr. Ajit Kr. Jain	2002	65
3.	Effect of Se and Vitamin E supplementation on the blood chemistry of growing Hariana calves	Dr. Pawanjit Singh	Dr. Rajesh Nigam	2004	66
4.	Molecular characterization of myofibrillar protein isoforms and MYF-5 gene in Indian goats	Dr. Pranjal Pratim Saikia	Dr. Rajesh Nigam	2005	66
5.	Comparative studies on uterine and fetal fluid proteins in pregnant and non-pregnant buffalo (*Bubalus bubalis*) using one and two dimensional electrophoresis	Dr. Reetu Raj Gogoi	Dr. Kranti Dev	2005	68
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9.	Studies on effect of enrofloxacin on semen quality and enzyme profile of Barbari buck	Dr. Chandrima Sinha	Dr. Kranti Dev	2008	70
10.	Studies on lipid and enzymatic profile of ovarian follicular fluid and luteal tissue during estrous cycle in buffalo (*Bubalus bubalis*)	Dr. Santosh Srivastava	Dr. Rajesh Nigam	2008	71
11.	Changes in Enzymatic and protein profiles in granulosa cells of oocyte in buffalo	Dr. Ajay Yadav	Dr. Rajesh Nigam	2008	72
12.	A Study on comparative protein profile in the pre-ovulatory follicles vis a vis serum of Buffalo during different stages of estrus cycle	Dr. Dalip Kumar Baitha	Dr. Rajesh Nigam	2010	73

13.	A study on evaluation of vitamin C and chlorophytum borivilianum extract supplementation for adaptogenic activity in stress induced rats	Dr. Narjeet Singh Rajput	Dr. Rajesh Nigam	2011	74
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15.	SDS-PAGE Analysis of Seminal Plasma Proteins of Bhadawari Buffalo and Their Association with Oxidative Status and Semen Characteristics in different seasons	Dr. Lokesh Sharma	Dr. Vijay Pandey	2012	75
16.	Circulating estradiol 17β and progestrone vis a vis nitric oxide (no) and nitric oxide synthase (nos) levels related to estrus behaviour in cycling buffaloes and cows	Dr. Shailaza Sharma	Dr. Rajesh Nigam	2013	76

M.V.Sc.

1. Changes in concentration of certain blood biochemical constituents in indigenous and cross-breed cattle during late pregnancy and early lactation in relation to milk yield

Brijesh Rawat and Rajesh Nigam

A study was conducted on six indigenous (Hariana) cow, a dual purpose breed and six cross breed (Hariana & Jersey) cows, a high milk potention breed during 3 months before and after calving. The study was aimed to explore a possible relationship between certain relevant serum biochemical constituent and the milk yield. The biochemical serum parameter namely phosphoglucoiomerase (PGI), lactate dehydrogenase (LDH), Alkaline phosphate (AKP), Acid phosphoatase (ACP), Glucose, total protein, Albumin, globulin, A:G ratio, urea, cholesterol, calcium, phosphorus, sodium and potassium were intimated in the samples obtained at fortnightly intervals. It can be concluded that serum levels of the enzymes estimated in the present study could not show any relationship with milk production. However, among the various serum metabolites studies, calcium levels revealed a negative relationship with milk yield, both during late pregnancy and early lactation. The serum glucose level and serum potassium level were found to be negatively correlated with milk yield during early lactation on the other hand, serum albumin, A:G ratio with milk yield during early lactation.

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2. Standardization of biological and immunological assays of pregnant mare serum godanotropin (PMSG) in immature rats

Uzma and Ajit Kr. Jain

Pregnant mare serum gonadotropin was purified from pregnant mare's serum and its biological activity was estimated by conducting biological assay in immature rates. For testing the biological activity test serum, first the biological assay with commercially available PMSG was performed for the standardization of techniques. The present study showed that serum of pregnant mare from about 40-110 days of gestation contains large amounts of gonadotropic hormone and also showed that pregnant mare serum gonadotropin has follicle stimulating hormone like activity in heterogeneous species. In his manner PMSG was performed in immature rats. On administrating PMSG ovarian and uterine weights in rats were increased. The increase in ovarian weight rat was more in the month of March as compare to February because of change in environmental conditions. During this study 40 i.u. dose of PMSG in considered best for ovulation in rats. Ovarian weights were significantly higher (at 5% level) on second and third day of treatment then it went down on fifth and seventh day of treatment. Histopathology of ovarian tissue was also performed to see the ovarian function on administration of PMSG. Coupus luteum and mature follicles could be seen more with dose of 50 i.u., ovulation was seen maximum on the third day treatment, ovum was also present in the ovary with this dose but with higher doses i.e. 100&200 i.u. PMSG, degenerating follicles were more. Ovum and corpus luteum were also absent in ovarian section of rat on administering 200 i.u. of PMSG. To overcome long and tedious method of biological assay, immunological assay was also performed in this assay there is no need to sacrifice the rats. Enzyme immunoassay was performed to estimate progesterone concentration in serum after administration of PMSG. The progesterone estimation can be a better tool than taking the ovarian and uterine weights that is more tedious and time consuming. Progesterone concentration was significantly different at 5% level after administration of 40 i.u. over administration of 4 &20 i.u. of PMSG. Progesterone concentration increased up to 400ng/ml with 40 i.u. on day 5 & 7 of the treatment, while it was around 50 ng/ml in control group of rats injected with 4 i.u. Haemagglutination inhibition assay

was also performed as a better tool for quantification of PMSG present in test serum from pregnant mare. Anti-PMSG antisera were raised in rabbits. Antibodies taken from rabbits were absorbed with non-pregnant mare serum to overcome the cross reaction of PMSG with non-specific antibodies against horse serum albumin. Turkey antisera do not contain non-specific antibodies against horse serum proteins, so use of turkey anti-PMSG antisera is more beneficial over rabbit antisera. After standerization of techniques for estimation of biological activity of PMSG, purification of pregnant mare's serum was performed by the method of ethanol 75% precipitation and biological was tested in rates by the same method given above. Administration of test serum showed activity of PMSG in rats by showing increase in ovarian and uterine weights. For testing the activity of test serum, pooled serum, deep frozen serum, liquid nitrogen frozen serum and lyphoilized serum were tested in rats and the maximum activity was seen on administering lyophilized test serum. It can be said that somehow activity may be lost during storage and processing for quantification of PMSG in test serum haemagglutination inhibition assay was performed. In this test titer of test serum was found 1:2000 for agglutination of PMSG sensitized RBCs. In this manner, PMSG can be considered as better tool to induce estrus and ovulation as to FSH, because superovulation with PMSG is much cheaper and it can be imported. As compare to FSH, the uses of PMSG are simple and possess less stress on the cows.

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3. Effect of Se and Vitamin E supplementation on the blood chemistry of growing Hariana calves

Pawanjit Singh and Rajesh Nigam

The study was conducted on twenty growing Hariana calves below one year age. The calves were divided into two groups viz control and treatment comprising of ten calves each. The treatment group was given supplementation dose of vitamin E and selenium combination intramuscularly at fifteen days interval. The blood samples were collected fortnightly. The samples were analysed for metabolites namely creatinine, calcium, phosphorus, urea nitrogen, glucose, albumin, total protein, and enzyme profile consisting of LDH and ferroxidase and TBARS concentration for determination of lipid peroxides. The creatinine, calcium, urea nitrogen, albumin, total protein recorded normal serum values, except glucose and phosphorus, whose values were found to be significantly higher in the treatment group as compared to the control. The ferroxidase activity and TBARS values did not record any significant differences among the control and treatment groups. However, LDH activity were found to differ between the treatment (881.93 I.U./l) and control (805.35 I.U/l)groups. The present study is an effort to come out with a metabolic and enzymatic profile in growing Hariana calves to assess the effect of antioxidants namely vitamin E in association with selenium, on the oxidative stress of growing calves. The present study concluded that the vitamin E and selenium supplementation had little effect on the blood metabolites except in the case of glucose and phosphorus. The LDH enzyme did reveal the effect of supplementation in the calves, Ferroxidase activity and TBARS values too could not reveal the supplementation effect.

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4. Molecular characterization of myofibrillar protein isoforms and MYF- 5 gene in Indian goats

Pranjal Pratim Saikia and Rajesh Nigam

Goat meat is one of the widely consumed meat in the world. Goat meat is a high source of essential amino acids, carbohydrate, nitrogenous compound, minerals and vitamins. So the study and characterization of muscle (meat) specific proteins and genes to manipulate the favourable gene expression is warranted to understand meat of specified quality. Muscle protein

can be divided into three groups, Sarcoplasmic, Myofibrillar and Connective tissue proteins. Myofibrillar and connective tissue proteins create the structure of meat. The most important myofibrillar proteins are Myosin, Actin, troponin, tropomyosin, M and C proteins, α-actinin and cytoskeletal proteins. Various isoforms of these myofibrillar proteins are found in skeletal muscle of goat. Meat and blood samples were collected from a total 195 numbers of goats containing Barbari (102), Sirohi (30), Jamunapari (32) and Marwari (31) maintained at the farm of C.I.R.G. Makhdoom. From the above population, some animals were categorized as high and low growth, depending upon their body weight at three months of age. Various molecular biological and biochemical techniques were applied to observe the expression patterns of muscle specific proteins as well as genes in high and low growth animals. The isoformes of myofibrillar proteins and their molecular weight were determined by Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) in four Indian goat breeds. Two isoforms of myosin heavy chain (myhc) were observed in 10% SDS-PAGE and designated as type-I (208 kd) and type-II (192kd). Three fastest migrating myosin light chains were also identified and designated as light chain-1 (LC-1), Light chain-2 (LC-2), and light chain-3 (LC-3) according to their increasing electrophoretic mobility on polyacrylamide gel. The molecular weight of LC-1, LC-2 and LC-3 of goat skeletal myosin calculated using SDS-PAGE were 26.3 kd, 17.26 kd and 14.92 kd respectively. The SDS-PAGE pattern of actin showed only one isoform and molecular weight was found to be 44.78 kd. Two isoforms were detected for tropomyosin in SDS-PAGE and designated as α, and β. The molecular weight calculated for β-tropomyosin and 'α' tropomyosin was 38.56 kd and 35.66 kd respectively. Depending upon the migration pattern on SDS-PAGE, three isoforms of troponin were detected in SDS-PAGE and designated as Troponin T, having molecular weight 30.96 kd, Troponin-I, having molecular weight 22.01 kd and Troponin C having molecular weight 16.18 kd. SDS- PAGE patterns of skeletal muscles from different Indian goats breeds showed variation in the myhc and MLC portion. SDS-PAGE showed a number of bands in the MLC region, indicating polymorphism of myosin light chain protein. Quantification of these isoforms in different breeds was found to be different. The expression pattern of myofibrillar protein isoforms in a population of Barbari goats having high and low growth was examined. But no variation in expression pattern of myofibrillar protein isoforms could be observed. It may be due to the variation of age at the time of slaughter or may be because of higher age of animals at the time of slaughter. Since different isoforms of myofibrillar proteins are expressed during the different developmental stages, so stydy of these isoforms during different growing stages may be more justified. The PCR-RFLP of MYF-5 gene revealed two alleles, A and B. The frequency of B allele (0.79) was found to be higher than A allele (0.21) in the population of Barbari goats tested. The homozygous BB (0.80) genotype was observed to be much higher in high growth animal population and heterozygous AB (0.54) genotype was found to be predominant in low growth animal population. This observation indicates a significant affect of BB genotype of myf-5 gene in growth and development of Barbari goats at three months of age. Polymerase chain reaction for Myogenin gene was carried with specific primers designed for porcine Myo-G locus. All these primers were used for several times to amplify the myogenin gene, but no amplification was found. However further characterization of myogenin gene with specific primers is necessary to study the affect of myogenin on muscle growth.

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5. Comparative studies on uterine and fetal fluid proteins in pregnant and non-pregnant buffalo (*Bubalus bubalis*) using one and two dimensional electrophoresis

Reetu Raj Gogoi and Kranti Dev

Gross composition and the changes of proteins during estrous cycle and early pregnancy have been studied in cattle, horse, sheep and pig. However, no information is available on biochemical composition and type of proteins present in uterine fluid of cycling and pre-implantation buffaloes. Similarly, report is not available on protein constituents of any of the fetal fluids of pre-implantation buffaloes. Therefore, the experiment is designed with the objectives to detect the pregnancy or uterine specific proteins through one and two dimension electrophoresis analysis. Uterine washing of the early, mid, late luteal stages of the estrous cycle, pre-implantation stage of pregnancy and the foetal fluid were collected and compared using one and two dimensional electrophoresis including isoelectric focusing techniques under non-reducing condition. Result of one dimension gel comparison indicated a band in serum proteins that was not available in uterine secretion and there were at least three uterus-specific protein bands of approx. 40.7, 29.6 and 27.3kDa molecular weight those were not present in serum. A band of 11.5 kDa was found to be unique for mid luteal sample that was not observed in any of the other phases of the cycle. Similarly 14.3 kDa band in late luteal and 9.9 kDa bands in early luteal were not observed in other stages of the cycle and serum sample. Comparison of bands in pregnant and non-pregnant luteal phase sample did not show any difference in banding pattern in one dimension gel detected with coomassie blue or silver stain. However, comparison of bands in isoelectric focused gel showed four unique bands having pi values of 6.6, 6.7, 7.0 and 7.2 in pregnant uterine fluid sample and three unique bands with pi values 6.8, 7.4 and 7.5 in luteal phase sample. Two-dimension electrophoresis analysis of pregnant and non-pregnant luteal sample revealed at least four high molecular weight proteins spots (154.8, 123, 114.8 and 97.7 kDa) between 5 to 5.5 pi those were not present in luteal phase sample. Similarly, two clusters of different protein spots were observed after 6.7 to 7.8 pi region with one cluster with 10 spots (125.8, 123, 123, 115.3, 107.1,104.7, 91.2, 91.2, 84.1 and 81.2 kDa) in relatively high molecular weight region and other one with nine different spots in the low molecular weight region (35.4, 28.8, 27, 29.7, 34.6, 37, 35.4, 32.6, kDa) in pregnant sample not present in non-pregnant luteal phase sample. Comparison of both allantoic and amniotic fluid protein band in one dimension non-reducing PAGE method showed three bands of 20.6, 10 and 8.1 kDa were present in allantoic fluid those were not present in amniotic fluid. Similarly a band of 6.8 kDa was observed in amniotic fluid that was not present in allantoic fluid. Isoelectric focusing of mixture of protein analysis showed 8 unique bands in allantoic fluid in the acidic ph range out of these 3 bands were having pi values of 5.4,5.7 and 5.9 rest 5 bands had pi values of 6.4,6.5,6.6,6.8 and 6.9. Two dimension comparisons of protein spots between allantoic and amniotic fluid revealed about four higher (138, 127.6, 122.1 and 117.2 kDa) and two lower molecular weight bands (47.6 and 47.6 kDa), different spots in allantoic fluid proteins those were not observed in amniotic fluid. However, only one spot (99.5 kDa) was found unique for the amniotic fluid protein that was not present in allantoic fluid.

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6. Cloning and characterization of goat enteric β-defensin

Asish Kumar and Kranti Dev

Beta-defensin are key components of innate immunity of animals. They provide defence against microbes as they are available within minutes to hours after the first contact with pathogen. Beta -defensins are widely distributed in epithelial tissues and leukocytes of

mammals, insects, plants, molluscs and birds. At mucosal surface intestinal epithelial is very much prone to many extrinsic microorganisms. The present assignment was undertaken to clone and characterize the enteric Beta -defensin mRNA from an Indian nondescript male goats distal ileum as goat is resistant to many diseases than sheep. Total cellular RNA was isolated using TRI REAGENTTM method immediately after collection of ileac epithelium from a local abattoir under ice. 0.15 μg RNA was yielded per mg of tissue and the A260/A280 ratio was found to be 1.84. Integrity of RNA was confirmed by Agarose gel electrophoresis. cDNA was synthesized by reverse transcription using 200 ng of goat EBD RNA, 2 μl of reverse primer and 2 μl of omniscript and sensiscript reverse transcriptase. Amplification of cDNA was done by designing a specific set of primer on comparing bovine EBD mRNA and goat BD2 mRNA sequence to amplify 253 bp fragment using Hotstart Taq DNA polymerase. The amplified PCR product was purified from gel and ligated into linearized TA cloning vector. The ligated product was transformed into the XL-blue strain of *E. Coli* competent cell. The transformed cells were allowed to grow in ampicillin containing LB plate at 37oc overnight. IPTG and X-Gal were used as an inducer and substrate respectively for blue and white colony screening of recombinant TA plasmid. The white positive colonies were selected and subcultured in ampicillin containing LB-broth tube and plasmids were isolated by aupre P (life technologies) kit. The isolated plasmids were digested by *nco*i restriction endonuclease to release the insert for confirmation of recombinants. As expected an insert of 253 bp was released following digestion. The recombinant plasmid was prepared as stab culture to be sent for DNA sequencing at DNA sequencing facility, Division of Biochemistry, University of Delhi South Campus, New Delhi by an automated sequencer. The sequence of goat EBD was compared with published beta-defensin nucleotide sequence of different species. There were 26, 16 and 5 nucleotide substitution in goat EBD as compared to cattle EBD, buffalo EBD and goat BD2, respectively. Goat EBD was found to be closer to BD2 with 97.4% homology. The deduced amino acids sequence of goat EBD from the nucleotide sequence showed a homology of 70.80%, 80% and 93.8% to cattle EBD and buffalo EBD and goat BD2, respectively. The phylogenetic tree drawn from the available beta -defensin sequnce showed that the beta -defensin of ruminants form one group that implies they evolve from the same precursor molecule.

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7. Molecular studies on goat antimicrobial peptides

Ambika Sharma and Kranti Dev

Antimicrobial peptides play an important role in providing innate immunity to the host against pathogenic microbes. These peptides include mainly defensins and cathelicidins. Defensins provides protection at the epithelial surface and has been isolated and characterized from neutrophils and epithelial surfaces of different species including bovine, ovine, caprine, porcine and avian. While cathelicidins have been isolated mainly from neutrophil granules and are synthesized by bone marrow cells. In the present study, we have characterized goat Lingual antimicrobial peptide and myeloid cathelicidin. Immediately after collection of tissues (tongue and bone marrow) RNA was isolated using TRI REAGENTTM. 0.24 μg and 0.28 μg RNA was isolated per mg of tissue having A260/280 ratio of 1.82 and 1.84 for goat LAP and myeloid cathelicidin respectively. Integrity of the RNA was checked by Agarose gel electrophoresis. cDNA was synthesized using BD Biosciences protocol. 12-18 μl of RNA sample was dissolved in 500 ng of oligo (dt) primers. DEPC water was added to obtain final volume of 20 μl and protocol was followed. cDNA was amplified using Taq DNA polymerase to amplify 272 bp and 297 bp using different set of primers for goat LAP and myeloid cathelicidin respectively. cDNA was cloned in pgemt-Easy cloning vector. The ligated product was transformed and plasmids were isolated. The plasmids were digested by *eco*ri enzyme to release the insert for confirmation of

recombinants. The recombinant plasmids were then sequenced. The goat LAP has 99.5%, 94.9%, 94.4% homology at nucleotide level and 98.5%, 87.7%, 86.2% homology at amino acids level with goat BD1, Goat EBD and goat BD2 respectively. The goat cathelicidin has 89.2%, 77.8%, 85.9% homology at nucleotide level and 81.8%, 74.7%, 74.7% homology at amino acid level with cattle CATHL-7, cattle CATHL-4 and goat bac 7.5 respectively. The phylogenetic tree analysis both at nucleotide and amino acid showed goat LAP and goat BD1 are related closely while goat myeloid cathelicidin is closest to cattle CATHL-7. The study provides basic information about expression of lingual antimicrobial peptide and myeloid cathelicidin in goat which is helpful in understanding innate immunity in goats compared to other domestic animals.

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8. Cloning and sequence analysis of α-subunit of goat's luteinzing hormone

Jakeerhusen A Olekar and Kranti Dev

Luteinizing hormone (LH) plays a critical role in ovulation and maintenance of pregnancy in female and gamete production in case of male during fertile phase of life. It acts through a G-protein coupled receptor to influence steroidogenesis and gemetogenesis. Physiological disturbance of this hormone leads to conditions such as delayed ovulation, an ovulation and cysticovarian disease and lack of sexual desire in male. Since there had been no report of molecular characterization of beta subunit of luteinizing hormone of Indian goat, the present study focussed on cloning and sequencing of goat LH-subunit. Genomic DNA was extracted from goat blood and amplified using specific LH gene primers. LH-gene was cloned in T/A cloning pgemt-Easy vector. After transformation, the recombinant clones produced white and blue colonies on LB agar plate containing ampicillin, X-gal and IPTG. Plasmids were isolated from randomly selected white colonies. Presence of insert was confirmed by restriction enzyme digestion of plasmid by *eco*ri as well as *not*i enzyme. It also was confirmed by PCR in which isolated plasmids acted as template for amplification of insert gene. After confirmation, plasmids were sent for sequencing to DNA sequencing facility, South Delhi Campus. Analysis of sequence revealed the size of insert was 1006 bp as expected. Comparison of nucleotide and amino acid sequence with that of other mammalian species revealed the cloned gene to be is LH encoding 141 amino acids. The similarity expressed as percent identity was, China goat (99.6%) > sheep (97.7%) > cattle (92.3%) > horse (64.5%) > pig (63.2%) > human (54%) > mouse (51.9%); values that are generally consistent with phylogenetic relationship. Inferred amino acid sequence shows absolute (100%) similarity with China's goat and sheep. The common and essential features such as twelve cysteine molecules, a single potential N-glycosylation site, the CAGY region (tetrapeptide essential for biological activity) and another tetrapeptide CGPC (integral part of active site and unique to mammals) are all found in the goat sequence also.

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9. Studies on effect of enrofloxacin on semen quality and enzyme profile of Barbari buck

Chandrima Sinha and Kranti Dev

Enrofloxacin is a broad spectrum fluoroquinolone group of antibiotic, is routinely used for treatment of various bacterial infections. Further, enrofloxacin was reported to have an inhibitory effect on libido and service behaviour while reaction time was increased following parentral administration of the drug in buffalo bull. Enrofloxacin has a tendency to get accumulated in the germ cells, spermatogonia or spermatids and accessory sex glands and may exert chemosterilizing effect on the testes. The present study was undertaken firstly to perform pre-treatment grading of bucks by subjective evaluation then to assess the effect of enrofloxacin

on various physical and biochemical attributes of selected bucks both subjective (manually) and objective (CASA). Six apparently healthy Barbari bucks aged 1.5 to 2.5 years fed as per NRC recommendations stationed at the experimental sheds of department of Physiology, DUVASU, Mathura, were used for this study. The bucks were maintained under similar management conditions. Enrofloxacin was administered at the dose of 5mg/kg body wt. Intra-muscularly daily for 7 days. Semen was collected by A.V. method biweekly using a non-oestrous doe as dummy. A total of eighteen ejaculates were collected from each buck after the drug administration. Seven pre-treatment ejaculates were taken from each buck before the start of experiment. Volume, Mass motility, Progressive motility, Sperm Concentration, Per cent Live Spermatozoa, Morphological Abnormalities on Analysis (ANOVA) did not find any significant difference both prior and after enrofloxacin administration. Sperm concentration on analysis (ANOVA & DMRT) of data revealed highly significant difference ($P \leq 0.01$) between bucks prior to the administration of drug. Total motility, Progressive motility, Rapid motility,Medium motility,Static spermatozoa on analysis (ANOVA & DMRT) of data revealed highly significant difference ($P \leq 0.01$) between bucks prior to the administration of drug. Straight-line velocity (VSL), Curvilinear velocity (VCL), Average-path velocity (VAP), Linearity, Straightness on analysis (ANOVA & DMRT) of data revealed highly significant difference ($P \leq 0.01$) between bucks prior to the administration of drug. Head area, Head length, Head ellipticity, Head elongation on analysis (ANOVA & DMRT) did not reveal any significant difference prior to enrofloxacin administration. Head width, Head perimeter, Head rugosity, Head regularity on analysis (ANOVA & DMRT) revealed significant difference ($P \leq 0.05$) prior to enrofloxacin administration. Mid-piece area, Mid-piece length, Tail length on analysis (ANOVA & DMRT) did not reveal any significant difference prior to enrofloxacin administration. Mid-piece width on analysis (ANOVA & DMRT) revealed significant difference ($P \leq 0.05$) prior to enrofloxacin administration. Aspartate animotransferase (AST) or Glutamate Oxaloacetate Transaminase (GOT), Alanine aminotransferase (ALT) or Glutamate Pyruvate Transaminase (GPT), Alkaline Phosphatase (AKP) Acid Phosphatase (ACP) all shows highly significant ($P \leq 0.01$) increase in AST activity of bucks after drug administration was observed in different ejaculates. Total Protein showed highly significant ($P \leq 0.01$) decrease in total protein concentration of bucks after drug administration was observed up to 12^{th} ejaculate. Concentration, Motility on analysis revealed non-significant difference between two methods of evaluation (t value=2.262).

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10. Studies on lipid and enzymatic profile of ovarian follicular fluid and luteal tissue during estrous cycle in buffalo (*Bubalus bubalis*)

Santosh Sriwastava and Rajesh Nigam

This study investigated various lipid content and enzymes in buffalo follicular fluid and luteal tissue in relation to ovarian estrous cycle stage. 96 ovaries obtained from slaughter house buffaloes, were classified as early luteal stage, late luteal stage, regressing luteal and follicular stage on the basis of shape color and consistency of corpus luteum. Activity of four enzymes, namely Alkaline phosphatase (AKP), Acid phosphatase (ACP), Lactate dehydrogenase (LDH), 3β-hydroxy steroid dehydrogenase (3β-HSD) and lipid profiles cholesterol, triglycerides and HDL, LDL,VLDL were estimated. It was observed that in the follicular fluid the ALP of the different ovarian stage did not significantly but in the luteal tissue was highly significant. The ACP activity in the follicular fluid at late luteal stage revealed higher significant than those of early luteal stage, regressing luteal stage and follicular stage. The LDH activity observed in to the follicular fluid did not vary significantly in respect to ovarian estrus cycle stages. The activity of 3β-HSD did not vary significantly, in respect to ovarian estrus stages. In the luteal tissue stage the activity varies higher significant in respect to ovarian estrus stages. In the follicular fluid

value of cholesterol in relation to ovarian stages did not vary significantly and also in the luteal tissue. In the follicular fluid value of Triglycerides in respect to ovarian stages did not vary significantly, similarly in the luteal tissue. In the follicular fluid value of HDL in relation to ovarian stages varies higher significantly. In the luteal tissue in respect to ovarian stages did not vary significantly. In the follicular fluid value of LDL in respect to ovarian stages varies highly significantly. In the luteal tissue in relation to ovarian stages did not vary significantly. In the follicular fluid value of VLDL in respect to ovarian stages varies significantly but not in the luteal tissue. In the follicular fluid total protein content differ highly significantly in relation to ovarian stages. In the luteal tissue total protein content differ highly significantly in relation to ovarian stages. Hence, finding of present study suggested that the change in the activity of enzyme and lipid profiles during the folliculogenesis and steroid genesis during the ovarian estrus cycle stages. The information obtained from this study can be used in formulating the culture media for oocyte maturation *in vitro*.

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11. Changes in Enzymatic and protein profiles in granulosa cells of oocyte in buffalo

Ajay Yadav and Rajesh Nigam

The study investigated enzymatic and protein profiles in the buffalo granulosa cells in relation to ovarian cyclic stages. Ninety six Ovaries obtained from a slaughtered house were classified as stage I (early luteal stage), stage II (Late luteal stage), Stage III (Regressing luteal stage) and Stage IV (follicular stage), on the basis of shape, colour and consistency of corpus luteum. Activity of four enzymes namely alkaline phosphatase, acid Phosphatase, Lactate dehydrogenase and 3β -Hydroxy steriod dehydrogenase and total protein contents (ng/cell) and protein profiles by electrophoresis were studied. The study observed that the alkaline phophatase in granulosa cells in relation to ovarian stages did not differ significantly, however, activity did show an increase from regressing luteal stage(1.795±0.0143 miu/mg of protein) to late luteal stage (1.852±0.0315 miu/mg of protein). Acid phosphatase activity in granulosa cells differed significantly among ovarian stages. The late luteal stage (3.593±0.092 miu/mg of protein) revealed highest ACP activity and lowest ACP activity recorded in early luteal stage (1.418±0.0048 miu/mg of protein). LDH activity differed significantly ($p<0.01$) among different ovarian cycle stages in granulosa cells. The LDH values were found to be highest in follicular stage (163.880±0.423 miu/mg of protein) and lowest in early luteal stage (46.302±0.633 miu/mg of protein). The 3β-Hydroxy steriod dehydrogenase activity observed for late luteal stage (67.833±3.381 miu/mg of protein), revealed highly significant ($P<0.01$) values as compared to regressing luteal stage (33.167±2.810 miu/mg of protein), follicular stage (34.333±3.621 miu/mg of protein) and early luteal stage (34.333±1.726 miu/mg of protein). Total protein concentrations in granulosa cells (ng/cell) differed significantly ($p<0.01$) among ovarian stages. Highest concentration of protein was observed in follicular stage (0.184±0.037 ng/cell) and lowest values were observed in late luteal stage (0.074±0.0024 ng/cell). Protein profiles of granulosa cells revealed nine bands on the basis of percentage of protein contents in stage I. Stage II showed eight bands of protein with missing protein band number nine. Stage III showed eight bands but protein no. One band was lacking. Stage IV showed only seven bands of protein with protein no. Eight and nine bands were missing. It was observed that Protein band number four with highest protein percentage and protein number while the sixth band was the next. The enzymatic profile and protein profile observed in granulosa cells for four estrous cycle stages revealed biochemical nature of the biological activities occurring during maturation of follicles in relation to metabolic and steroidogenic events in the buffalo ovary. The study may be considered as a pioneer work on the biochemical nature of granulosa cells in buffalo ovary since the literature is scanty about

such information. However further studies involving granulosa cells from buffalo and other domestic species are very much required to obtain a complete biochemical picture and comparative data. Findings of such studies may be utilized for inclusion of granulosa cells in the medium for *in vitro* culture of buffalo oocytes aimed to get both its cytoplasmic and nuclear maturation.

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12. A Study on comparative protein profile in the pre-ovulatory follicles vis a vis serum of Buffalo during different stages of estrus cycle

Dalip Kumar Baitha and Rajesh Nigam

The present study was conducted on follicular fluid, granulosa cells and corresponding blood samples of buffaloes obtained from local abattoir. Six samples from four ovarian stages each were taken for the study. The ovarian stages were determined on the basis of corpus luteum morphology. In the present study, highest numbers of granulosa cells were found in the stage IV i.e. follicular stage (4.1817±0.1609) as compared to stage III i.e. regressive luteal stage (3.6733±0.025489), stage II i.e. late luteal stage (2.7783±0.04254) and stage I i.e. Early luteal stage (1.5383±0.14041). The total protein contents (gm/dl) in follicular fluid differed significantly among all the four ovarian stages and exhibited an increasing trend from stage I (3.4867±0.27658) to stage IV (7.4167±0.3137). The total protein values (mg/ml of homogenates) in the homogenate of granulosa cells for stage II (2.758±0.0969) was the lowest when compared to other three ovarian stages. Similarly the protein concentration (ng/cell) per granulosa cell in stage II revealed the lowest value (0.076±0.00504). The highest total protein levels in granulosa cells were observed for stage IV (4.4683±0.24456). In corresponding serum samples of buffaloes, the protein values (gm/dl) recorded for stage I (6.9567±0.6085) and stage II (8.0333±0.40139) were found to be significantly lower than the values recorded for stage III (10.2383±0.5294) and stage IV (9.7333±0.3783). The electrophoretogram of follicular fluid obtained on silver stained gel from ovarian stage III and IV revealed fifteen protein bands with almost similar molecular masses ranging from 5.92 kDa to 203.61 kDa. However the gels stained with Coomasie Brilliant Blue-G could reveal only seven protein bands of protein bands of molecular weight ranging from 19.99 kDa to 220kDa in the follicular fluid of stage III and only five protein bands of molecular weight ranging 20.98 kDa to 65.89kDa in the follicular fluid of stage IV. In the silver stained electrophoretograms of granulosa cells obtained for stage III eleven protein bands of molecular weight 6.45 kDa to 202.31 kDa could be detected where as the granulosa cells of stage IV revealed nine protein bands of molecular weight ranging from 17.16 kDa to 183.99 kDa. The protein bands of 24.36 kDa 14.45 kDa and 6.45 kDa, could not be detected in the granulosa cells of stage IV. However the gels stained using Coomasie Brilliant Blue-G could reveal only four protein bands in both the ovarian stages III and IV. The silver stained electrophoretograms of serum of buffaloes at stage III and stage IV showed seven protein bands of similar molecular weight ranging from 10.43 kDa to 190.5 kDa in both the cases. From the present study it may be concluded that protein contents increased from ovarian stage I to ovarian stage IV indicating the gradual increase in the quantity of peptidergic ovarian factors in all the ovarian tissues including follicular fluid and granulosa cells. In turn this trend may be correlated with the process of follicular maturation. Further, the silver staining clearly came out as more sensitive staining technique for the polyacrylamide gels as compared to Coomasie Brilliant Blue- G. The study could reveal that the three protein bands of molecular weight 6.45 kDa, 14.45 kDa and 24.36 kDa were found missing in granulosa cells of stage IV. When compared with that of stage III However the findings need to be confirmed by an *in vivo* study in the buffaloes.

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such information. However further studies involving granulosa cells from buffalo and other domestic species are very much required to obtain a complete biochemical picture and comparative data. Findings of such studies may be utilized for inclusion of granulosa cells in the medium for *in vitro* culture of buffalo oocytes aimed to get both its cytoplasmic and nuclear maturation.

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12. A Study on comparative protein profile in the pre-ovulatory follicles vis a vis serum of Buffalo during different stages of estrus cycle

Dalip Kumar Baitha and Rajesh Nigam

The present study was conducted on follicular fluid, granulosa cells and corresponding blood samples of buffaloes obtained from local abattoir. Six samples from four ovarian stages each were taken for the study. The ovarian stages were determined on the basis of corpus luteum morphology. In the present study, highest numbers of granulosa cells were found in the stage IV i.e. follicular stage (4.1817±0.1609) as compared to stage III i.e. regressive luteal stage (3.6733±0.025489), stage II i.e. late luteal stage (2.7783±0.04254) and stage I i.e. Early luteal stage (1.5383±0.14041). The total protein contents (gm/dl) in follicular fluid differed significantly among all the four ovarian stages and exhibited an increasing trend from stage I (3.4867±0.27658) to stage IV (7.4167±0.3137). The total protein values (mg/ml of homogenates) in the homogenate of granulosa cells for stage II (2.758±0.0969) was the lowest when compared to other three ovarian stages. Similarly the protein concentration (ng/cell) per granulosa cell in stage II revealed the lowest value (0.076±0.00504). The highest total protein levels in granulosa cells were observed for stage IV (4.4683±0.24456). In corresponding serum samples of buffaloes, the protein values (gm/dl) recorded for stage I (6.9567±0.6085) and stage II (8.0333±0.40139) were found to be significantly lower than the values recorded for stage III (10.2383±0.5294) and stage IV (9.7333±0.3783). The electrophoretogram of follicular fluid obtained on silver stained gel from ovarian stage III and IV revealed fifteen protein bands with almost similar molecular masses ranging from 5.92 kDa to 203.61 kDa. However the gels stained with Coomasie Brilliant Blue-G could reveal only seven protein bands of protein bands of molecular weight ranging from 19.99 kDa to 220kDa in the follicular fluid of stage III and only five protein bands of molecular weight ranging 20.98 kDa to 65.89kDa in the follicular fluid of stage IV. In the silver stained electrophoretograms of granulosa cells obtained for stage III eleven protein bands of molecular weight 6.45 kDa to 202.31 kDa could be detected where as the granulosa cells of stage IV revealed nine protein bands of molecular weight ranging from 17.16 kDa to 183.99 kDa. The protein bands of 24.36 kDa 14.45 kDa and 6.45 kDa, could not be detected in the granulosa cells of stage IV. However the gels stained using Coomasie Brilliant Blue-G could reveal only four protein bands in both the ovarian stages III and IV. The silver stained electrophoretograms of serum of buffaloes at stage III and stage IV showed seven protein bands of similar molecular weight ranging from 10.43 kDa to 190.5 kDa in both the cases. From the present study it may be concluded that protein contents increased from ovarian stage I to ovarian stage IV indicating the gradual increase in the quantity of peptidergic ovarian factors in all the ovarian tissues including follicular fluid and granulosa cells. In turn this trend may be correlated with the process of follicular maturation. Further, the silver staining clearly came out as more sensitive staining technique for the polyacrylamide gels as compared to Coomasie Brilliant Blue- G. The study could reveal that the three protein bands of molecular weight 6.45 kDa, 14.45 kDa and 24.36 kDa were found missing in granulosa cells of stage IV. When compared with that of stage III However the findings need to be confirmed by an *in vivo* study in the buffaloes.

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13. A study on evaluation of vitamin C and *Chlorophytum borivilianum* extract supplementation for adaptogenic activity in stress induced rats

Narjeet Singh Rajput and Rajesh Nigam

The present study was conducted in Wistar albino rats to evaluate adaptogenic effect of *Chlorophytum borivilianum* and Ascorbate after inducing chronic cold restraint stress. The study comprises of four groups of rats namely Groups I (Control), Group II (stress), Group III (stress with *C. borivilianum*) and Group IV (Stress with Ascorbate). The blood plasma, liver tissue and kidney tissue samples were analyzed for different biochemical parameter related to stress. The Triglyceride and Cholesterol concentration in all the samples revealed a similar trend recording significantly higher values in the stress group II when compared to the control. The values for the treatment group III and IV reduced significantly almost equal to the control. However when compared statistically, the values obtained for treatment groups differed significantly from the control value for both Triglyceride and Cholesterol. Triglyceride values in the blood plasma (mg/dl), liver tissue (mg/g wet tissue) and kidney tissue (mg/g wet tissue) were found to range between 81.01±0.83 to 112±2.20, 14.48±0.64 to 19.34±1.16, 6.45±0.17 to 9.44±0.49 respectively. Cholesterol values in the blood plasma (mg/dl), liver tissue (mg/g wet tissue) and kidney tissue (mg/g wet tissue) were found to range between 74.77±5.19 to 96.78±3.67, 4.45±0.18 to 8.81±0.99, 4.15±0.29 to 7.23±0.25, respectively. The Lipid peroxidation (LPO) measured in terms of MDA produced, showed similar trend recorded for Triglyceride and Cholesterol. The LPO values in blood (nm MDA/ml) ranged between 4.74±0.34 to 8.89±0.30, in liver tissue (nm MDA/mg protein) 2.62±0.09 to 3.98±0.21, in kidney tissue (nm MDA/mg protein) 1.81±0.11 to 3.00±0.11 were recorded. The enzyme SOD recorded a significant fall in the values under stress in all the three samples in blood, liver and kidney tissue. After treatment with *C. borivilianum* and Ascorbate the values rose to near control. In blood, the range for SOD activity (U/mg Hb) was between 22.29±1.33 to 30.51±1.19, where as in liver tissue (U/mg Hb) recorded the values between 37.78±0.92 to 47.70±2.79 and in kidney tissue (U/mg Hb) recorded values between 8.17±0.40 to 11.78±1.24. The Catalase activity determined in the blood and liver tissue under stress exhibited a similar trend when compared with SOD. However, the kidney tissue revealed significantly higher values under stress as compared to the control values. Upon supplementation with *C. borivilianum* and Ascorbate the Catalase activity in the kidney tissue was found to reduce statistically equal to control. In blood the range for Catalase activity (mm H_2O_2 utilized/min/mg Hb) was between 114.27±1.96 to 133.56±2.91,where as in liver tissue (mm H_2O_2 utilized/min/mg protein) recorded the values between 266.45±4.14 to 296.73±4.80 and in kidney tissue (mm H_2O_2 utilized/min/mg protein) recorded values between 216.05±05.23 to 241.26±5.05. The Vitamin C levels recorded a significant fall in the values under stress in blood plasma and liver tissue. The values rose near to control after treatment with C. *borivilianum* and Ascorbate. The range of Vitamin C concentration (mg/dl) in blood plasma was between 0.66±0.12 to 1.44±0.24, and in liver tissue (µg/ mg protein) between 1.54±0.15 to 2.64±0.20. However, in kidney tissue Vitamin C concentration did not show significant variation as compared to other groups. A significant decline was observed in the Vitamin E values in blood plasma and liver tissue under stress and treatment with Ascorbate showed significant effect on Vitamin E in liver tissue whereas no effect of treatment was observed in blood plasma. However no significant effect of stress as well as treatment was observed in kidney tissue. The values of Vitamin E level were observed between 0.95±0.006 to 1.13±0.04 in blood plasma (mg/dl), 1.17±0.019 to 1.62±0.09 in liver tissue (µg/ mg protein) and 0.76±0.04 to 0.91±0.04 in kidney tissue (µg/ mg protein). The protein concentration in blood plasma, liver tissue and kidney tissue revealed no significant differences among all the four groups. The range of protein concentration (g/dl) in blood plasma was between 6.06±0.09 to 6.21±0.10, liver tissue (mg/ g wet tissue) between 96.82±3.29 to

97.82±2.51 and in kidney tissue (mg/ g wet tissue) between 65.21±3.8 to 66.66 to 2.94. The *in vitro* antioxidant activity 0f *Chlorophytum borivilianum* showed 50% inhibition at the dose rate of 1750 µg/ml for DPPH and 300µg/ml for TBARS. The observations recorded in the present study concluded that both *Chlorophytum borivilianum* and Ascorbate brought about similar adaptogenic effects in the rats after chronic cold restraint stress and can be used as effective antioxidants. However, further studies are required to evaluate the efficiency of the herb, *Chlorophytum borivilianum* and Ascorbate to prove their antioxidant status in domestic species.

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14. A study on status of lipid peroxidation and antioxidant system when supplemented with vitamin C and *Andrographis paniculata* extract in stress induced rats

Dhirendra Singh and Vijay Pandey

The study investigated the antioxidative activity of aqueous *Andrographis paniculata* extract and Ascorbate supplementation in rats using chronic cold restraint stress model. The study comprised of four groups of experimental male wistar albino rats; group I (control), group II (stress), group III (stress with *Andrographis paniculata* @ 250 mg/kg body weight), group IV (stress with Ascorate @ 200 mg/kg body weight). The blood and tissue homogenate (Liver and kidney) samples were analysed for different biochemical parameters. The Triglyceride, Cholesterol, and Lipid peroxidation in all the samples increased significantly in the stress group when compared to the control. The values for the treatment groups reduced to almost near the control showing the effect of the herb and Ascorbate. The enzyme Superoxide dismutase (SOD) decreased significantly in stress among all three samples. After treatment with *Andrographis paniculata* and Ascorbate, the values rose near to the control. The Catalase activity in blood and liver tissue under stress exhibited similar trend as SOD. However, the Catalase activity in the kidney tissue showed significantly higher values under stress as compared to the control values and found to reduce significantly near to the control after treatment with *Andrographis paniculata* and Ascorbate. The Vitamin C levels reduced significantly under stress in all the samples and rose to near control after treatment with *A. paniculata* and Ascorbate. The Vitamin E levels reduced significantly under stress in liver and kidney tissue and rose to near control after treatment with *A. paniculata* and Ascorbate however, no significant effect of stress as well as treatment was observed in blood plasma regarding Vitamin E. *In vitro* DPPH and TBARS assay of *Andrographis paniculata* extract considerably inhibited in a dose dependent manner, the levels of DPPH free radicals and TBARS, respectively thus showing significant antioxidant properties. The findings indicated that both *Andrographis paniculata* and Ascorbate brought about similar antioxidative effects in the rats after chronic cold restraint stress and can be used as effective antioxidants.

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15. SDS-PAGE Analysis of Seminal Plasma Proteins of Bhadawari Buffalo and Their Association with Oxidative Status and Semen Characteristics in different seasons

Lokesh Sharma and Vijay Pandey

The present study was designed to investigate the influence of season on semen characteristics, oxidative status and protein composition of seminal plasma of Bhadawari buffalo bulls. Six sexually mature Bhadawari buffalo bulls having age of 2-4 years were used as semen donor. The study was divided into three season viz. July to September, December to February and April to June (S3). Six ejaculates from each bull were collected in each season in morning hours using artificial vagina. Immediately after collection, the semen was brought to the laboratory and divided into two parts. The semen characteristics (volume of each ejaculate,

sperm concentration, mass motility, progressive motility, live-dead percentage, HOST percentage, and percent acrosomal integrity) were determined in the neat semen. Simultaneously, another part of neat semen was centrifuged for harvesting the seminal plasma. The influence of season on oxidative status was measured by estimating the level of MDA, catalase and SOD activity in seminal plasma. The results of the study showed significant effect of season on ejaculate volume, sperm concentration, progressive motility, HOST percent and acrosomal integrity. The highest ejaculate volume, sperm concentration, Live-Dead percentage and HOST% were observed in summer season where as highest progressive motility, and percent AI were observed in rainy season. The significant seasonal effect was observed on level of total protein and SOD activity in seminal plasma of buffalo bulls. The peak values of these biochemical attributes were observed in summer season as compared to other season of the year. SDS-PAGE analysis of seminal plasma proteins revealed 20 protein bands in rainy season, 23 bands in winter season and 25 bands in summer season. Out of these bands 18 protein bands were observed common in semen samples of all three seasons. The protein bands of 46, 55, 58, 144 and 160 were found in seminal plasma of rainy and summer season but not observed in winter season. Likewise protein bands of 48 and 60 kDa were observed only in winter season whereas 184 and 200 kDa were reported in semen samples of summer season only. The protein fractions (protein%) of common protein bands observed in three seasons revealed significant effect of season on protein bands of 24.5, 66, 70, 72, 84 and 86 kDa protein bands. The sperm concentration showed correlation with 70, 72and 86 kDa proteins; progressive motility showed correlation with 24.5, 70, 72, 84 and 86 kDa proteins, AI showed correlation with 18.5, 20, 24.5, 44.5, 70, 72 and 84 kDa proteins. The total protein of seminal plasma showed correlation with 66 kDa proteins, LPO values exhibited correlation with 70, 72and 86 kDa proteins, Catalase activity of seminal plasma revealed correlation with 70 kDa and 86 kDa proteins, and SOD activity showed correlation with 24.5, 70 and 72 kDa proteins. It can be concluded from the study that season significantly influences the semen quality by affecting the semen characteristics, oxidative status and differential expression of seminal plasma proteins of Bhadawari buffalo bull semen.

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16. Circulating estradiol 17β and progesterone vis-à-vis nitric oxide (NO) and nitric oxide synthase (NOS) levels related to estrus behaviour in cycling buffaloes and cows

Shailaza Sharma and Rajesh Nigam

The present study was designed to measure circulating Estradiol 17β and Progesterone vis-à-vis Nitric oxide (NO) and Nitric oxide synthase (NOS) levels related to estrus behaviour in cycling cows and buffaloes. Blood collection was done at -2,-1, 0, +1, +2 days of estrus cycle of cows and buffaloes. The findings of the study revealed a uniform trend of nitric oxide levels from day -2 to day+2 of estrus cycle showing peak levels at estrus in cows. The levels ranged between 6.98 - 24.1 μm/l during estrus cycle. The levels in buffaloes showed a completely different trend. The levels fell gradually from day -2 - day +2. This ranged between 11.4 - 26.4 μm/L in buffaloes. The profile of NOS concentration at and around estrus showed similar pattern as exhibited by NO in cows and buffaloes respectively.NOS levels ranged from 0.56 to 1.28 U/L in buffaloes and 0.32 - 1.18 U/L in cows. The Estradiol 17β in cows and buffaloes peaked at day of estrus and ranged between 17.31 - 23.14 pg/ml and 1.9 - 29.32 pg/ml respectively. The pattern observed for E2 was similar to those of NO and NOS in cows and buffaloes. In cows, progesterone levels ranged between 0.11 - 0.54 ng/ml at and around estrus. Progesterone concentration in buffaloes was found to be lower as compared to cows. It can be concluded that in cows there was a positive correlation among NO, NOS vis a vis Estradiol 17β and same was not found for buffaloes.

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1. Studies on seroepidemiology and impact of vaccination in calves for control of bovine brucellosis

Naresh Kumar and B.C. Pal

In the study eight strains of Brucella organism were isolated from 133 samples of vaginal discharge, placenta and aborted fetus of cattle and buffaloes. Two isolate of *Brucella abortus* were isolated from vaginal discharge of serologically positive cow and one isolate each from placenta and aborted fetus of cow. Two isolate were from vaginal swabs of buffalo and two strains from placenta of serologically positive buffalo. All the isolates were identified on the basis of their microscopic morphology, cultural characteristics and bio chemical examination. A total of 781 blood sera samples from bovines (483 cattle, 191 buffalo, 79 cow breeding bulls and 28 buffalo breeding bulls) of different organized farms, guashala, rural herd and Veterinary hospitals of state. These serum samples were analyzed by using rose Bengal pate agglutination test (RBPT), Standard tube Agglutination test (SAT) and Avidin-Biotin ELISA . 46 animals were positive for RBPT, 47 for SAT and 61 positive for A-B ELISA. The sero prevalence in organized farm, gaushala, rural herd and Veterinary Hospitals of the state were observed 6.6% in cattle, 7.5% in breeding bulls, 5.7% in cattle and 5% in breeding bulls, 5.2 % and 4.4% respectively.The sero prevalence was observed in buffaloes and buffalo breeding bulls maintained at organized farms, 7% and 9.5% respectively, in rural herd of buffalo 6.1%, while in Veterinary hospitals the prevalence was recorded 4.7%. In this study 236 milk samples of cattle and buffalo collected from organized farm, gaushala, rural herd and Veterinary hospitals and tested using milk ring test (MRT) revaealing the of prevalence of 5.8% in cattle and 6% in buffaloes. The antibody response against *Brucella abortus* strain -19 vaccine were observed in group I calves which are free from infection, group II calves whose mother were found positive during screening by serological tests and III group have calves which having antibody titer against brucellosis infection monitored using RBPT, Standard tube Agglutination test (SAT) and Avidin- Biotin ELISA (A-B ELISA). At 30 days of post vaccination female calves of group first demonstrated 832 IU mean titer, group second 736 IU and group third 284IU and it started decline the mean titer at 60 days post vaccination of group first, second and third were found 388IU, 336IU and148IU respectively, subsequently, At the 90 days of post vaccination the mean titer of group first was118 IU, group second 122 IU, it started decline the mean and group third102 IU at 90 days post vaccination. All calves become negative after 6 months of vaccination. At 30 days mean pp value of group first was 90.2, group second 83.65 and group third 76.95 and 90 days of post vaccination pp value of A-B ELISA were found group first 50.3, group second 61.1 and group third 52.65. Calf hood vaccination of female calves at least at organized farms needs to be practiced besides regular screening and removal of positive reactors

2. Epidemiological Studies of Gastrointestinal parasites of buffaloes

Dileep Kumar and B.C. Pal

The study was conducted between December, 2003 and August, 2004, on survey, incidence, intensity of infection, seasonal prevalence, morphology, in respect of various trematodes, cestodes and nematodes of buffaloes, the chief dairy animal in India. For this present epidemiological investigation various parts of the gastrointestinal tract of buffaloes viz. rumen (1681), abomasum (1279), small intestine (1164), large intestine (1147), bile ducts (1830), liver (2112) and peritoneal cavity (1886) procured from different abattoirs located in Agra and were screened for the presence of trematode, cestode and nematode parasites. Some faecal samples were also examined for confirmation of procured parasites. Helminth infection were detected as

trematode 364 (6.47%), cestode 91 (7.81%) and nematode 905 (16.52%). Overall incidence rate of helminths were detected 12.20% in different age groups of buffaloes and in different seasons.The collection of helminths, in the present epidemiological survey, comprised of 15 genera and 17 different species. These were with prevalence rate: *Paramphistomum cervi* (5.89%), *Cotylophoron cotylophorum* (1.30%), *Gastrothylax crumenifer* (3.45%), *Gigantocotyle explanatum* (7.54%), *Fasciola sps.* (2.69%), among trematodes; *Moniezia sps.* (4.98%), *Avitellina sps.* (2.83%), among cestodes; *Haemonchus contortus* (7.66%), *Trichostrongylus axei* (8.91%), *Paracooperia nodulosa* (10.22%), *Bunostomum phlebotomum* (5.84%), *Toxocara vitulorum* (9.69%), *Oesophagostomum radiatum* (14.38%), *Trichuris discolor* (9.24%) and *Setaria digitata* (11.72%), among nematodes. The overall prevalence rate of trematode was 6.47%. Their salient information on morphology, intensity of infection and seasonal variation have been discussed in respect of five trematode species found during post mortem examination. The prevalence rate of cestode infections was 7.81%. Salient information on their morphology, intensity of infection, seasonal variations have been analysed in respect of the three cestode species encountered. Their pathogenic role in heavy infections or in conjunction with other helminths has also been discussed.Among nematodes, data on toxocarid (*T. vitulorum*), trichostrongylid (*T. axei, H. Contortus*), nodule forming worms (*P. nodulosa and O. radiatum*), hookworms (*B. phlebotomum*) and setarid worms (*S. digitata*) have been presented in respect of their morphology, incidence, intensity of infection, seasonal fluctuation and pathogenic propensities. *T. vitulorum* infection, detected in 9.69% young calves, showed maximum prevalence in winter (1.96%) followed by rainy (1.55%) and summer (1.38%) seasons. similarly, relevant information on other nematode infections have been presented and discussed.

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3. Epidemiological studies on food and mouth disease and its vaccination strategy in buffaloes

Chandra Pal Singh and S.K. Yadav

Food and mouth disease (FMD) is one of the most economically important disease of Indian livestock its presence in developing countries is imposes serious limitations on the development of livestock industries and creates restrictions upon international trade in livestock products. In India, the disease is endemic. Therefore, to study the dynamics and distribution of virus types must form an important component of the control programmes of the disease. Keeping this in view the study was carried out to generate a base line epidemiological data regarding the prevalence of different serotypes of FMD. During this study a total of 244 specimens were collected from 404 outbreaks from the different districts of U.P. during 10 years at Regional Centre Mathura of All India Coordinated Research Project for Epidemiological Studies on Foot-and- Mouth disease. The serotyping of specimen was done by sandwich ELISA, which is more specific and easy to perform. 64.02% samples shows types O and 18.90%samples shows types A and 17.07% samples shows type Asia-1. In general type O continued its predominance though with lesser degree followed by type A. The Asia-1 was also present in the region but with subdued activity. This probably showed that the existing population maintains both types of virus because of previous exposures. The type O had all among predominated except in 1999 and 2000 when type Asia-1 was the dominant type. The epidemiological data generated during this study is just an initiative and emphasizes the need for intensive and planned programmes for generation of a comprehensive epidemiological data base which bridge between many information gaps and can also contribute significantly in various strategies for the control of the disease. The present investigation was undertaken to measure the antibody titre against FMD virus (FMDV) antigens in buffalo heifers vaccinated with Raksha O vac trivalent foot and mouth disease vaccine. The study was conducted in buffalo heifers of 1-2 years age, two groups were selected containing 30 buffalo heifers one from organized herd and other from field

both groups were immunized with oil adjuvanted FMD trivalent vaccine. The blood was collected from all the buffalo heifers of each group on 0, 30, 90 and 180 days post vaccination (DPV) for analysis of antibody titre against type O, A and Asia-1. The antibody titre was analyzed by using liquid phase blocking ELISA to determine the specific serum antibody titres against FMDV antigens. The results of 0 and 180 days showed that the vaccine used for immunization to buffalo heifers did not give enough protection for 6 months but with regular vaccination (3 times for the first year and twice annually) better protection level can be obtained. The protection rates of the three serotypes were not same, type O being the least protective one and much potent vaccine should be used for type O, the protection rate was higher in group I than that of II. A training programme for the organization and execution of the serosurvey is essential to obtained better results and to decrease variations between epidemiological unit vaccination team should be trained to minimize vaccination failures. For control of FMD systemic vaccination programmes followed by regular monitoring of serum antibody levels is required. A battery of tests is available for monitoring and measuring the serum antibody levels. However none of the available tests measure all the components of the humoral immune response that might be playing an important role in protection against FMDV in vivo. The present work attempts to study the antibody level in organized and field buffalo heifers after vaccination.

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4. Mycoplasma capricolium: Isolation and molecular diagnosis

Bhawana Gupta and B.C. Pal

During the present study, a total of 493 samples (nasal, ocular and cervicovaginal swabs, milk, semen) were collected from goats (94), sheep (74), cattle (60) and 265 buffaloes. A total of 138 (28%) mycoplasma isolates recovered 107 nasal swabs (61 goats and 46 sheep), milk (16 buffaloes) and 15 buffalo semen samples with clinical history of respiratory distress, mastitis and with low mortality of sperms. Mycoplasma organisms were isolated from 61 nasal swabs, out of 94 (64.8%) collected from diseased goats. Of 74 healthy and diseased sheep, 46 (62.16%), were positive for mycoplsma organisms. Sixteen mycoplasma isolates could be recovered from 23 milk samples from mastitic cases of buffaloes, where as 15 mycoplasma isolates could be isolated from 44 semen samples from buffalo breeding bulls. All mycoplasma isolates were characterized by morphological, cultural and biochemical tests. The digitonin sensitivity test was performed for differentiation of mycoplasmas and acholeplasmas. In this study 107 mycoplasmas were identified as *Mycoplasma capricolum* subsp. using the earlier mentioned tests. The remaining 31 isolates could not be identified by these tests. All the 107 *Mycoplasma capricolum* subsp. were further speciated, in view of antigenic differentiation by using PCR, SDS-PAGE and western blot. For serological testing whole cell antigen (WC) was prepared. Growth inhibition test was performed by using homologous antiserum. Antibiotic sensitivity of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) *Mycoplasma capricolum* subsp. *capricolum* against six antibiotics, erythromycin, spiramycin, sparfloxacin, tylosin, enrofloxacin and tetracycline revealed sensitivity for all the above antibiotics, where as bovine strains were found to be resistant for spiramycin, sparfloxacin and tylosin. The majority of mycoplasma isolates were characterized by using polymerase chain reaction.Among total 138 isolated mycoplasma species, *Mycoplasma capricolum* subsp. *capripneumoniae* was the most frequently prevalent species. The SDS polyacrylamide gel electrophoresis was carried out on two isolates of Mccp and two isolates of Mcc and two unclassified isolates from bovine one each from milk and semen were analyzed to detect protein profile, and this revealed 22 to 33 discrete bands ranging from 14.3 to 97.4 kDa. The prominent amongst these were 14, 17, 19, 20, 63, 66, 68, 32, 35, 43, 45, 75, 80 proteins. Three protein bands of molecular weight viz., 43, 19 and 66 kDa. were present in Mccp. but was absent

in Mcc. Three representative isolates of Mccp, two from goat and one from sheep of identified by biochemical and growth inhibition test were used in western blotting test. The protein profile was analyzed by using whole cell antigen and standard antiserum. Both the species from sheep and goat revealed similar protein profile. Mccp revealed about 24-protein bands including four major bands 98, 85, 78 and 52 kDa. Among these 52 kDa protein band was the intense and prominent. A transmission study was also carried out by inoculation of *Mycoplasma capricolum subsp. capripneumoniae* in kids. On postmortem lungs were seen highly congested with consolidation. The isolation procedure was used for detection of the presence of *Mycoplasma capricolum subsp. capripneumoniae.* The pieces of lungs were collected for histopathological examination. The lungs from dead kids revealed acute serofibrinous pleuropneumonia with infiltration of neutrophils.

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5. Studies on seroepidemiology and molecular diagnosis of PPR in goats

Pankaj Kumar and B.C. Pal

In present study, two outbreaks were studied viz. one from Malhoo village of Mathura district and the other of the Jamunapari Goat Farm Etawah. The infected goats were having clinical signs like high fever (104°F-105°F), necrotic stomatitis, erosions on gums, white pulpy coating with wheat bran appearance on tongue, sticky ocular and mucopurulent nasal discharges, crust around nostrils, coughing, pneumonia, arched back and streamy diarrhea and the morbidity, mortality and case fatality rates were 60%, 40% and 66.67%, respectively. On post-mortem observation, there was inflammation of gastro-intestinal tract, hemorrhagic liver and enlarged gall bladder in kids, congested and consolidated lungs, zebra stripping over large intestine, hemorrhagic kidneys, cyanotic spleen and congested intestinal mucous membrane. Virus isolation attempted from five samples (spleen, lung, lymph nodes, tissue mixture) and a rectal swab, using Vero and B-95a cell lines applying both viz. co-cultivation and absorption methods of virus inoculation. The CPE on B-95a cell lines were developed 4th day post-infection, the CPE was more observable after third passage and characterized by syncytia formation. The virus isolation was confirmed by using RT-PCR with amplicon size of 372 bp. The antigen detection of PPR virus was conducted by using sandwich-ELISA. Out of total 33 samples (18 tissues, 12 swabs and 3 of whole blood) were processed. 27 (17 of tissue, 7 of swabs and all the 3 of whole blood samples) were having PPRV antigen. The 41 sera samples were collected from the outbreaks during three phases viz. 0, 15 and 30 days post-infection. From the five sera samples collected during outbreaks, 3 were positive for PPRV antibodies as they showed the percentage inhibition (P.I.) in c-ELISA ranging 45-75%. Similarly of 18 samples collected 15 dpi, 17 were positive (P.I. ranging 46-87%). Similarly, 18 sera samples collected 30 dpi, 17 were found positive and only one sample was found negative for PPRV antibodies. For seroprevalence of PPRV antibodies, five nearby districts of outbreaks were selected viz. Mathura, Agra, Aligarh, Hathras and Etah. The PPRV antibodies were maximum in Mathura district (63.64%) and minimum in Agra district (18.60%). Thus, the overall prevalence of PPRV antibodies was found to be 34.03%. The maximum prevalence was found in goats with age group 6-12 month (40.96%) and minimum for the age group below 6 months (26.32%). prevalence was more in Jamunapari goats (40.54%) than Barbari goats (29.91%). The maximum prevalence of PPRV was found in the Jamunapari ((75%))and Barbari goats ((57.12%)) of Mathura district and minimum in Jamunapari of Agra district (8.33%). And Barbari of Aligarh district (13.04). the females were more affected than males except, the goats of Aligarh district where the males were more affected (26.09%) than females (16.67%). The sexwise prevalence was more in Mathura district for both male and females viz. 42.85% and 69.23%, respectively and the overall prevalence during present study

was 21.31% for males and 32.81% for females, respectively.The serum neutralization test (SNT) was also done on 20 sera samples, The 14 were able to neutralize the PPR viruses and no cytopathic effects were observed on Vero cell lines. The 4 sera samples have higher neutralizing antibodies and their maximum dilution (even > 1:128) can neutralize the PPR virus infected Vero cells and 6 sera samples were negative for SNT. It was concluded that, though all the sera samples selected for SNT were showed high P.I. values in c-ELISA (ranging from 95-98%), but 6 of them were unable to neutralize the virus. Hence, SNT was found much specific test for the detection of PPRV antibodies. The molecular characterization of PPRV was done using RT-PCR for F-gene during this study. The specimens viz. spleen, lung, liver, tissue mixture and rectal swab, were tested positive for s-ELISA and virus isolation were subjected for RT-PCR. Only 3 specimens, a rectal swab and two isolate of lung and spleen tissues were found showing high amplicon with 372 bp, which is similar to PPR virus. The characteristic bands were found on agarose gel electrophoresis for F-gene specific PCR product with primer pairs (F1 and F2).

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6. Studies on seroprevalance by different serological tests and PCR for diagnosis of bovine Brucellosis

Manisha Chaudhary and S.K. Yadav

Eleven isolate of B. *abortus* were isolated from 200 samples of vaginal discharges, placenta and aborted fetus of cattle and buffaloes. A total of 718 sera sample from bovines (515 cattle and 203 buffaloes) located at different organized farms, gausalas, rural heads and veteribnary hospitals of UP were analyzed by RBPT, STAT and indirect ELISA. 75 animals (60 cattle and 15 buffaloes) were positive to STAT and 123 animals (85 cattle and 38 buffaloes) were positive to I-ELISA. A comparative efficacy of RBPT, STAT and ELISA was determined by chi-square test. ELISA was found to be most significant test as compared to RBPT and STAT. Overall seroprevalance of cattle and buffaloes were 6% and 7.3% respectively. The seroprevalance in cattle and buffalo was 6.3% and 7.8% respectively in organized herd 5% and 8% respectively in rural herd, 6.19% and 0% respectively in gaushalas and 6% and 4.8% respectively in veterinary hospitals. 260 milk samples of cattle and buffalo were also collected and tested using MRT. The percentage positivity was 5.33% and 5.45 % in cattle and buffalo respectively. A PCR analysis targeting BCSP-31 gene encoding 31 kDa OMP of brucella was conducted and detected 4 out of 20 vaginal discharge as positive and also showed the presence of amplicons of 135 bp in 16 out of 28 serum samples indicating presence of B. *abortus*.

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7. Impact of RB-51 vaccination in control of Bovine Brucellosis

Ramesh K. Singh Yadav and B.C. Pal

A total of 146 cows and 69 buffaloes were taken to know the impact of RB-51 vaccination in control of bovine brucellosis. After vaccination there was no side effects like fever, swelling pain at the site of injection, decrease in feed intake, abnormal behavior except only slight drop in milk yield in lactating animals. No abortion has been found in brucellosis positive and brucellosis negative cows and buffaloes that were vaccinated at different stage of pregnancy from 6-8 months in cows and 6-9 months in buffaloes. During this trial, the four animals (2 cows and 2 buffaloes) excreted the *Brucella* strain RB-51 organisms in milk and lochial fluid. The strain was identified on the basis of cultural, morphological and biochemical characteristics. All the 94 animals (pregnant and non-pregnant), which were found sero-negative with RBPT and STAT remained sero-negative at 1 month, 2 month, 3 month, 4 month and 5 month of RB-51 vaccination. It was also found that there was no change in the titre of brucellosis positive cows against brucellosis after 5 months of vaccination with RB-51 vaccine.The milk Ring Test was

performed on 50 milk samples (20 Cows + 30 buffaloes) milk samples of lactating seronegative animals at two months of RB-51 vaccination and only 3 samples (1 Cow + 2 Buffaloes) were found positive, while 47 samples (19 Cows + 28 Buffaloes) were negative.Skin test was performed on 35 animals at 3 months of post vaccination. Out of 30 animals, 10 animals were buffaloes and all were found sero-negative and rest of 20 animals were cows in which 10 were seronegative which were from Brucella free herd DDD farm Mathura and 10 cows were seropositive which were from brucellosis infected herd Military dairy farm Agra. It was also observed that the thickness of cervical fold of vaccinated cows before intradermal skin test were in the range of 4-6 mm and after intra dermal skin test, skin thickness were increased in the range of 9-17mm. In buffaloes the thickness of cervical fold before and after intradermal skin test were in range of 8-9mm and 15-22mm, respectively. Increase in thickness was due to hot, red, painful and diffused oedematous swelling.In the present study, 100 sera of animals were tested for humoral response by DOT ELISA before and after RB-51 vaccination. Dot ELISA responses of sera from seronegative animals (35 cows and 35 buffaloes) vaccinated with a reduced dose of Strain RB-51 (10^9C.F.U.) did not differ from non vaccinated animals except slight indication, which may be either due to non-specific reactions or low dose of vaccination. Dot ELISA responses of sera from seropositive animals (30 cows) at a dose of 10^9 C.F.U. were similar to pre-vaccinated sera of the seropositive animals. In present study, it was found that vaccine induces good humoral and cell mediated immune response.

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8. Study on Canine Mycoplasmas

Vikendra Singh and B.C. Pal

During the present study, a total of 141 samples were collected from domestic dogs (89) and street dogs (52). The samples include nasal, ocular and vaginal swabs. A total of 7 (4.96%) mycoplasma isolates were recovered from 41 nasal swabs (domestic dogs (20) and street dogs (21)), 82 genital (domestic dogs (48) and street dogs (34)) and 18 oculars domestic dogs (11) and street dogs (7) with clinical history of respiratory distress, healthy, arthritis suspected, infertile, pneumonia, parvovirus suspected, fever and digestive problem, abortion and paralysis suspected. Mycoplasma organisms were isolated from 1 nasal swabs, out of 41 (2.45%) nasal swabs collected from healthy and diseased dogs. Six mycoplasma isolates could be recovered from 82 (7.32%) genital samples from healthy and diseased dogs. No mycoplasma were isolated from 18 ocular samples. All mycoplasma isolates were characterized by using morphological, cultural and biochemical tests. The digitonin sensitivity test was performed for differentiation of mycoplasmas and acholeplasmas. To minimize the use of standard antiserum, biochemical tests were used viz., glucose and mannose catabolism, arginine hydrolysis, phosphatase production, formation of film and spot, tetrazolium chloride and hemadsorption. In this study 7 mycoplasmas were identified as *Mycoplasma canis* (1), *Mycoplasma gateae* (2), *Mycoplasma arginine* (2) and two samples remained unidentified for serological testing whole cell antigen (WC) was prepared. Growth inhibition test was performed by using homologous antiserum. These isolates of canine mycoplasmas were tested against six antibiotics, erythromycin, spiramycin, sparfloxacin, tylosin, oxytetracycline and enrofloxacin. Tylosin, enrofloxacin and spiramycin were found to be sensitive for all these *Mycoplasma* isolates above antibiotics.In the present study, isolate (*M. canis*) was inoculated in three experimental dogs via different route viz., oral, nasal and intramuscular. The respiratory symptoms like coughing, nasal secretions, high temperature were observed.One pup was sacrificed for histopathological examinations. On gross examination, lungs showed congestion and mild to moderate consolidation. Histopathological changes include massive infilteration of lymphocytes and macrophages around the bronchioles, alveoli and blood vessels.There was development of lymphoid hyperplacia in the peribronchial

and perivascular areas. Brochial epithelium showed hyperplasia with lymphocytic and infilteration macrophages. Alveoli showed coupious serocellular exudates consisting primarily of desquamated epithelial cells, mononuclear cell infilteration. Alveolar duct, sacs and alveoli were highly enlarged throughout the lung (Emphysema). Inter alveolar septa become thickened with abundance of mononuclear cells. Fibrin was also noticed in the lumen of blood vessels. The lumen of the blood vessels contained eosinophilic mass having few mononuclear cells. in the lumen of these vessels plasma cells within the wall was also noticed.

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9. Studies on Heamoprrhagic Septicemia: molecular characterization of the serotype

Avinash K. Singh and B.C. Pal

A total of 440 animals from an outbreak and healthy were taken for isolation and identification of ***Pasteurella multocida*** from different specimen viz blood, oedomatous fluid and morbid material like spleen, lung and liver). Of 368 animals were found positive for hemorrhagic septicemia on their history and clinical symptom like high rise of temperature, presence of edematous fluid in intermandibular space, swelling in brisket, gargling sound during inspiration etc. Out of 368 selected animals 25 isolate were isolated which were characterized on cultural, morphological and biochemical characteristics. The blood smear revealed the presence of bipolar organism. The characterized isolates were subjected to pathogenicity test in rabbit which revealed that tested isolates were pathogenic indicating severity of the organism varied from individual to individual isolate. Twenty four isolates were subjected to antibiotic sensitivity by using 12 commonly used antibiotics by disc diffusion method. The majority of isolates were found sensitive to Chloramphenicol (100%), Ciprofloxacin(91%), Enrofloxacin(83%), Gentamicin(75%), Amoxicillin(62.5%), Oxytetracyclin (54%), and Streptomycin(54%). slight sensitivity was observed with Nitrofurantoin(50%), Ampicillin(42%), Erythromycin(33%) and Co-triamoxazole(25%). A rapid slide agglutination test was used for identification of the organism with standardized antiserum obtained from FAO regional reference laboratory (Asian region) Peradeniya, Sri-Lanka. Agar gel immunodiffusion was the method for antigenic characterization of the isolate by using slandered hyper immune serum for observing precipitin band. Counter current immunoelectrophoresis was used for the screening of the animal for the disease. It was also used for the capsular and somatic typing of the isolate in short. For this test 45 sera samples were taken for screening of the animals against disease, out of which 11 sera sample were found positive. It can be concluded that the rapid slide agglutination AGID and CCIE were good technique but were time consuming. Out of 24 isolates 12 representative and fully characterized isolates were subjected for PCR by using culture lysate as DNA template. The amplified product was approximately 620 bp was obtained. This result showed that PCR is a good test for diagnosis of hemorrhagic septicemia as it detected only hemorrhagic septicemia causing B:2 serotype of *Pasteurella multocida.* Restriction endonuclease analysis was also performed using ECORI endonuclease enzyme. ECORI digestion gave a good pattern. The DNA fingerprinting was done and it was observed a good tool to differentiate the isolate in the epidemiological study.

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10. Sero-epidemiology and molecular diagnosis of foot and mouth disease in Cattle and buffalo

Arbind Singh and S.K. Yadav

In this cross sectional study, a total of 800 serum samples collected from cattle and buffalo of different sex, age at different places (districts Ghaziabad, Gautam Budh Nagar, Aligarh and Mathura) were screened for seroprevalence of FMD using LPB-ELISA. An overall sero-prevalence of FMD serotypes O, A and Asia-1 in cattle and buffalo was calculated to be 65.25%, 58.75% and 54.75%, respectively. Analysis of sero-prevalence of FMD serotypes O, A and Asia-1 with respect to sex, age and place of cattle and buffalo was determined and study revealed that the place and age seems to influence the distribution of antibodies against the O, A and Asia-1 serotypes. The sero-prevalence of antibodies against serotypes O, A and Asia-1 was the highest in Mathura which was 91.67%, 88.33% and 78.33%, respectively. It was reported that Mathura is having sugar mill, holy river Yamuna, market and state boundary may be causes for higher prevalence. Age wise sero-prevalence of antibody against serotype O, A and Asia-1 were high in cattle and buffalo of ≥3 years indicated that either vaccination or repeated clinical and/or subclinical infection with the prevalent serotype boost to keep the antibody titer high enough to be detected positive. Sex and species (cattle and buffalo) had no significant influence on the sero-prevalence of antibodies against FMD serotypes O, A and Asia-1. A total of 50 tongue epitheliums from different districts of Uttar Pradesh were processed for the different types of virus viz., type O, A, C and Asia-1 by using sandwich ELISA test, of which 32 specimens were found positive for the presence of FMD virus. The overall typeability was 64.00%. In the scenario of overall distribution pattern of FMD virus types, type O (62.50%) was the predominant type, followed by serotype A (37.50%), Type C and type Asia-1 could not be recovered. FMD virus could be isolated (showed cytopathic effect on BHK-21 cells) from only 10 of the 50 samples (tongue epithelium), however, 38 of the 50 samples (tongue epithelium) yielded positive results using PCR technique. Of the ten FMD virus isolates, three were characterized as FMD serotype O and seven as FMD serotype A. Sensitivity and specificity of PCR was found to be 100% and 66.67%, respectively. These findings reassert the usefulness of PCR in diagnosis of FMD and can complement isolation in detecting carriers in cattle and buffalo. The study concludes that RT-PCR can be used as modern tool for screening the cattle and buffalo for different types of FMD virus.

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11. Studies on Contagious Agalactia in small Ruminants

Pramod Kumar and B.C. Pal

During the present study, a total of 190 samples were collected from sheep and goats. The samples include nasal, vaginal, preputial, lung and milk live and slaughtered animals. A total of 70 (36.84%) mycoplasma/acholeplasma isolates recovered. Out of 70 mycoplasmas/ acholeplasma, 31 [38.75%] isolates were from sheep and 39 (35.45) goats. Mycoplasma/ Acholeplasma organisms were isolated from 77 nasal swabs, 46 vaginal swabs, 36 preputial swabs,14 lung pieces, and 17 milk samples, which collected from diseased and apparently healthy goats. Out of 70 [38.75%] isolates of mycoplsma/acholeplasma from sheep and goats were 28 (36.36%) from nasal swabs, 16 (34.78%) from vaginal swabs, 14 (38.88%) preputial swabs, 5 (35.71%) from lung pieces and 07 (41.71%) milk samples of healthy and diseased sheep and goats. All mycoplasma/acholiplasma isolates were characterized by using morphological, cultural, biochemical and serological tests. Thus above 70 isolates were identified as the *Mycoplasma mycoides subsp. mycoides LC (10), Mycoplasma capricolum capripneumoniae* (10), *Mycoplasma capricolum capricolum (9), M. agalactiae (17), M. bovis (4), A. laidlawii (15), and A.*

granularum (5). from sheep and goats. The digitonin sensitivity test was performed for differentiation of mycoplasmas and acholeplasmas. In this study 70 mycoplasmas/acholeplasma were grouped as 40 isolates of Mycoplasma/Acholeplasma in group I including *Mycoplasma mycoides subsp. mycoides LC, capricolum subsp. capripneumoniae and Acholeplasma sps.* In group II, 9 isolates of *Mycoplasma capricolum capricolum* and in group III, 21 isolates of mycoplasma including *M. agalactiae* and *M. bovis.* For serological testing whole cell antigen (WC) was prepared from *Mycoplasma mycoides subsp. mycoides LC, Mycoplasma capricolum capripneumoniae, Mycoplasma capricolum capricolum, M. agalactiae, M. bovis, A. laidlawii, and A. granularum.* All 70 isolates of mycoplasma and acholeplasma were tested by growth inhibition test, using standard homologous antisera. *Mycoplasma mycoides subsp. mycoides LC (10), Mycoplasma capricolum capripneumoniae* (10), *Mycoplasma capricolum capricolum (9), M. agalactiae (17), M. bovis (4), Acholeplasma laidlawii (15), and A. granularum (5)* produced a good zone of inhibition around homologous antiserum disk with respective antiserum while very slight or no zone of inhibition with non-homologous antiserum. The cross reaction was found between *Mycoplasma mycoides subsp. mycoides* and *Mycoplasma capricolum capripneumoniae, Mycoplasma capricolum capricolum* and *capricolum subsp. capripneumoniae* and *M. agalactiae* and *M. bovis. Mycoplasma mycoides subsp. mycoides LC* isolates were found very sensitive to enrofloxacin, erythromycin and sensitive to tetracycline but resistance to sparflxacin, spiramycin and tylocin. *Mycoplasma capricolum capripneumoniae* isolates were found very sensitive for erythromycin, tetracycline, spiramycin and tylosin but were resistant for sparfloxacin and enrofloxacin. *Mycoplasma capricolum capricolum* isolates were found sensitive for tetracycline, erythromycin, sparfloxacin, tylosin but were resistant for spiramycin and enrofloxacin. *Mycoplasma agalactiae* and *Mycoplasma bovis* revealed sensitivity to erythromycin, enrofloxacin, tetracycline and tylocin but was resistant to sparfloxacin The first Polymerase chain reaction was performed with "Mycoplasma mycoides cluster" specific primers, which revealed 240bp amplicon in 29 isolates and characterized as the members of "Mycoplasma mycoids cluster".The second Polymerase chain reaction was performed with *Mycoplasma agalactiae* specific primers, which revealed 375bp amplicon in 17 isolates and third Polymerase chain reaction was performed with *M. bovis* specific primers, which revealed 412bp amplicon in 4 isolates. Among total 70 isolated mycoplasma/acholeplasma species, *Mycoplasma agalactiae* was the most frequently prevalent species in sheep and goats. This was "classical" aetiological agent of "contagious agalactia" in sheep and goats.

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12. Studies on seroepidemiology and molecular disagnosis of IBR virus in cattle

Tribhuwan Kumar and S.K. Yadav

Out of 341 samples, one hundred samples were processed for virus isolation in MDBK cell line 11 samples could showed cytopathic effect (CPE), with characteristics viz. rounding of cells (grapes like), degeneration and detachment of the MDBK cell monolayer from surfaces. Of these 11 positive samples, 15.63% samples were positive of breed hariana followed by 12.5% from Jersey, 9.09% from Sahiwal and lowest prevalence 6.67% was noted in non descriptive breed and the over all 6 (12.77%)were recovered from vaginal swabs, 3 (9.09%) were from nasal swabs and 2 (10%) were from semen. The sensitivity of the tests were also evaluated in relation to location. By using i-ELISA 62.16% in the cows maintained at Government livestock farms followed by (43.96%) cows with clinical history of infertility brought to the veterinary hospitals. The lowest (25%) of IBR antibodies prevalence was noted in the breeding bulls maintained at A.I. centers. A close related IBR antibodies prevalence percentage was noted in the cows maintained at private Gaushalas and rural areas say 33.33% and 33.71% respectively. By using c-ELISA 54.95% in cows maintained at Government farms while lowest 20% prevalence was observed in

bulls of A.I. centers, 42.86% was noted in the cows maintained at veterinary hospitals followed by cows at rural areas 31.46% and 26.67% from cows of private Gaushalas. With virus neutralization 52.25% in cows maintained at Government farms while lowest 15% was observed in bulls of A.I. centers, 38.46% prevalence was noted in the cows maintained at veterinary hospitals followed by cows at rural areas 29.21% and 23.33% from cows of private gaushalas. It can be seen from this table that highest IBR prevalence 62.16% was found in the cattle with i-ELISA while lowest 52.2% with VNT. It was also noted that lowest 15% was in the bulls of A.I. centers. The seroprevalence in the animals of veterinary hospital with i-ELISA, c-ELISA and VNT revealed approximately similar percentage viz 43.96, 42.86 and 38.46 respectively. The breed wise percentage (%) for detection of IBR antibodies by using indirect ELISA test was highest (67.60%) in the cows of Hariana breeds followed by Sahiwal (46.67%) and Jersey (45.45%) respectively. The lowest (31.03%) IBR antibodies prevalence was noted in non descriptive cattle. With c-ELISA test in relation to breed and showed that 52% sera could revealed highest prevalence in the cattle breed of Hariana while lowest 28.45 % was observed in non descriptive cattle followed by Sahiwal 42.22% and 41.82% from cows of Jersey breed. With virus neutralization test in relation to breed and concluded that 48% sera could revealed highest prevalence in Hariana breeds of cattle while lowest 25.86% was observed in non descriptive cattle followed by Jersey 40% and 37.78% from Sahiwal breed of cattle's. For comparison indicating that breed wise seroprevalence of IBR infection in the Hariana breeds revealed highest antibodies with all three tests while non descriptive cows revealed lowest seroprevalence with all three tests, the breeds revealed in Sahiwal 46.67%, 42.22% and 37.78% respectively and in jersey breed of cattle 45.45%, 41.82% and 40% respectively. Whereas least, 31.03%, 28.45%, 25.86% were detected in sera from non descriptive cattle. It can be showed that overall age wise IBR antibodies prevalence was highest (55.96%) in the cattle of 4 years or above age group, followed by 48.91% in 2-<4 years age group of cattle. The lowest prevalence percentage (34.29%) of IBR antibodies was noted in the age group of 0-<2 years by using i-ELISA. The screening results of c-ELISA showed that 51.38% sera could revealed highest prevalence percentage in the cattle of 4 years or above age group followed by 44.57% from the cattle of age group 2-<4 years while lowest 30.71% prevalence percentage was observed in the cattle of age group 0-<2 years and with virus neutralization test concluded that the 48.62% sera could revealed highest prevalence percentage in the cattle of 4 years or above age group followed by 43.48% from the cattle of age group 2-<4 years while lowest 25.71 % prevalence percentage was observed the in cattle of age group 0-<2 years. The results of the age wise prevalence percentage indicated that the cattle at the age of 4 years or above found more susceptible to the IBR infection. However, the virus specific antibodies detected by i-ELISA, c-ELISA and VNT are 34.25%, 30.82% and 26.03% respectively in the age group of 0-<2 years, 48.75%, 45% and 43.75% respectively in the age group of 2-<4 years and 56.52%, 51.30% and 48.69% in 4 years or above age group of cattle respectively.Of 50 samples (swabs and semen), were analyzed with PCR, the amplicon (468 bp, which is similar to IBR virus) could be amplified from 11 (22%) specimens and concluded that 2 (8%) were from field sample, 3 (20%) were from serologically positive samples and 6 (60%) were from cell adopted samples. The higher number of PCR positive results were obtained in samples, which showing cytopathic effect in MDBK cell lines and least in field samples i.e. 8% only. Comparison of virus isolation and PCR results (of 50 different samples) revealed that all the 6 samples positive for IBR virus by virus isolation were positive with PCR.

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13. Heamoprrhagic Septicemia in Bovines: Its Epidemiology and Diagnosis by molecular tools

Alka Manisha and B.C. Pal

A total of 516 samples from healthy and with clinical history of respiratory infection from cattle and buffaloes were collected from four districts viz Mathura, Agra, Aligarh, Etah. These samples comprises of blood, nasal swabs, nasopharyngeal swabs, edematous fluid, morbid material (lung, liver, spleen etc.) out of these 46 *Pasteurella multocida* organisms could be identified with a prevalence of 8.91%. No significant difference in the prevalence of organism among different districts. It was also noticed that buffaloes were more susceptible than the cattle as the prevalence rate were 10.14% and 6.43% respectively. From buffaloes total samples collected were 345 out of which organism was isolated from 35 samples while in cattle out of 171 samples only 11 samples revealed the organism. The samples were collected from different age group of animals such as 0-6 months; 6-24 months; more than 24 months. It was observed that the animals with age group 6 months to 2 years were most susceptible as compared to younger and older animals. This may be due to maternal antibody and acquired immunity in youngs and adults respectively. The blood and impression smears fro spleen, lung and liver on staining with Gram's, Leishman's and Methylene blue revealed gram negative, bipolar, short bacilli revealed clear bipolar organism, indicating *P. multocida.* The smears stained with MB were more distinct and clear. The blood, spleen, lung were inoculated on blood agar organism shows smooth, grayish, glistening, translucent colonies measuring approximately 1 mm in diameter, after 24 hours in incubation at 37°C. The organism fails to grow on McConkey's lactose agar. This indicates that it is *P. multocida.* Biochemical identification indicates the slight variation in the isolates. As few isolates shows dissimilarity on MR, VP tests and sugar fermentation. Virulence of the isolates can be judged by biological or pathogenecity test, when processed morbid material is inoculated in rabbit (0.2ml subcutaneously) virulent strain kills the rabbit within 24 hours. Drug sensitivity was performed by single disc methods using 12 commonly used antibiotics it was found that isolates were All 30 isolates were 100% sensitive for Ciprofloxacin, Enrofloxacin and Amoxicilline; 93.33% isolates were sensitive for Chloramphenicol, 90% was sensitive for. Oxtetracycline, 86.67% were found sensitive for Gentamicin, isolates were slightly sensitive for Co-Trimaoxazol, Nitrofurantoin and Ampicillin all the isolates were resistant for Trimethoprim and Streptomycin.A rapid slide agglutination test was used for identification of the organism with standardized antiserum obtained from FAO regional reference laboratory (Asian region) Peradeniya, Sri-Lanka. A positive agglutination indicates the *P. multocida* organism. PCR is the gold standard assay by which the organism can be detected within a very short time. In this piece of work eleven representatives isolates, identified on the basis of morphological and biological identification, different morbid tissues lung, spleen, trachea and liver pieces which are suspected to HS were put for B serotype H.S specific PCR. The amplified product was obtained approximately 620 bp. Present study indicates that the PCR is more sensitive than other conventional test as a diagnostic tool.26 nasopharyngeal swabs along with some nasal swabs edematous fluid were also examined by PCR. The amplified product obtained was approx. 620 bp DNA fingerprint shows similar profile for all the isolates indicating the closeness of the organism. Genomic fingerprinting method have been proved to be valuable in characterization of bacteria including *P. multocida* particularly in differentiation between strain of similar type. DNA fingerprinting of 7 *P. multocida* strains generated by REP-PCR, were shown to contain multiple amplification products ranging in size from approximately 350 bp. The homogeneity of REP-PCR fingerprints in HS- causing isolates of *P. multocida* provides support for the existence of a disease- associated REP profile that is distinct from other pasteurellosis. It is also evident that this technique is the novel method to identify a particular organism as an HS-causing strain.

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14. Molecular epidemiology of IBR virus infections in buffaloes of U.P.

Priyanka Shukla and S.K. Yadav

In this study, a total of 462 female buffaloes having reproductive problems were selected from 10 different districts of U.P. to dtect IBR antibodies in their sera by I-ELISA. out of 468i sera, 208 (45.02%) were found positive for IBR antibodies. prevalence was highest (63.41%) in Etawah and lowest (26.66%) in Ghaziabad dairy farm murrah was found to have highest seropositivity (48.19%) while Bhadawari was found to be least infective (34.42%). usualy buffaloes above 4 years had more sero-prevalence (58.19%). prevalence was lower in females (45.02%) than male animals (48.00%). out of 20 nasla and vaginal samples, only 2 samples were positive (10% prevalence). to reveal CPE on MDBK cell line, 17 samples were positive by PCR using gB primer showing overall prevalence of 64.7%. out of 50 semen samples from breeding bulls, only 2 wre postive to show CPE on MDBK. PCr detected BHV-! directly from 24 (48%) semen samples out of 50. senstivity and specificity of PCR was found to be 100% and 16.66% respectively.

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15. Detection of IBR virus and development of multiplex PCR

Rashmi Singh and S.K. Yadav

In this study total 492 samples from different organized herds and unorganized herds in U.P. were taken to study the seroepidemiology and antigen detection studies and viral isolation for correct diagnosis of the disease. Out of 492 serum samples 312 serum samples were taken from organized herds and 180 serum samples were taken from unorganized herds. Out of 312 serum samples, 135 serum samples were found positive with an over all prevalence of 43.26% in organized herd. Out of 180 serum samples from unorganized herds 24 samples were found positive with an overall prevalence of 13.2%.A total of 483 nasal swabs of cattle were screened for IBR antigen detection studies, using antigen detection sandwich ELISA kit. Out of 483 samples, 303 samples were taken from organized herds and 180 samples from unorganized herds. Out of 303 samples of organized herds 37 samples showed positive reaction i.e. 12.2%. Out of 180 nasal swabs of unorganized herds only 7 samples showed positive reaction i.e. 3.9% IBR antigen presence in unorganized herds. Over all IBR antigen presence in nasal samples were found 8.7% in this study. A total of 123 semen samples from different organized herds and veterinary hospitals were screened by antigen based sandwich ELISA. Result showed that out of 123 samples, 8 semen samples were found positive with overall 6.5% antigenic presence. Like wise 50 milk samples were also screened for antigen detection studies by sandwich ELISA. Out of these 50 milk samples, 6(12%) samples were found positive for IBR antigen by sandwich ELISA. In this study for IBR virus isolation 44 nasal samples, which were found positive in antigen detecting sandwich ELISA, were tested. Of these 44 samples, 12 samples showed the Characteristic cytopathic effect in MDBK cell line. Likewise 123 semen samples were tested from different herds, out of these, 8 samples were found positive by virus isolation .6 milk samples were also tested for virus isolation but no sample showed the CPE. These 20 virus isolates from cell line were further subjected to multiplex pcr and were found positive.A total of 44 nasal samples which were found positive in antigen detection ELISA were processed for multiplex PCR. Out of 44 samples, 12 samples (27.3%) were found positive with gB and gC gene based multiplex PCR, 6(13.6%) samples were found positive with only gB gene based primer of multiplex PCR and 3 (6.8%) samples were found positive gC gene based primer. Over all 47.7% tested nasal samples were found positive with multiplex PCR.123 semen samples from different organized herd were tested for multiplex PCR. Out of them 10 (8.1%) samples were found positive with both gB and gC gene primers of multiplex PCR. 4(3.3%) samples were found positive with only gB gene primer of multiplex PCR and 4 (3.3%) samples out of 123 samples

were found positive with only gC gene primer of multiplex PCR. Over all 14.6% semen samples were found positive by multiplex PCR. 6 milk samples were also tested for multiplex PCR, but none of them found positive.In this study, two sets of primer were designed one from gC gene and one from gD gene. These Primers have been properly checked after blast and running the PCR reaction. These primers were given light bend in electrophoresis, require some standardization. One of our PCR products, we got sequenced from "Banglore Gene" .That report after analysis with BLAST showed that our PCR product is 100% similar to BHV-1 of genebank.

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16. Seroprevalence of FMDV Type A and the seroconversion ratio in different commercial vaccines in India

Herpal Singh and S.K. Yadav

In present study the seroconversion after vaccination in the districts under FMD control programme with special reference to highly mutable serotype type A and another experimental study with two vaccine Raksha Triovac (FMD,HS,BQ),Raksha Ovac(only FMD) was conducted. A total of 2202 bovine serum samples, collected from different districts under Control programme, in different phases were screened for antibodies against FMD virus using LPB-ELISA at serial dilutions of 1:16, 1:32, 1:64 and 1:128. The study showed that Liquid phase ELISA was a suitable test for the detection of vicinal immune response. The results of seroservillance study revealed that the highest number of animals (28.14%) showed protective titre against **type A** found in Agra followed by Gautam Budh Nagar (15.75%), Firozabad (4.28%), Bulandshehar (4.23%) before the vaccination started. While after vaccination the protective antibody titre increased and recorded in large number of animals, showed highest in Bulandshehar(4.23-37%) followed by Firozabad (4.28-24.39%), Gautam budh Nagar (15.75-30.5%) where as in Agra it was increased from 28.14 to 39.6% in prevaccination and post vaccination,respectively.Out of 1070 pre-vaccination samples, 914 showed the antibody titre below protection level (< 1.8) against **serotype "A"** i.e. 85.42 % (914 out of 1070) and in post-vaccination sera samples showed antibody titre below protection (< 1.8) i.e. 65.90 % (476 out of 1132).Where as type O ranged from 2.3 to28.8% from Bulandsaher and Agra, respectively in prevaccination where as it was increased after 4-5th phase 30.75to 74.3% from Bulandsaher and FIrozabad, respectively.Type Asia1 ranged from 13.8 to 36.2 and 31.5 to 76.8% in prevaccination and post vaccination, respectively.In present study, in both two vaccination experiment viz; Raksha Triovac trivalent contains FMD along with HS, BQ and Raksha- O vac detectable immune response developed at 7 day post vaccination (dpv) and reached up to protective level on 21st dpv and maintained up to 28th dpv than started to decline from 60th to 180th dpv. It was also observed that in first vaccine experiment the calves (no.6 and 7) showed prevaccination tirte 1.5 at 0 day, after vaccination it was increased up to protective level (2.1) at 7dpv, indicated strong booster effect on previously activated immune response. The percentage of animals showed level of antibodies against type O, A, Asia1 in sera samples of calves at 0 day 13%, 13%, 20% increased and reached to protective level at 21 dpv as 86%, 86.6%, 86.6%,respectively in I group (Raksha trivac). Further it was maintained up to 28dpv as 73%, 83%, 73%, respectively then decreased in consecutive days fall up to 33%, 40%, 40% respectively at 180dpv. Similarly, in II group (Raksha Ovac) 0 day 13%,6%,20% increased and reached to protective level at 21 dpv as 80%,86.6%,73.3%,respectively in I group (Raksha trivac). Further it was maintained up to 28dpv as 67%, 87%, 73%, respectively then decreased in consecutive days and fall up to 27%, 47%, 40% respectively at 180dpv.The individual animal variation also observed within each group as antibody titre increased at 14 -21 dpv, in 7 out 15 calves it was reached up to 2.1 and above where as 6 calves showed 1.8 protective titre. The two calves failed to showed protective titre

and therefore remain at 1.5. This variation in the response of immune system to vaccination is general feature in the field condition. It was also observed from results that the combination of FMD, HS, BQ vaccine also produced same level of response as detected by FMD alone which was used for in the control programme.

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17. Studies on molecular epidemiology of Foot and Mouth Disease virus in bovines

Malik Raies Ul Islam and S.K. Yadav

During the present study period, a total of 40 samples (including 3 tongue epitheliums and 37 OPF samples) were collected from cattle and buffalo from different parts of Uttar Pradesh showing clinical signs viz. vesicle formation on dorsal surface of tongue and interdigital spaces, salivation and anorexia. Of these, 3 were typed as type 'O' and 2 as type 'Asia-1' and 1 untypable by indirect sandwich ELISA test. The untypable sample could only be typed by mPCR as type 'O'. All the 37 OPF samples were processed for virus isolation. Out of these samples, only 4 samples could be revived on cell line. Of these, 2 were typed as FMDV type 'O' and 2 as FMDV type 'Asia-1' by using indirect sandwich ELISA test. By the use of mPCR, 06 samples were positive. From the results it can be seen that mPCR worked more efficiently on clinical samples compared to the sandwich ELISA. In our study, it is plausible that some factors played a crucial role in virus degradation, as samples on most occasions had to withstand high environmental temperature during the transition for more than 10–15 days. Similarly, drop in pH of the buffer due to bacterial growth or incorrect pH during the buffer formulation also might have contributed to virus degradation. All the samples were tested for the reverse transcription polymerase chain reaction using multiplex PCR. Of these, 4 amplicons of 249bp (type "O") and 2 amplicons of 537bp (type "Asia-1") could be amplified. These six positive samples (by multiplex PCR) were processed for the PCR using primers for 1D gene and sequencing. Out of these, only two samples (IND 121/09 and 1MTH/09) could be processed further for sequencing. In the present study, nucleotide sequence of 1D region for the isolate FMD type 'Asia-1' (IND121/09) was generated. For phylogenetic analysis of this recent isolate with the vaccine strain (IND 63/72) and the previous isolates such as IND 490/97, IND 140/01 and IND 260/00 included in the tree construction the sequences were retrieved from local database of project directorate on foot and mouth disease (PDFMD), Mukteshwar, District Nanital, Uttarakhand. Phylogenetic analysis was conducted using MEGA version 4. Tamura Nei (1993) model of nucleotide substitution with gamma-distribution of among-site rate heterogeneity (with 8 categories) (termed as TrN+G model) available in MEGA was used to construct the trees. The aminoacid divergences between the recent isolate (IND 121/09) and vaccine strain (IND63/72) was 15.9%, while aminoacid divergences between the recent isolate (IND 121/09) and previous isolates (IND 490/97, IND 140/01 and IND 260/00) were 10.7%, 9.1% and 9.1%, respectively. The nucleotide and amino acid sequence alignment study showed that, the receptor binding motif RGD was found to be conserved in FMD type Asia-1 viruses compared. In all the viruses compared in this study, majority of changes observed in amino acid and nucleotide sequence alignment were found in the mobile GH loop, which is considered as most variable in the immunogenic protein VP1. In the dendogram, the newly sequenced isolate was clustered with lineage C and subcluster CI. The vaccine strain was clustered with lineage B and subcluster B1 confirming the continuous dominance of genotype VII in the field. Finally, the present study sheds some light on molecular epidemiological situation of the disease in India due to type Asia-1. Thus continuous monitoring of the field strains help in selection of vaccine strain and evolving a proper control strategy for the disease in the future.

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18. Prophylactic and therapeutic effect of Johne's disease vaccine in cattle

Abhisek Kumar Srivastava and S.K. Yadav

High prevalence of JD has been reported in domestic livestock using indigenous, sensitive and MAP specific tests in North India with sero-prevalence up to 31.9% and 23.3% in UP and Punjab, respectively. In our study, there was slight improvement in the body weight of the vaccinated animals in comparison to sham immunized animals in both the trials ($p<0.05$). There was no indication of any short term adverse clinical effect of vaccination. Vaccination site lesions were persistent, but were not a major concern to processors. There was very low mortality rate in vaccinated group (1.5%) as compared to sham immunized (10.0%). This data alone justify the use of vaccination, providing immediate benefits to producers who are experiencing significant OJD-mortality in their flocks. In our study, proliferative response was greater in PBMCs from the vaccinated group which was stimulated with Protoplasmic antigen of MAP at all the sampling periods after 30 DPV, with respect to sham-immunized group stimulated with MAP antigen. The stimulative index was always higher in vaccinated cattle than in sham-immunized. The stimulative index value of vaccinated cows was significantly higher ($p<0.05$) at 30 DPV & onwards. Therefore on the basis of lymphocyte transformation test, it was clear that vaccine had induced effective CMI response during monitoring period. The significant reduction in percentage of fecal shedding of MAP was observed in vaccinated animals in comparison to sham immunized animals in both the trials (trial 1 and 2). All the 21 microscopy positive samples (17 vaccinated and 4 sham-immunized groups) were subjected to IS 900 based PCR amplification. A total of 17 animals (14 vaccinated and 3 sham-immunized) showed an amplicon of 413bp at 0 DPV (fig--) but after 4 month post vaccination, when all the 14 microscopy positive samples (9 vaccinated and 5 sham-immunized) were subjected to IS 900 based amplification, then the amplicons of 413bp was amplified in 12 animals (9 vaccinated and 3 sham-immunized) which confirms that the reduction of Mycobacterium paratuberculosis in animals from vaccinated group, while no such reduction was found in the animals of sham immunized group. The sensitivity for microscopy was slightly high than blood PCR at different time interval. The high detection by microscopy may be due low specificity of test i.e. false positive diagnosis by microscopy or detection of other acid fast bacilli. Overall data showed the therapeutic and prophylactic efficacy of Johne's disease vaccine in protection from MAP infection as indicated by the significant increase ($p<0.05$) in CMI response and humoral immunity response. The antibody titer in vaccinated animals was found to be enhanced after 30 and 60 DPV respectively. After 6 month of post vaccination, the significant reduction in shedding of MAP was observed only in vaccinated group which indicate 28.57% protection in Trial I, with respect to 33.63% in trial II. Study also suggests the long term monitoring of the vaccinated animals to analyze the proper effect of vaccine.

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19. Development of sandwich ELISA for the detection of IBR virus

Shahnaz Bashir and S.K. Yadav

In this study, a sandwich ELISA was developed for the detection of BHV-1. IBR virus (source of antigen) was isolated from an aborted foetus (7 months). Large scale isolation of BHV-1 was done in MDBK cell line. Polyethylene glycol precipitation (PEG) method was adopted for the concentration of virus from cell culture harvest. Purification of virus was carried out by continuous sucrose gradient (20% - 60%) ultracentrifugation. Assesment of the purified virus was done by protein assay and evaluation of the development of cytopathic effect in MDBK cell line. Hyperimmune sera were produced in rabbits and guinea pigs by injecting purified virus

with Freund's adjuvant. The antibody titre of antisera, as estimated by ELISA test, was found to be 1:3200 and 1:2000 for rabbit and guinea pig antisera, respectively. IgG of high purity was extracted by serum purification. Optimum dilutions of various reagents to be used in the preliminary ELISA were determined by their titrations. Dilutions of 1:20 for rabbit 'capture' antisera and 1:10 for guinea pig 'detector' antisera were chosen as the optimum working dilutions to be used in the preliminary ELISA. Optimum dilution of conjugate (HRP –conjugated rabbit anti-guinea pig immunoglobulin) was determined by the serial dilutions. Conjugate dilutions of 1:2000 were selected for the preliminary ELISA test. In this study, the value of the cut-off point was fixed at 0.15 (A_{490}) for a positive reaction. A_{490} of >0.15 and a P/N ratio of >1.30 indicated a positive reaction. OD (A_{490}) of the purified virus was greater than 1.5 and that for culture positive samples was >1.2. OD of the negative controls was less than 0.2 (below the cut-off point). The (A_{490}) values of the background were <0.07 in all the cases (in the preliminary ELISA test).

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20. Studies on molecular characterization of Methicillin resistant Staphylococcus aureus (MRSA) in Bovine mastitis

Kiran Kutar and Amit Kumar Verma

In the study, a total of 125 milk samples, collected from the clinical and sub clinical cases of mastitis from cows (109) and buffaloes (16) in and around the Mathura city were processed for isolation of methicillin resistant *Staphylococcus aureus.* Overall incidence of *S. aureus* in clinical as well as sub clinical mastitis was found 41.60% (52/125). Incidence of clinical mastitis was 56.00% (42/75) and sub clinical mastitis was 20.00%. The results also revealed that the incidences of *Staphylococcus aureus* in clinical as well as sub-clinical mastitis were higher in cattle (43.11%) in comparison to buffaloes (31.25%). All the 52 isolates of *S. aureus* produced black colonies on the potassium tellurite agar and 39 (75.00%) isolates showed fermentation on mannitol salt agar where as 13 (25.00%) isolates were mannitol non-fermentatative *S. aureus.* All the 52 *S. aureus* isolates were tested for coagulase activity by using rabbit plasma. Of these, 30 (57.69%) were found positive for coagulase production whereas 22 (42.30%) were found negative for coagulase production. Majority of *S. aureus* isolates exhibited α haemolysin 33 (63.46%) which is most potent membrane damaging toxin of *S. aureus,* whereas 8 (15.38%) isolates showed β haemolysin and 11 (21.15%) isolates were non-haemolytic on 5% SBA. Out of 52 isolates of *S. aureus,* 22 (42.30%) isolates were found to be positive for lipase production and 32 (61.79%) were found negative for lipase activity. 3 (6.97%) were found positive for the TDNAase production. Among 52 *S. aureus* isolates, 43 (82.69%) *Staphylococcus aureus* produced slime, while 9 (17.30%) isolates were negative for slime production on CRA media. Antibiotic sensitivity patterns of 52 *Staphylococcus aureus* isolates revealed that the resisance antibiotics were cotrimoxazole (63.46%), followed by streptomycin (57.69%), Gentamycin (55.76%), cephalexine (42.30%), amoxicillin (38.46%) and Erythromycin (36.53%). Molecular characterization of the isolates by PCR assay was applied for species specific detection of methicillin resistant *S. aureus* as well as detection of virulence associated genes. Template DNA obtained by boiling method yielded good results in PCR. This technique proved to be very simple and rapid technique for template DNA preparation. PCR amplification of *Staphylococcus aureus* specific part of gene encoding the MecA of S. *aureus* isolates yielded an amplification product of 310bp for all the isolates, specific for *S. aureus* species.

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Ph.D.

1. Seromonitoring of PPR infection in small ruminants and evaluation of synthetic antigen in solid phase enzyme immuno assay

Shweta Raghuvanshi and B.C. Pal

A total of 1707 non-vaccinated 7 296 sera samples were collected for testing by competitive ELISA to determine epidemiological parameters affecting the occurrence of PPR in animal on the basis of age and sex. Experimentally 14 mer overlapping peptides were designed and synthesized in the region 421 to 490 of N-protein of PPRV. Only 2 peptides (463-483) reacted specifically with PPr positive sera. A 27 mer amino acid stretch at C-terminal of N-protein (463-489) was selected to be used as antigen in sero diagnosis. Of 296 samples from vaccinated population, 70.955 were positive by C-ELISA whereas 82.77% were positive by P-ELISA. In unvaccinated population, out of 1707 samples, the percentage of positive samples was 32.8 in sheep and 19.47 in goats using C-ELISA where as it was 21.5 in sheep and 27.02 in goats using P-ELISA. Sensitivity of P-ELISA with C-ELISA as reference was found to be 90% and specificity was found to be 85.5% in total population. With C-ELISA 22.8% sheep and 19.9% goat serum samples revealed anti PPR antibodies. By P-ELISA goats showed higher prevalence (27%) than sheep (21.5%). The synthetic peptide antigen used in ELISA in this study was highly sensitive in detecting PPR specific antibodies in vaccinated animals and did not cross react with RP virus.

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2. Studies on epidemiology of mycoplasmal pneumonia in goats

Udit Jain and B.C. Pal

The present study was undertaken to ascertain the epidemiology of mycoplasmal pneumonia in goats. Out of total 1910 samples from goats with different clinical history, 150 *mycoplasmas* and 42 *acholeplasmas* were isolated. The 38 samples were having the mixed infections. Overall 10.5% M/A prevalence was observed in the Barbari goats. The highest prevalence of M/A 15.84% was found in the samples processed from postmortem cases followed by 12.85% from samples collected from goats with history of respiratory problem and 8.87% from fresh slaughtered goats. The lowest prevalence 6.18 was recorded from healthy goats. The overall prevalence in relation to the age from the respiratory tract of Barbari goats was found highest in the goats of Mathura while lowest 7.00 was in the goats of Hathras. The rate of prevalence was followed 7.46, 8.75 and 8.0 Agra, Aligarh, and Etah respectively. The percentage of isolation of M/A from the respiratory tract of Barbari goats having different viz. age group below six month (Kids) was 8.31, between 6-12 months of age group was 5.08 percent and above 12 month of age group it was highest i.e. 10.00 percent. Similarly, the prevalence was more in the winter 10.85 of Distt. Mathura and Etah 10.63. While lowest prevalence was observed 3.57% in summer in Agra and Hathras Distt. The overall percentage of isolation of M/A from Barbari breed of goats was found 8.11. The overall percentage of prevalence in Jamunapari goats was (10.0) of M/A. A comparison was done with the demographic data's viz. breed, sex, age, location and environment of Barbari, Jamunapari and other non-descript breed of goats. The overall maximum prevalence (63.5%) was observed in the female in comparison to male where prevalence was 36.4%. When the prevalence in relation to breed was evaluated, the non-descript goats were having highest prevalence (73.9%). Age wise comparison shows that the highest prevalence (52.6%) was observed in the age group of 6 Month or below in comparison to 6-12M age in which it was lowest (18.2%). When the prevalence in relation to breed was evaluated, the non-descript goat kids were having highest prevalence (56.5%). shows the overall prevalence of M/A was highest in winter season (60.9%) in comparison to summer (10.9%). The breed Barbari shows highest prevalence of M/A (63.2%) in

winter season in comparison to non descript breed (56.5%). The overall prevalence in relation to location was observed highest in Mathura district(31.2%) in comparison to Etah in which it was lowest(11.4%). In relation to breed it was highest in Non descript breed (34.7) than Jamunapari (28.2%). Of 134 sera samples, 8 sera samples from apparently healthy and 15 from pneumonic goat, from them isolation of *Mycoplasma capricolum* subsp. *capripneumoniae* (causative agent of CCPP) was done showing titre range 1:128 to 1:256. Sero epidemiological analysis of sera samples in relation to district, breed and age was done by using ELISA test. On 1181 sera samples 434 were found positive giving overall 36.7% prevalence against members of "Mycoides cluster" with different antigens. *Mccp* isolates were found sensitive for erythromycin, tetracycline, spiramycin and tylosin in that order but were resistant for sparfloxacin and enrofloxacin. Mcc isolates were found sensitive for tetracycline, and erythromycin but were resistant for spiramycin, sparfloxacin tylosin and enrofloxacin. Other isolates like Mycoplasma agalactiae and M. bovis, revealed high sensitivity to erythromycin and enrofloxacin, slight sensitivity to tetracycline but were resistant to sparfloxacin. On doing PCR of 134 biological samples by using specific primers of *M. genus, M. mycoides cluster and M. capricolum subsp. Capripneumoniae,* 129 were positive for Mycoplasma genus, 108 for "Mycoides culture" group and 81 for *M. capricolum subsp.* Of 52 biological material collected, 6 were found culture positive and 19 PCR positive.

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3. Studies on molecular epidemiology of FMD in bovines with reference to serological diagnosis of vaccinated and infected animals

Amit Kumar Verma and B.C. Pal

During the study period, a total of 15 samples of vesicular epitheliums of tongue were collected from cattle and buffalo showing clinical signs viz., vesicle formation on the mucous membranes of the tongue, inter-digital spaces, salivation and anorexia from Uttar Pradesh state. Of these, two were typed as FMDV type 'O', nine as FMDV type 'A' and four as FMDV type 'Asia-1' by using indirect sandwich ELISA test. These data of results obtained were compared with the previous year's results available with the AICRP, RRC, Mathura, Uttar Pradesh; for determining the annual prevalence, seasonal occurrence of FMD virus type in the state. In the present study a total of ten recently collected samples (vesicular epithelium of tongue) of cattle and buffaloes of different parts of India were processed for virus isolation, sandwich ELISA and reverse transcription polymerase chain reaction (RT-PCR). Out of these samples, amplicon of 866 bp could be amplified from 3 specimens (IND 49/07, IND 195/07 and IND 196/07). In the present study, nucleotide sequence of 1D region for three FMD type 'A' viruses (IND 49/07, IND 195/07 and IND 196/07) isolated during 2007 was generated and compared with the rest of the previous isolates sequences available with the local database of Project Directorate on foot-and-mouth disease (PDFMD), Mukteswar, Distt. Nainital, Uttarakhand. For phylogenetic analysis of these recent isolates with Phylogenetic analysis was conducted using MEGA version 4. Tamura Nei (1993) model of nucleotide substitution with gamma-distribution of among-site rate heterogeneity (with 8 categories) (termed as TrN + Gmodel) available in MEGA was used to construct the trees. In the Phylogenetic tree, all the field isolates of 2007 were grouped in genotype VII indicating the incessant supremacy of that genotype in the field in recent times, while the older vaccine strain IND17/77 and in-use vaccine strain IND 490/97 were grouped in genotype IV and VI respectively. In this study, the nucleotide (nt) divergence among the recent field isolates (IND 49/07, IND 195/07, IND 196/07) sequenced, vaccine strains (IND 17/77, IND 490/97) and other representative isolates (IND 258/99, IND 81/00, IND 270/03) were compared and found that nucleotide divergence between the recent isolates

and vaccine strain (IND 490/97) is higher (22.0%) compared to that among recent field isolates (0.00%). amino acid divergence among the recent isolates and vaccine strain (IND 490/97) is higher (15.6%) compared to that among recent field isolates (0.00%). The polyprotein of FMD virus can be divided into four elements: L, P1, P2 and P3. During the present study a total of 470 sera of apparently healthy cattle from districts (Agra, Mathura, Gautambudh Nagar, Ghaziabad and Bulandsahar, where FMD control programme is going on since 2004) of Uttar Pradesh State were screened by using 3ABC-ELISA against 3ABC proteins. Out of 470 sera samples screened, 136 (28.93%) sera were detected as positive, 286 (60.85%) as negative and 48 (10.21%) as suspected. The sero-prevalence was highest in Mathura (52.13%), followed by Gautam Budh Nagar (47.87%), Agra (35.105), Buland Sahar (5.32%) and Ghaziabad (4.26%). Finally, the present study sheds some lights on molecular epidemiological situation of the disease in India due to type A which is considered to be antigenically and genetically most diverse. Such continuous monitoring of the field strains help in selection of vaccine strain and evolving a proper control strategy for the disease in the future.

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4. Studies on molecular epidemiology and genetic variations among FMD-V of livestock in UP

Govind Yadav and B.C. Pal

A total of 100 specimens from FMD outbreak from 7 out of 9 agroclimatic zone of UP over a period of 6 years (2002-07). Distribution of FMD-V serotype was accessed using sandwich ELISA. Studies showed that majority of outbreaks (57%) were due to type O, 39% due to type A and only 4 due to Asia 1. Of these, the highest (39) type O were from south western followed by western (4) mid eastern (4), central zone (3) and lowest one in each mid western and north eastern zone. Serotype Asia 1 was observed only in Bundelkhand zone. Thus FMD is endemic in different climatic zone of UP. Disease occurrence is highest in winter (78%) followed by summer (12%) and rainy season (100%). For molecular characterization 12 FMD outbreak strains were characterized by different tests. Multiplex PCR was found to be more sensitive (8/12) than complete ID gene amplification (6/12) and virus isolation in cell culture (3/12). Phylogenetic analysis indicated that genotype VII of type A continuously showed its dominance since last few years in UP. In this study 3A NSP ELISA was found to be a suitable test for detecting antibodies against non-structural proteins of FMD-V.

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Veterinary Gynaecology and Obstetrics

SNo.	Titles	Name of Scholars	Name of Major Guide	Years	Page No.
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1.	Hormonal induction of ovarian cyclicity in post partum cattle with special reference to mineral and metabolic profile	Dr. Chandra Pal Singh	Dr. Atul Saxena	2003	101
2.	Studies on performance of Sirrohi bucks and estimation of enzyme in certain biological fluid	Dr. Nagender Singh	Dr. Atul Saxena	2004	101
3.	*In vitro* fertilization and cleavage rate of *in vitro* matured pubertal goat oocytes	Dr. Roopali Yadav	Dr. Atul Saxena	2004	101
4.	Studies on certain aspects of post-partum anestrus in cattle with special reference to metabolic, hormonal and mineral profile	Dr. Pankaj Kumar Yadav	Dr. Atul Saxena	2004	102
5.	Studies on development competence of *in vitro* fertilized goat oocytes in different culture system	Dr. Poonam Uniyal	Dr. Atul Saxena	2005	102
6.	Cryopreservation of Frieswal bulls	Dr. Raghubir ~~Yadav~~ Singh	Dr. Atul Saxena	2005	103
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8.	Studies on development competence of *in vitro* matured goat oocytes in medium with different supplementation	Dr. Kaushlendra Pratap Singh	Dr. Atul Saxena	2005	104
9.	Studies on cryopreservation of Bhadawari bull semen	Dr. Dhananjay Mishra	Dr. Atul Saxena	2006	104
10.	Studies on improving postpartum anestrus in cattle and buffaloes using Buserelin acetate (GnRH) and Tiaprost tromethamine ($PGF_{2\alpha}$)	Dr. Jaiprakash Yadav	Dr. Atul Saxena	2006	105
11.	Studies on induction of estrus in post partum Murrah buffaloes using different hormonal treatments	Dr. Satyendra Kumar Bhati	Dr. Atul Saxena	2007	105
12.	Studies on collection and grading of cumulus oocyte complexes from buffalo slaughter house ovaries and their culture *in vitro*	Dr. Vinod Verma	Dr. Atul Saxena	2007	106
13.	Studies on performance of Murrah bulls following cryopreservation of their semen and effect of post-thaw incubation of semen on certain seminal attributes	Dr. Adesh Kumar	Dr. Atul Saxena	2008	106

14.	Studies on comparison of different doses of gonadotrophin releasing hormone (Busereline) on the fertility status of post partum anestrus buffaloes employing ovsynch protocol	Dr. Ravinder Kumar	Dr. Atul Saxena	2008	107
15.	Studies on induction of estrus and fertility with controlled internal drug release (CIDR) device and other hormonal combination in buffalo heifers	Dr. Atul Kumar Verma	Dr. Atul Saxena	2010	107
16.	Studies on comparison of fertility in different ovulation synchronization protocol for fixed time insemination in anestrus cows	Dr. Brijesh Kumar	Dr. Atul Saxena	2010	108
17.	Studies on induction of estrus and fertility in anestrus buffaloes using progesterone releasing intravaginal device (PRID) and other hormonal combination	Dr. Ram Shyam Singh	Dr. Atul Saxena	2010	108
18.	Studies on cryopreservation of Hariana Bull semen	Dr. Arun Kumar	Dr. Atul Saxena	2011	108
19.	Studies on effect of progesterone releasing intravaginal device and other hormonal combination on the reproductive performance of bovines	Dr. K. Sai. Gunaranjan	Dr. Atul Saxena	2011	109
20.	Studies on reproductive management of dairy heifers using progesterone releasing intravaginal device along with other hormonal combination	Dr. Brijesh Kumar Singh	Dr. Atul Saxena	2011	109
21.	Studies on certain seminal attributes and effect of dilutors on cryopreservation of Hariana bull semen	Dr. Ashok Kumar Singh	Dr. Atul Saxena	2012	109
22.	Studies on semen characters of Bhadawari bulls and its freezability under semi-arid conditions	Dr. Alok Kumar Sahrawat	Dr. Atul Saxena	2012	110
23.	Studies on semen quality, freezability and fertility performance of Murrah bulls	Dr. Devendra Singh	Dr. Atul Saxena	2012	110
24.	Studies on effect of different concentrations of egg yolk and glycerol in tris based extender on cryopreservation of Bhadawari bull semen	Dr. Vipin Sonker	Dr. Atul Saxena	2013	111
25.	Studies on effect of different concentrations of egg yolk and glycerol in tris based extender on cryopreservation of Hariana bull semen	Dr. Vikas Sachan	Dr. Atul Saxena	2013	111

1. Hormonal induction of ovarian cyclicity in post partum cattle with special reference to mineral and metabolic profile

Chandra Pal Singh and Atul Saxena

The study was designed with the objective of inducing ovarian cyclicity in true anestrus animals using hormone GnRH (buseraline acetage) and P4+E2 (17α-hydroxy caponate and estradiol valerate) and to compare the metabolic and hormonal profile of anestrus animals with animals in natural estrus. Seventeen true anestrus animals were divided into two groups. Group 1 (n=8) animals were treated with a single intramuscular dose of 20 ug GnRH while Group 2 animals were treated with combination therapy using P4 @.25 g/ml (intramuscular injection in two equally divided dose) and E2 @500 ug (single intramuscular injection) after 48 h of P4 treatment. In Group 1 treatment resulted in induction of estrus in 62.5% (5/8) animals after 88.80±25.0 h of treatment resulting in 40% (2/5) pregnancies. In Group 2, 100% (9/9) exhibited estrus after 57.44±9.47 h of treatment resulting in 66.67% (6/9) pregnancies. No significant difference was observed for concentration of hormone progesterone and estrogen at different stages of treatment neither any difference could be observed with the values reported in natural estrus animals. Anthelmentic treatment did not results in any significant improvement in various metabolic profile (Hb, glucose, total protein, Ca, P, Ca: P ratio) however, compare with natural estrus animal there occurs a significant difference.

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2. Studies on performance of Sirrohi bucks and estimation of enzyme in certain biological fluid

Nagender Singh and Atul Saxena

The study was aimed with the objective of analyzing certain seminal attributes of Sirrohi bucks and estimation of enzymes in certain biological fluids. The semen from seven sirrohi bucks was collected adopting standard AV method. The overall (7x10=70) mean seminal volume was found as 0.96±0.08 ml, gross motility, 4.10±0.07, sperm concentration, as 4074.62±57.89 millions/ml and percent live spermatozoa as 69.68±0.44. Except for sperm concentration, no significant difference was observed for other parameters. In the fresh seminal plasma, the GOT and GPT were found as 159.50±4.83 units/ ml, and 30.87±1.30 units/ ml. The AKP and ACP were found as 208.68±2.81 KA units/ml and 14.11±0.33 KA units/ ml. There was a significant difference in all the enzyme levels amongst different bucks. In various biological fluids (seminal plasma, uterine fluid, follicular fluid and cervical mucous) the GOT and GPT were found to be 162.46±6.47, 25.96±0.79, 41.8±1.73, 27.88±1.07 units/ml and 33.00±1.52, 31.68±1.15, 40.42±1.45, 33.14±1.08 units/ ml. The AKP and ACP were 217.67±3.39, 52.68±1.75, 63.7±2.17, 79.75±1.94 KA units/ ml and 14.17±0.38, 3.07±0.19, 3.58±0.13, 5.58±0.34 units/ ml.

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3. In-vitro fertilization and cleavage rate of *in vitro* matured pubertal goat oocytes

Roopali Yadav and Atul Saxena

The study was designed with the objective of comparing methods of oocyte recovery their maturation and fertilization rate of different grade recovered oocytes from pubertal goat's ovaries. Fifty ovaries collected from slaughter house were subjected to oocyte retrieval by the technique of (1) Slicing (2) Aspiration and (3) Puncturing. The oocyte recovery rate per ovary by slicing was 7.9±0.53 of which grade 'A' was 12.66%, 'B' was 20.25%, 'C' was 30.63% and 'D' was 36.46%. By aspiration technique the recovery rate was 4.76±0.45 oocytes per ovary and the

respective grade oocytes were 10.50%, 17.65%, 30.26% and 41.18%. For the method of puncturing the recovery rate was 8.04±0.61 oocytes per ovary with respective grade oocytes as 15.42%, 27.36%, 28.36% and 39.55%. The method of slicing and puncturing yielded a significantly higher oocyte recovery. Further, the method of puncturing yielded a significantly higher number of grade 'B' oocytes. Out of the 426 oocytes collected through puncturing method and subjected to three different medium i.e. M1 (Basic media + EGS), M2 (Basic media + ESS) and M3 (control with no serum supplementation) the maturation rate were found as 85.78%, 79.7% and 12.14% with cleavage rate as 67.26%, 36.36% and 17.64%. Further, when 537 oocytes collected through puncturing were the maturation rate in Fert talp was 78.53%, in BO medium as 76.47% and in SOF as 81.87%. The respective cleavage rates were 60.67%, 32.48% and 36.71% which differed significantly.

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4. Studies on certain aspects of post-partum anestrus in cattle with special reference to metabolic, hormonal and mineral profile

Pankaj Kumar Yadav and Atul Saxena

The study was designed with the objective of comparing the metabolic, hormonal and mineral profile of anestrus cows with animals in normal estrus and their response to hormonal treatments. Thirty five lactating cows with normal genitalia and having history of anestrus since last six months were taken for study. All animals were subjected to single Intramuscular injection of cloprostenol ($PGF_{2}\alpha$ @ 500ug/animal) treatment with the intension of segregating true anestrus animals. As a result of the treatment 22 animals return to estrus and segregated. The remaining 13 animals were divided into two groups as Group 1 (n=7) treated with single intramuscular injection of GnRH (@20 ug/animal) and Group 2 (n=6) treated with intramuscular injection of progesterone (@ 200 mg in two dividing dose) for two days followed by an intramuscular injection of estrogen (@ 500 ug as single dose). Besides, these animals were screened for metabolic (glucose, total protein and Hb), hormonal (progesterone and estrogen) and mineral (Cu, Zn, Fe, Ca and P) profile. Additionally, 5 normal estrus animals were also taken for comparing the various profile results. The results revealed a significantly lower Hb (9.65±0.05 vs 10.20±0.17 g/dl) and a higher Ca (2.49±1.11 vs 1.31±0.09 mg/dl) levels in anestrus animals compared to normal cyclic animal in estrus. No difference could be observed for hormonal profile between anestrus and normal cyclic animal in estrus. Following anestrus treatment in Group 1, 85.71% (8/7) animals reported in estrus resulting in 50% (3/6) pregnancy. For Group 2, the respective values were 100% (6/6) and 33.33% (2/6).

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5. Studies on development competence of *in vitro* fertilized goat oocytes in different culture system

Poonam Uniyal and Atul Saxena

The aim of the present study was to compare the efficacy of two maturation media and two culture system on the developmental competency of *in vitro* fertilized goat oocytes. Oocytes obtained from the ovaries of slaughtered prepubertal and pubertal goats adopting method of follicle puncturing. Out of 338 prepubertal and 649 pubertal ovaries, 819 and 1150 (Grad A and B) oocytes were recovered with a recovery rate of 2.42 and 1.72 oocytes per ovary. The recovered oocytes were subjected to maturation in two maturation media M1 (TCM-199 + L-glutamine + gentamycine + sod pyruvate + 20% EGS) and M2 (TCM-199 + NaHCo3 + L- glutamine + gentamycine + sod pyruvate + FSH+ LH+ E2). In maturation medium M1, out of 454 prepubertal and 687 pubertal oocytes the maturation rate were 90.5% and 86.3%. In medium M2, out of 365 prepubertal and 463 pubertal oocytes the maturation rates were 95.06% and 95.03%. No

significant differences were observed for the two medium used neither any significant effect were notice with regard to maturation of prepubertal and pubertal goat oocytes. The *in vitro* matured oocytes were subjected for fertilization and further development into two culture system ie CS 1 (Graulosa cell monolayer) and CS2 (Oviductal epithelial cell). In CS1, out of 120 prepubetal and 250 pubertal matured oocytes the cleavage rate were found to be 41.6% and 15.6% where as in CS 2 out of 284 prepubertal and 259 pubertal matured oocytes the cleavage rates were 34.5% and 21.62%. No significant difference was observed in the cleavage rate between two culture system and the two types of oocytes (prepubertal and pubertal) used. However, in CS2 more oocytes reached to >8 celled stage of development.

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6. Cryopreservation of Frieswal bulls

Raghubir Yadav and Atul Saxena

The study was designed with the objective of ascertaining the freezability of Frieswal bull semen in tris egg yolk citrate extenders and to observe the effect of age, season, vaccination and post-thaw incubation on certain seminal attributes. Seven Frieswal bulls between 18-42 months of age were used as semen donors. Based on 60 ejaculates from each bulls, the overall (60 x7=420) mean volume was found as 4.14±0.08 ml, mass motility as 2.77±0.03, concentration as 1348.45±26.64 millions/ml and progressively motile sperm as 56.10±0.60 per cent. A significant difference was observed for these parameters amongst the bulls. Age of the bull has a significant ($P<0.01$) effect on semen volume with maximum ejaculated volume evident between 31-36 month aged bulls. Summer and winter season had a significant ($P<0.05$) effect on mass motility compare to rainy season. FMD and HS vaccination had a significant effect on percent live spermatozoa and total damaged acrosome. The effect of vaccination last upto 90 days for live spermatozoa and 60 days for acrosomal damage thereafter recovery were observed for these parameters. Following freezing and thawing (37°C for 1 minute), progressive motility was found as 43.13±1.37 per cent. The respective values for percent live spermatozoa, HOS positive sperm and acrosomal damaged sperm were 68.43±1.35, 54.56±1.22 and 18.25±0.77 per cent. After incubation of thawed semen to 1 h, 2 h and 3 h the deterioration in per cent progressive motility were 35.64±1.43, 22.69±1.18 and 9.06±0.55. The respective drop in per cent liver spermatozoa, HOS positive spermatozoa and damaged acrosome were 62.75±1.33, 47.73±1.62, 38.20±1.64 and 49.59±1.14, 37.68±1.19, 29.74±1.06 and 20.80±0.71, 24.75±0.91, 27.49±1.18.

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7. Synchronization, Multiple Ovulation and Embryo Transfer in Frieswal Cows

Srawan Kumar Maurya and Atul Saxena

The study was designed to compare the superovulatory responses of two gonadotrophins (FSH and eCG) and pregnancy rate following transfer of recovered embryos in Frieswal Cows. Twenty eight healthy frieswal cows with normal genitalia were used as Donors (n=10) and recipient (n=18) were synchronized using single injection of PG injected intramuscularly started 60 hr of superovulatory treatment in donor animals and 24 hr before in recipient animals. The donor were divided into groups, group 1 (n=5) animals were superovulated with FSH (280 mg) intramuscularly twice daily in multiple and descending doses for 4 days while group 2 (n=5) animals were superouvlated with eCG (1500 IU) intramuscularly as single dose. Estrus was induced using PG (tiaprost tromethamol @ 0.75 mg) after 60 hr of respective treatments. The donors were inseminated at induced estrus with 0.25 ml semen dose at 12 hr interval till the signs of estrus were present. Embryos collected non-surgically on $6^{th}/7^{th}$ day post insemination. The superovulatory response (>2CL) was 80% (4/5) in group 1 and 100% (5/5) in group 2. The

viable embryos recovery per donor was 2.8±0.37 in group 1 and 2.0±0.55 in group 2. The embryo quality graded as good, fair and poor in group 1 were 28.57%(4/14), 35.71%(5/14) and 35.71% (5/14) and in group 2 were 40% (4/10), 40%(4/10) and 20%(2/10). The stage of the recovered embryos in group 1 were 9 morulla, 1 compact morulla and 4 early blastocyst and in group 2 were 8 morulla, 1 early blastocyst and 1 hatched blastocyst. The concentration of hormone estradiol 17 beta and progesterone did not differ significantly between donor and recipient and no significant difference was observed between donor and recipient in term of degree of synchrony (time taken for induction of estrus). Out of the total 24 embryo recovered from two groups, 17 good and fair types were transferred to 17 synchronized recipients resulting in 4 (23.12%) positive pregnancy.

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8. Studies on development competence of *in vitro* matured goat oocytes in medium with different supplementation

Kaushlendra Pratap Singh and Atul Saxena

The study was aimed with the objective of observing the effect of different supplementation in the basic maturation media (TCM-199) on maturation rate of goat oocytes and the effect of two culture system i.e. CS1 (Granulosa cell monolayer) and CS2 (oviductal epithelium cells co culture) on the developmental competency of *in vitro* matured and fertilized oocytes. The oocytes were recovered from the prepubertal and pubertal goat ovaries collected from slaughter house and adopting the technique of puncturing of follicles. The collected oocytes were subjected to maturation in four maturation medium i.e. M1 (TCM-199 + $NaHCO_3$ + L-glutamine + Gentamycine + sod pyruvate + 20% estrus goat serum (EGS)), M2 (TCM-199 + $NaHCO_3$ + L-glutamine + Gentamycine + sod pyruvate + 20% NCS), M3 (TCM-199 + $NaHCO_3$ + L-glutamine + Gentamycine + sod pyruvate + 10% NCS) and M4 (TCM-199 + NaHCO3 + L-glutamine + Gentamycine + sod pyruvate + 20% CFF). The maturation rate for prepubertal and pubertal oocytes were 86.98% and 89.76% in M1, 88.35% and 88.02% in M2, 97.31% and 92.48% in M3 and 88.99% and 89.56% in M4. The respective cleavage rates were 43.30% and 26.68%, 41.08% and 41.95%, 42.07% and 44.06% and 44.27% and 40.37% for medium M1, M2, M3 and M4 respectively. The *in vitro* fertilized oocytes were cultured on culture system CS1 and CS2. The prepubertal and pubertal oocytes which reached to >8 celled stage were 5.64% and 1.89% whereas in CS2 the values were 6.48% and 6.57%. It was concluded with the study that all medium and culture system behaved similarly for the development of embryos.

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9. Studies on cryopreservation of Bhadawari bull semen

Dhananjay Mishra and Atul Saxena

The study was aimed with the objective of observing the effect of addition of gluatamic acid and different concentrations of glycerol on cryopreservation of Bhadawari bull semen. Further comparisons were made for suitability of a hypo-osmotic solution for conducting HOS test. Semen was collected bi-weekly from six Bhadawari bulls using standard AV method and evaluated for various seminal attributes. The overall mean semen volume was 2.62±0.17 ml, mass motility, 3.28±0.10, sperm concentration, 619.54±32.68 millions/ml and live sperm as 71.85±1.54%. Except for sperm concentration no significant difference was observed for other parameters between bulls. After diluting the semen in EYT extender supplemented with glutamic acid (@0.704 mM), the overall live sperm percentage following dilution, glycerolization and equilibration were 69.27±20, 66.01±1.95 and 60.26±1.92 which differed significantly with non-supplemented extender with respective values as 67.77±1.93, 63.56±1.90 and 53.50±1.93 percent. The percent progressive motility also differed significantly with values as 69.43±2.22, 65.38±2.43

and 58.65±2.26 in supplemented compare to 68.84±2.17, 62.31±2.27 and 53.26±1.80 percent in non-supplemented extenders. The post thaw results of percent live spermatozoa and progressive motility in glutamic acid supplemented and non-supplemented EYT extender differed significantly with respective values as 52.47±1.57, 45.21±1.58 against 40.10±1.40, 28.75±1.10 percent. For HOS positive spermatozoa at 150 mOsm in supplemented vs non- supplemented the values were 44.87±1.48 and 31.95±1.28 percent. Amongst the three hypoosmotic solution used maximum HOS positive spermatozoa were observed with 150 mOsm (66.84±1.52%), followed by 100 mOsm (66.75±1.42%) and 75 mOsm (42.40±1.08%). Amongst the three percentage of glycerol used, 7% level was found to be best compared to 6% or 8%.

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10. Studies on improving postpartum anestrus in cattle and buffaloes using Buserelin acetate (GnRH) and Tiaprost tromethamine ($PGF_2\alpha$)

Jaiprakash Yadav and Atul Saxena

The study was designed with the objective of improving the postpartum anestrus in cattle and buffaloes with the use hormonal treatments. Fifty-five parous animals comprising of 24 cattle and 31 buffaloes between the age group of 5-11 years were selected for the study. These animals were divided into groups as Group 1 (n=24) comprising of cows which were given anthelmentic (Fenbendazole feed pellets @ 1500 mg) treatment followed by single $PGF_2\alpha$ (total dose 0.75 mg/animal) intramuscular injection, Group 2 (n=15) comprising of buffaloes having good BCS were given $PGF_2\alpha$ injection as of group 1, Group 3 (n=9) comprising of buffaloes having moderate BCS were treated similar to group 2 animals and Group 4 (n=7) comprising of buffaloes with smooth ovaries and were treated with $GnRH^7$-PG-$GnRH^2$ (Ovsynch protocol) using burseraline acetate (@20 ug) and $PGF_2\alpha$ (@0.75mg). In Group 1 animals after anthelmentic treatment two animals reported in heat within 30 days and were inseminated separately. Out of the 22 Group 1 animals, 40.90% (9/22) reported in estrus within mean time interval of 49.68±3.23 h resulting in 55.5% (5/9) pregnancies. Progesterone (P4) assay of these animals revealed that 6 animals had a mean concentration of less than 1.0 ng/ml while others had concentration of 5.84±0.69 ng/ml of which the responder animals were having a concentration of 6.78±0.96 ng/ml. In Group 2 animals, the response was 46.66% (7/15) with a mean time interval of 56.75±3.96 h and 42.85% (n= 3/7) pregnancies. The progesterone assay results were 6 with less than 1.0 ng/ml while other had a mean concentration of 4.42±0.63 ng/ml of which the responders had a concentration of 5.18±0.48 ng/ml. In Group 3, the response was 66.66% (n=6/9) within a mean interval of 56.75±3.96 h resulting in 83.3% (5/6) pregnancies. The progesterone concentrations were, 2 animals with less than 1.0 ng/ml, while remaining had a mean concentration of 3.43±0.44 ng/ml and the responder animals having concentration of 3.46±0.62 ng/ml. Group 4 animals were inseminated at fixed time (12-16 h of last GnRH) resulting in 71.5% (5/7) pregnancies.

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11. Studies on induction of estrus in post partum Murrah buffaloes using different hormonal treatments

Satyendra Kumar Bhati and Atul Saxena

The study was designed with the objective of inducing estrus in post partum Murrah buffaloes using different hormonal combination. Thirty one parous buffaloes between 1287-4891 days of age were subjected to three different hormonal treatments by forming groups. Group 1 animals (n=6) received treatment with Crestar + eCG (Crestar ear implant inserted subcutaneous in the ear for 10 day and simultaneously an Intramuscular injection of norgestomate 3mg and estrogen 5 mg + 500 IU of eCG I/M on day 9, removal of implant on day

10 + GnRH injection @10ug on day 12, AI on induced estrus). Group 2 animals (n= 7) received treatment with Crestar + eCG + Ovsynch (Crestar and eCG as of Group 1 + ovsynch using two GnRH injection @ 10ug on day 3 and day 12 and $PGF_{2}\alpha$ injection @0.75mg on day 10). Group 3 animals (n=6 cyclic group of ovsynch) received ovsynch treatment with same dose of GnRH and $PGF_{2}\alpha$ as in Group 2 following day 0, 7 and 9 protocol). Group 4 animals (n=7 acyclic group of ovsynch) received treatment as of Group 3. Group 5 animals (n=5) were kept as control group. The treatment resulted in 50% (3/6) pregnancies in Group 1, 71.42% (5/7) in Group 2, nil (0/6) in Group 3, 14.29% (1/7) in Group 4 and nil (0/5) in Group 5 (control group). The concentration of hormone progesterone and estrogen evaluated at different stage between treatment and control group did not differ significantly except for Group 2 where it differed significantly.

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12. Studies on collection and grading of cumulus oocyte complexes from buffalo slaughterhouse ovaries and their culture *in vitro*

Vinod Verma and Atul Saxena

The study was designed with the objective of comparing the oocyte recovery rate retrieved from slaughter house buffalo ovaries employing different techniques and their maturation and fertilization in different culture conditions. Forty ovaries collected from non-pregnant slaughtered buffaloes were subjected to five different techniques namely (1) aspiration (2) aspiration + slicing (3) puncturing (4) puncturing + slicing (5) slicing alone for the purpose of oocyte retrieval. The average oocyte recovery per ovary for these employed method methods were 2.28, 7.73, 2.30, 5.83, and 5.15 which differed significantly. Although maximum oocyte recovered was from apiration + slicing technique, however, the culturable oocyte (grade A and B) were more from the technique of puncturing. Condition like pregnancy/ non-pregnancy, presence of CL/ absence of CL and Right ovary/ left ovary do not have any effect with regard to recovery of oocytes within a technique. Upon culturing the oocyte for maturation in five different medium viz (1) TCM-199 + 10% FBS + FSH + LH + estrogen (2) TCM-199 + 20% BFF + EGF + Cystein (3) TCM-199 + 20% FCS (4) TCM-199 + 10% FCS + FSH + LH + EGF + cystein (5) TCM-199 + 20% EBS, the respective maturation rate were 88.4%, 84.4%, 85.2%, 87.8% and 89.1% with no significant difference observed with different medium. The developmental competency of matured and fertilized oocyte in medium 1 and 2 were 42.9% and 54.4% up to the 8 celled stage with only 6.3% and 3.5% oocytes reached up to morulla stage.

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13. Studies on performance of Murrah bulls following cryopreservation of their semen and effect of post-thaw incubation of semen on certain seminal attributes

Adesh Kumar and Atul Saxena

The study was aimed with the objective of successful cryopreservation of Murrah bull semen and to observe the effect of post-thaw incubation of semen on certain seminal attributes. Semen was collected from three Murrah bulls in the age group of 3-6 years, biweekly adopting standard procedure of AV method. The various seminal attributes in the fresh semen viz semen volume (ml), mass motility (0-5 scale), sperm concentration (millions/ml), live spermatozoa (%) were in the range of 3.09±0.17 to 1.92±0.07, 3.72±0.10 to 3.34±0.13, 979.20±13.33 to 723.88±2.1.27, 75.36±0.75 to 75.01±1.19. Except for percent live spermatozoa all other parameter differed significantly. For HOS positive test, 150 mOsm solutions was found best compared to 100 mOsm and 75 mOsm. At 150 mOsm the HOS positive spermatozoa ranges between 63.99±1.84 to 60.51±1.87 percent amongst the three bulls. Semen was cryopreserved under the liquid nitrogen vapors and thawed at 37°C for 40 seconds. The thawed semen was than evaluated for different

seminal attributes at 0, 15, 30, 45 and 60 minutes. Immediately after thawing (0 mts) the live sperm percent ranges between 51.08±4.8 to 51.91±1.34 with overall drop (0-60mts) ranges between 36.80±1.8 to 38.20±1.16 percent. The percentage of progressively motile spermatozoa ranges between 55.63±1.10 to 58.13±1.43 with overall drop (0-60 mts) ranges between 37.50±1.68 to 38.62±1.51. The HOS positive sperm ranges between 37.24±1.25 to 38.29±0.89 with overall drop (0-60mts) ranges between 17.50±0.93 to 20.23±1.07 percent.

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14. Studies on comparison of different doses of gonadotrophin releasing hormone (Busereline) on the fertility status of post partum anestrus buffaloes employing ovsynch protocol

Ravinder Kumar and Atul Saxena

The study was aimed with the objective of comparing the two doses of buseraline (GnRH) for the treatment of post partum anestrus in buffaloes using ovsynch protocol. Seventeen parous buffaloes with the history of post partum anestrus were divided into two groups. Group I (n=10) animals received ovsynch treatment as per the standard protocol i.e. first dose Gn-RH (day 0) followed by $PGF_{2}\alpha$ (day 7) followed by second dose of GnRH (day 9) using the dose of GnRH @ 20 ug. Group II (n=7) animals received the same schedule as of Group I except that the dose of GnRH was reduced to half i.e @ 10 ug. PGF2α was used as two different salts ie Tiaprost @ 0.75mg/ animal and cloprostenol @ 500 ug. All treated animals received two insemination dose consisting of 40 millions spermatozoa one at 18 hr of last GnRH injection and second 15-18 hr of the first AI. The treatment resulted in nil pregnancy in Group I and 42.86% (3/7) pregnancy in Group II. The average dominant follicle size in both the groups did not differ significantly (9.98 mm vs 9.99 mm). Progesterone concentration differed significantly between the two groups on day 7 and 9 and was higher in Group I animals. The BUN level in Group 1 animals was significantly lower compare to the level of pregnant animals. The Ca level was found to be significantly lower in Group I animals compare to the pregnant animals. No difference was found with concentration of inorganic phosphorus.

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15. Studies on induction of estrus and fertility with controlled internal drug release (CIDR) device and other hormonal combination in buffalo heifers

Atul Kumar Verma and Atul Saxena

The study was aimed with the objective of inducing estrus and fertility in buffalo heifers using CIDR along with different hormonal combination. Forty four heifers were divided into three groups as G1 (n=14), G2 (n=16) and G3 (n=14). G1 animals received progesterone in the form of CIDR inserted intravaginally for 6 days. G2 animals received progesterone in the same manner as of G1, additionally injection GnRH (20ug) and PG (@ 500 ug) were administered at the time of CIDR insertion and following its removal respectively. G3 animals treated similar to G1 with addition of injection of estradiol valerate (estrogen @ 1 mg) administered 24 h after removal of implant. Following treatment all animals were teased and those in heat were inseminated with liquid semen (25-30 million sperms) once at the time of detected estrus and other 24 h latter. Treatment resulted in 42.85 (06/14) pregnancy in G1, 50.0% (8/16) in G2 and 42.85% (06/14) in G3 animals. Progesterone hormone concentration measured at different stages of the treatment and their comparison with pregnant and non-pregnant animals did not revealed any significant difference. However, the follicle size and rate of growth of follicle between pregnant and non-pregnant animals differed significantly.

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16. Studies on comparison of fertility in different ovulation synchronization protocol for fixed time insemination in anestrus cows

Brijesh Kumar and Atul Saxena

The study was aimed with the objective of inducing estrus and fertility in anestrus cows using Ovsynch protocol along with combination therapy with progesterone and Progesteron + Estrogen. Fifty three anestrus cows between 4-11 years of age were divided into three groups as G1 (n=17), G2 (n=18) and G3 (n=18). Group 1 animals received standard ovsynch protocol (GnRH-PG-GnRH) using GnRH as I/M injection @20 ug and PG as I/M injection @ 500 ug. Group 2 animals received ovsynch similar to G1 with addition of progesterone implant (CIDR) inserted for 7 days started with the first injection of ovsynch. Group 3 animals received treatment similar to G2 animals in addition received an injection of progesterone (100 mg) and Estrogen (1 mg) at the start of ovsych treatment and an injection of estrogen (1 mg) on day 8 of the treatment. Treatment resulted in 88.23% (15/17) pregnancy in G1, 55.55% (10/18) in G2 and 77.77% (14/18) in G3 animals. Progesterone hormone concentration measured at different stages of the treatment and their comparison with pregnant and non-pregnant animals did not revealed any significant difference. However, the follicle size and rate of growth of follicle between pregnant and non-pregnant animals differed significantly.

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17. Studies on induction of estrus and fertility in anestrus buffaloes using progesterone releasing intravaginal device (PRID) and other hormonal combination

Ram Shyam Singh and Atul Saxena

The study was aimed with the objective of inducing estrus in anestrus buffaloes using PRID along with other hormonal combination. Forty nine parous anestrus buffaloes were divided into three groups as G1 (n=16), G2 (n=16) and G3 (n=17). G1 animals received progesterone in the form of PRID (sponges containing 900 mg of progesterone supplied by CSWRI, Avikanagar via Jaipur) inserted intravaginally for 14 days. G2 animals received progesterone in the same manner as of G1, additionally injection GnRH (20ug) was administered a day prior to removal of PRID. G3 animals treated similar to G1 with addition of injection of PMSG (FSH @ 500 IU) administered on the day of removal of implant. Following treatment all animals were teased and those in heat were inseminated with liquid semen (25-30 million sperms) once at the time of detected estrus and other 24 h latter. Treatment resulted in 31.25% (05/16) pregnancy in G1, 62.50% (10/16) in G2 and 76.47% (13/17) in G3 animals. Progesterone hormone concentration measured at different stages of the treatment and their comparison with pregnant and non-pregnant animals did not revealed any significant difference. However, the follicle size and rate of growth of follicle between pregnant and non-pregnant animals differed significantly.

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18. Studies on cryopreservation of Hariana Bull semen

Arun Kumar and Atul Saxena

This experiment was designed to compare GEYT extender with GEYC extender for cryopreservation of Hariana bull semen. For this purpose, ejaculates were collected from four Hariana bulls using artificial vagina at biweekly interval. Semen ejaculates were diluted (80×10^6 motile spermatozoa ml^{-1}) in GEYT and GEYC extender. Diluted semen was equilibrated for 5h at 5°C, filled in straws at 5°C, kept in liquid nitrogen vapours for 10 min and then stored in the liquid nitrogen. Thawing was performed after 12 h of storage, at 37°C for 45 s. Progressive motility, live spermatozoa, abnormal spermatozoa, plasma membrane integrity and acrosomal

integrity were assessed at different stages of cryopreservation (Start of equilibration, End of equilibration and post-thawing). Amongst the two extenders used, GEYT was found to be better as it preserve the maximum seminal attributes consider for the study better than GEYC extender.

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19. Studies on effect of progesterone releasing intravaginal device and other hormonal combination on the reproductive performance of bovines

K. Sai Gunaranjan and Atul Saxena

The study was carried out to study the fertility rate, growth pattern of the largest follicle and hormonal relationship (progesterone) during the treatment in two estrus synchronization protocols. Two estrus synchronization protocols were carried out on 24 parous bovines. In the first trial 17 parous bovines (n = 17) including 7 parous cows and 8 parous buffaloes were subject to ovsynch + PRID protocol. In the second trial 9 parous cows (n = 9) were subject to EV+P_4+$PGF_{2\alpha}$ + PRID protocol. In trial I, 57.14% and 50.00% pregnancies were obtained respectively in parous cows and in parous buffaloes. No significant difference was observed in the follicle size, rate of growth of follicle and progesterone concentration between pregnant and non-pregnant animals in either cattle or buffaloes. In trial II, 33.33% pregnancies were obtained in the parous cows and all the parameters considered in trial I also did not differ significantly between pregnant and non-pregnant groups of animals.

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20. Studies on reproductive management of dairy heifers using progesterone releasing intravaginal device along with other hormonal combination

Brijesh Kumar Singh and Atul Saxena

The success of dairy heifer economic lies in ensuring proper and optimal reproductive rhythm with in physiological limits. The synchronization protocol PRID+PMSG+EV and PRID+EV were used in 28 dairy heifers (Experiment I, n =16, Experiment II, n = 12) in order to obtain pregnancies. In both the protocols we obtained 25% pregnancies. In experiment I, no significant differences were observed in the follicle size, rate of growth of follicle and progesterone concentration between pregnant and non-pregnant animals. Effect of PMSG has resulted in early ovulation. In experiment II, all the parameters considered in experiment I also did not differ significantly between pregnant and non-pregnant animals. However, in non-pregnant group, no significant difference could be observed between different stages, suggesting, and persistency of follicle.

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21. Studies on certain seminal attributes and effect of dilutors on cryopreservation of Hariana bull semen

Ashok Kumar Singh and Atul Saxena

This experiment was designed to compare GEYT extender with GEYC extender for cryopreservation of Hariana bull semen. For this purpose, ejaculates were collected from four Hariana bulls using artificial vagina at biweekly interval. Semen ejaculates were diluted (80×10^6 motile spermatozoa/ ml.) in GEYT and GEYC extender. Diluted semen was filled in straws, equilibrated for 5 hrs at 5°C kept in biological freezer for 7.25 minutes and then stored in liquid nitrogen. Thawing was performed after 24 hrs of storage, at 37°C for 45 seconds. Progressive motility, live spermatozoa, abnormal spermatozoa, plasma membrane integrity and acrosomal integrity were accessed at different stages of cryopreservation (start of equilibration,

end of equilibration and post-thawing). Amongst the two extenders used, GEYT was found to be better as it preserve the maximum seminal attributes consider for the study better than GEYC extender. To further evaluate the breeding performance of bulls In-vitro fertility test (IVF) was conducted by co-incubating fresh semen with zona-free-hamster ova penetration test. The penetration rate and penetration index were in the range of 48.41 to 78.34 and 0.94 to 2.13 per cent. In fertility trials where A.I. was performed with frozen thawed semen straws, the conception rate ranged from 70.83 to 100 per cent with service per conception in the range of 1.5to 2.82.

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22. Studies on semen characters of Bhadawari bulls and its freezability under semi-arid conditions

Alok Kumar Sahrawat and Atul Saxena

This experiment was designed to compare GEYT extender with GEYC extender for cryopreservation of Bhadawari bull semen. For this purpose, ejaculates were collected from four Bhadawari bulls using artificial vagina at biweekly interval. Semen ejaculates were diluted (80 x 10^6 motile spermatozoa/ml) in GEYT and GEYC extender. Diluted semen was filled in straws, equilibrated for 5 hrs at 4°C, kept in Biological freezer for 7.25 minutes and then stored in the liquid nitrogen. Thawing was performed after 24 hrs. of storage, at 37°C for 45 sec. Progressive motility, live spermatozoa, abnormal spermatozoa, plasma membrane integrity and acrosomal integrity were assessed at different stages(half dilution of semen, full dilution of semen, end of equilibration and post -thawing). Amongst the two extenders used, GEYT was found to be better as it preserves the maximum seminal attributes consider for the study better than GEYC extender. To further evaluate the breeding performance of bulls, In-Vitro fertility test (IVF) was conducted by co-incubating fresh semen with zona free oocytes of Golden hamster (Zona Free Hamster Ova Penetration Test). The penetration rate and penetration index were in the range of 62.83 to 96.67 per cent and 3.10 to 5.69. This test further verified the fertilizing ability and quality of individual bull semen.

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23. Studies on semen quality, freezability and fertility performance of Murrah bulls

Devendra Singh and Atul Saxena

This experiment was designed to compare GEYT extender with GEYC extender for cryopreservation of Murrah bull semen. For this purpose, ejaculates were collected from three Murrah bulls using artificial vagina at biweekly interval. Semen ejaculates were diluted (80 x 10^6 motile spermatozoa/ml) in GEYT and GEYC extender. Diluted semen was filled in straws, equilibrated for 5 hrs at 4°C, freezing was carried out in Biological freezer for 7.25 minutes and then stored in the liquid nitrogen. Thawing was performed after 24 hrs. of storage, at 37°C for 45 sec. Progressive motility, live spermatozoa, abnormal spermatozoa, plasma membrane integrity and acrosomal integrity were assessed at different stages(half dilution of semen, full dilution of semen, end of equilibration and post-thawing). Amongst the two extenders used, GEYT and GEYC there was no significant difference found. To further evaluate the breeding performance of bulls, In-Vitro fertility test (IVF) was conducted by co-incubating fresh semen with zona free oocytes of Golden hamster (Zona Free Hamster Ova Penetration Test). The penetration rate and penetration index were in the range of 90.62 to 100.00 per cent and 1.40 to 5.24. The conception rate was in range of 46.66 to 66.66. These tests further verify the fertilizing ability and quality of individual bull semen.

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24. Studies on effect of different concentrations of egg yolk and glycerol in tris based extender on cryopreservation of Bhadawari bull semen

Vipin Sonker and Atul Saxena

This experiment was designed to study the effect of different concentrations of egg yolk and glycerol along with effect of two different thawing protocols on cryopreservation of Bhadawari bull semen. For this purpose, ejaculates were collected from four Bhadawari bulls using artificial vagina at biweekly interval. Ejaculates which confirms the basic criteria for cryopresvation were split into 6 equal parts for dilution with GEYT having six different combinations of egg yolk and glycerols i.e. 5% glycerol with 12% egg yolk, 5% glycerol with 16% egg yolk, 5% glycerol with 20% egg yolk, 7% glycerol with 12% egg yolk, 7% glycerol with 16% egg yolk, 7% glycerol with 20% egg yolk in order to achieve a final dilution of 100 millions sperms/ ml. Diluted semen was filled in straws, equilibrated for 5 hrs at 5°C and were kept in biological freezer for vapour freezing completing in 7.25 minutes. The cryopreserved samples were then stored in liquid nitrogen. After 24 hr of storage in liquid nitrogen the straws were thawed at 37°C for 45 seconds and also at 50°C for 15 sec. Parallel straws were thawed at two different thawing protocol and 8 observations from each bulls (8x4=32) were evaluated for Live spermatozoa, progressive motility, plasma membrane integrity, acrosomal integrity and abnormal spermatozoa at different stages of cryopreservation (After dilution, after equilibration and after thawing). Amongst the three egg yolk concentrations, 20% egg yolk was found better in preserving the semen character at all stages of cryopreservation and thawing. Amongst two glycerol concentrations, 7% glycerol proved better than 5% glycerol in preserving all seminal characters up to thawing. Amongst two thawing protocols, 50°C for 15 sec showed better results than 37°C for 45 sec at post thaw. Thus best combination was 20% egg yolk and 7% glycerol with thawing of semen at 50°C for 15 sec.

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25. Studies on effect of different concentrations of egg yolk and glycerol in tris based extender on cryopreservation of Hariana bull semen

Vikas Sachan and Atul Saxena

This experiment was designed to study the effect of different concentrations of egg yolk and glycerol along with effect of two different thawing protocols on cryopreservation of Hariana bull semen. For this purpose, ejaculates were collected from four Hariana bulls using artificial vagina at biweekly interval. Semen ejaculates were splitted into 6 equal parts to do dilution with GEYT having six different combinations of egg yolk and glycerols i.e. 5% glycerol with 12% egg yolk, 5% glycerol with 16% egg yolk, 5% glycerol with 20% egg yolk, 7% glycerol with 12% egg yolk, 7% glycerol with 16% egg yolk, 7% glycerol with 20% egg yolk up to 100 million sperms/ml. Diluted semen was filled in straws, equilibrated for 5 hrs at 5°C kept in biological freezer for 7.25 minutes and then stored in liquid nitrogen. Thawing was performed after 24 hrs of storage, at 37°C for 45 seconds and also at 50°C for 15 sec live spermatozoa, progressive motility, plasma membrane integrity, acrosomal integrity and abnormal spermatozoa were evaluated at different stages of cryopreservation (After dilution, after equilibration and after thawing). Amongst the three egg yolk concentrations, 20% egg yolk and 16% egg yolk were found better as they preserve the maximum seminal attributes after thawing consider for the study better than 12% egg yolk with significant difference. Simultaneously amongst two glycerol concentrations, 7% glycerol proved better than 5% glycerol in preserving all seminal characters up to thawing. Amongst two thawing protocols used, 50°C for 15 sec showed better results regarding seminal characters than 37°C for 45 sec at post thaw.

Veterinary Medicine

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M.V.Sc.

1. Studies on some aspects of post parturient hameoglobinouria in dairy animals and their interaction with soil and feeds

Manoj Kumar Gupta and B.B. Pandey

The present study was performed to conduct the haematobiochemical, enzymatic analysis and clinico-therapeutic trials in postpartum haemoglobinuric dairy animals and analysis of soil and feed from disease prone area of Mathura and their neighbour areas. In PPH buffaloes and cows haematological profile revealed severe degree of anaemia and the mean values of TEC, Hb and PCV before therapy were significantly ($P<0.05$) decreased as compared to healthy control. In PPH buffaloes and cows the mean value of TLC, MCV, MCH and MCHC before therapy was significantly ($P<0.05$) high revealing the macrocytic normochromic anaemia associated with leucocytosis. In PPH buffaloes and cows, significant ($P<0.05$) fall in serum inorganic phosphorus was noted (1.84+0.05 mg/dl in buffaloes and 2.60+0.04 mg/dl in cows), indicated the severe degree of hypo phosphataemia. The mean concentration of total protein in PPH buffaloes and cows revealed the significant ($P<0.05$) rise. Mean concentration of albumin shows significant rise in buffaloes and non-significant rise in PPH cows. Serum creatinine concentration in PPH buffaloes and cow was significantly high (1.58+0t1 mg/dl in buffaloes and 1.48+0.07 mg/dl in cows). The level of serum bilirubin was significantly increased (7.04+0.08 mg/dl in buffaloes and 3.39+0.10 mg/dl in cows) in PPH dairy animals. The blood glucose concentration in PPH buffaloes and cows was significantly high (89.56+0.23 mg/dl in buffaloes and 81.26+0.23 mg/dl in cows). The concentration of serum urea was also raised in PPH buffaloes and cows (40.44±0.29 mg/d1 in buffaloes and 29.50+0.03 mg/d1 in cows). The high level of serum urea and serum creatinine is indicative of renal impairment. In PPH buffaloes and cows the level of alkaline phosphatase, was significantly raised showing the skeletal tissue damage. There was also significant rise in the ALT and AST in PPH buffaloes and cows revealed the damage to vital organs viz. heart, liver, kidneys, and erythrocytes. The therapy was based upon restoring the normal blood constituents. All of the clinical cases (25 buffaloes and 12 cows) were administered Tonoricin (sodium salt of 4-dimethyl amino 2- methylphenyl- phosphonic acid) as basic therapy and supportive therapy consist of 5% dextrose normal saline, vitamin B-complex and Iron-dextran complex. The mineral mixture was recommended to prevent re-occurrence of hypophosphataemia after clinical recovery.

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2. Studies on opisthochiasis in dogs with special reference to biochemical alteration and its zoonotic importance

Sanjay Jain and B.B. Pandey

During the period from November 2001 to March 2002 regular collection of 4 Ophiocephalid pond fishes viz., *Ophiocphalus punctatus, Cirrhina mrigala, Lobeo rohita* and *Mystus seen ghala* was made from various fresh water source of Mathura district. In all, a total of 1588 specimens of fish were collected and examined for the presence of matacercarial cysts. Of these, 530 (29.96%) fish were found positive or infected of larval trematodes. Village wise incidence detected was in Naujheel, 70 (30.04%) out of 233 specimens; in Dautana 9 (29.03%) out of 31 specimen; in Sakraya 88(32.59%) out of 270 specimen and: in other places 154(31.16%) out of 480 specimens, were found to carry matacercarial cysts. 8 healthy and 24 clinical cases naturally infected with opisthorchias s in dogs were found in *Opisthorchis* prone area (areas where fish was found positive for opisthorchiasis) were examined. Symptoms of anemia, jaundice, ascites and diarrhea with frequent passing of clay colored greasy feces were observed in infected dogs. In opisthorchiasis infection, a significant ($p<0.01$) drop in TEC, Hb, PCV, MCV, MCH, and MCHC

values was observed in the present study. Leucocytois with lymphopenia and eonsinophilia occurred in infected animals and values differed significantly in infected stage of parasitism when compared with the normal values. Macroscopic pathological changes indicated enlarged congested liver with prominent bile duct showing necrotic foci on both dorsal and ventral surface of the liver. Histopathological necrotic patches were seen irregularly distributed in liver parenchyma with fibrosis tissue proliferation of the wall of bile duct with the presence of parasites in the lumen and liver parenchyma. The therapy was based upon restoring the normal blood constituents. All of the clinical cases (4 male and 4 female dogs) were administered praziquantel (Prazital-Sarabhai) at the dose rate of 60 mg/kg b.wt. in three divided doses for one day orally as basic therapy and supportive therapy consist of 5% Dextrose normal saline, Vit. B-Complex (Belamyl-Sarabhai) and Iron preparation (Imferon-Rallis) and showed more progressive recovery.

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3. Studies on prophylactic efficacy of herbal anticoccidial "IHZYSS-2" against induced coccidiasis with special reference to Immunomodulatory effect of "ImmuPlus" and "Kamdhenu ark" in chicks

Nagendra Gupta and B.B. Pandey

Prophylactic efficacy of a polyherbar anticoccidial `IFIZYSS-2' at two doses level, given alone or in combination with immunomodulants 'ImmuPlus' and`Kamdhenu ark' were studied against *Eimeria* infection in broiler chicks. The anticoccidial IHZYSS-2 was given in feed @ 0.1% and 0.05%, respectively from 7th to 40[t1] day of life, whereas, the immunomodulants, ImmuPlus was given @ 7.5 g/1000 birds in water from 7[th] to 20[th] day of life and Kamdhenu ark @ 1 ml/bird in water from 7[th] to 40[th] day of life. Moreover, the synergistic effects of ImmuPlus -IFIZYSS-2 (0.05%) and Kamdhenu ark + IHZYSS-2 (0.05%) were also studied. The birds were infected with 50,000 sporulated oocysts of *Eimeria* on 21 day of life, and the efficacy of the drug was assessed from body weight gain, feed consumption, fecd conversion ratio, clinical recovery, faecal score, OPG count, lesion score, mortality rate, gross and histopathological changes, performance index, immunity index and restoration of haematobiochemical profile at the end of study (day 21 PI). The results were compared with uninfected-unmedicated (healthy control) birds and infected-unmedicated birds. Medication with different polyherbal compounds, Kamdhenu ark and their combinations resulted in better body weight gain in the absence of infection and the treated birds showed significantly higher body weight and body weight gain prior to infection compared to unmedicated birds. Significantly higher body weight and body weight gain was observed in infected-medicated birds in comparison to infected-unmedicated birds on the 6[th] week, i.e., after 21 days of infection. Although there was reduction in final body weight in infected-medicated birds compared to healthy control birds, this reduction was found to be non-significant in IHZYSS-2 (0.1%), ImmuPlus + IHZYSS-2 (0.05%) medicated birds, less significant (1°10.05) in ImmuPlus and Kamdhenu ark + IHZYSS-2 (0.05%) treated birds and more significant (10.01) in IHZYSS-2 (0.05%) and Kamdhenu ark medicated birds.

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4. Studies on some aspect of subclinical and clinical mastitis in cow and buffaloes with special reference to incidence, etiology, diagnosis and treatment

Man Pal Singh and H.P. Lal

The present work embodies the study of clinical bovine mastitis including incidence, etiology, diagnosis with hematological changes in blood, biochemical changes in milk and therapeutic

trials. On month wise basis the highest incidence of clinical bovine mastitis recorded in October (9.58%) and lowest in June (3.37%) whereas season wise highest incidence was recorded in rainy season (9.71%) followed by winter (8.03%) and lowest in the summer (5.19%). Lactation number wise incidence of mastitis was found' to be 37.50% in the first lactation followed by 29.47% in second lactation and the gradual decrease with increase number of lactations. On the basis of lactation stage maximum number of mastitic cases was found in second month of lactation (37.50%) followed 33.92% in first month of lactation and 13.33% in third month of lactation then decrease with time. The infectious organisms were isolated from 112 milk samples belong to 112 lactating bovines, positive for mastitis. Among the cultural isolates Staphylococci 11(9.82%) Streptococcus 8 (7.14%) *E. coli* 9 (8.03%) Klebsiella 13 (11.61%) Brucella 8 (7.14%) pseudomonas 8 (7.14%) Proteus (6.25%) Corynebacteriun 29 (25.89%) Enterococci 8 (7.14%) Bacillu spp (3.97%) mix infection 3 (2.6%) and Fungal infection 3(2.6%) The milk composition in mastitic and healthy lactating bovine, were compared. It revealed that pH, titrable acidity, Fat%, total solids % and SNF % were 6.84±0.032, 0.3741 +.0521, 4.68 + 0.326% 11.70 f 5621% and 7.024 +0.3612°6 respectively in subclinical mastitis, and 6.988 + 0.213, 0.3771 + 0.121, 4.604+0.3316% 11.3385 +0.563% and 6.7805 + 1316% respectively in clinical mastitis. However no significant changes were observed in milk composition of healthy and subclinical mastitic animals. Whereas, a significant change was observed for SNF (%), total solid (%) and pH during clinical mastitis.

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5. Studies on tickcidal properties of Eucalyptus Hybrida, Lantana Camara, Embelia ribis and Eucalyptus oil in animals with phyto-chemistry

Durga Ram and H.P. Lal

Eucalyptus hybrida leaves, *Lantana camara* leaves, *Embelia ribes* seed and eucalyptus oil selected for assessment of their acaricidal property by both *in vitro* and *in vivo* studies. In both studies crude extracts were prepared by soxhlet extraction using methanol solvent. Subsequently solvents fraction of crude extracts was also done by using different solvents. Three crude extracts and their fractions were tested at concentration of 250 mg/ml, 500 mg/ml and 1000 mg/ml for *in vitro* studies and at the 250 mg/ml were tested for *in vivo* studies. The Acaricidal efficacy of *Eucalyptus hybrida* leaves (methonolic extract) were evaluated under *in vitro* design. The maximum mortality percentage recorded at 24 hours in three different concentrations (250 mg/ml, 500 mg/ml and 1000 mg/ml) were 50%, 60% and 80% respectively, in crude extract where as 100% mortality observed in petroleum ether fraction and Ethyl acetate fraction at 24 hrs at 500 mg/ml and 1000 mg/ml concentrations. Rest of the fractions showed lesser mortality at 24 hrs. Efficacy assessment was based on LC_{50}which showed lowest for solvents ether fraction (56.10) and Ethyl methyl ketone (97.20). Activity improvement has also observed in solvent fraction by calculating AIF in relation to crude extract. The highest AIF calculated in solvent ether and ethyl acetate fraction (4.86, 4.44) at 24 hrs and 2 hr time intervals'. Drug efficient index was calculated in relation to standard drug Cypermethrin. Among four selected plants extract and oil, the maximum tickicidal property was found in *Eucalyptus* oil, followed by *Eucalyptus hybrida, Embelia ribes, Lantana camara.*

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6. Studies on acaricidal property of *Tagatus erecta*, *Butea frondosa* and *Melia azedarach* plants with their phyotochemical analysis

Umakant Singh and H.P. Lal

The acaricidal properties of *Tagetus erecta* (flower), *Butea frondosa* (seed), *Melia azedarach* (leaves and seed) were studied by using methonolic crude extract and subsequently with solvent

fractioned of all plant crude extract. The percent yield of crude extract of *Tagetus erecta* (flower), *Butea frondosa* (seed), *Melia azedarach* (leaves) and *Melia azedarach* (seed), was 36.08%, 22.63%, 35.63%, and 22.60%, respectively. Under *in vitro* study, methonolic crude extract of *Tagetus erecta* resulted maximum mortality of 20, 40 and 100% at 250, 500, and 1000 mg/ml concentration respectively at 24 hrs intervals. The methonolic crude extract of *Butea frondosa* (secd) revealed maximum mortality percent of 40, 50 and 70 at 250, 500, and 1000 mg/m1 concentration respectively at 24 hrs interval. Crude extract of *Melia azedarach* (leaves) showed highest mortality percent of 70, 70 and 80 at 250, 500 and 1000 mg/ml concentration at 24 hrs respectively. *Melia azedarach* (seed) methonolic crude extract revealed highest mortality percent of 70, 90 and 100 at 250, 500 and 1000 mg/m1 concentration respectively at 24 hrs interval. The chemical analysis showed the presence of mainly flavonoids, alkaloids, tannins and phenolic compounds in *Tagetus erecta* (flower) crude extract; alkaloids, glycosides, fixed oil and fats in *Butea frondosa* (seed) crude extract; alkaloids, glycosides, tannins and phenolic compound in *Melia azedarach* (leaves) crude extract and alkaloids, fixed oil *and* fats in *Melia azedarach* (seed).

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7. Epidemiological, haemato-biochemical and therapeutic studies on anchylostomiasis in dogs

Ashok Kumar Sharma and H.P. Lal

The study was undertaken to find out the prevalence of hookworm diseases in dogs. Fecal samples were collected from clinical cases coming to Kothari veterinary hospital and from dogs of college campus and hostels, which were either pets or stray dogs. Prevalence rate at different age groups, sex, different months, and different breeds was studied from 1st December 2004 to 30th April 2005. During this period 384 dogs were examined, out which 234 were found positive for Ancylostomiasis. The prevalence rate of Ancylostomiasis was determined on the basis of EPG. Comparisons were made between breeds, groups, sex, and different months. Hematological and Biochemical analysis was carried out in 21 pups during 21days, at different time interval in pre and post treatment periods. Four different Anthelmintic preparations were tried. Out of four, two were chemotherapeutic (Ivermectin-Intas, Pyrantel pamoate-Venky,s pet) and two were herbal drugs. The herbal preparations were seed.extracts from *Butea frondosa* and *Melia azadirachta*. In hookworm infection a significant ($p<0.01$) drop in Hb., PCV, and TEC was observed. Leukocytosis with Neutrophilia, Lymphopaenia and Eosinophillia occurred in infected pups in clinical stage of parasitism. Significant fall ($p<0.01$) in Total Serum Protein, Serum Albumin and PIG ratio was recorded. Serum alkaline phosphatase (SAP), Serum glutamic oxalo-acetic transminase (SGOT) and Serum glutamic pyruvic transminase (SGPT) were significantly higher in clinical case ($P<0.01$). Ivermectin showed highest efficacy (99.27%) followed by Butea frondosa (98.27%), Pyrantel pamoate (97.27%), and Melia azedarachta (86.98%).

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8. Clinico- therapeutic studies on dermatosis with a trial of "Dermanol" skin cream in Canine, caprine and bovine and a note on epidemiology of canine diseases with special reference to dermatosis

Satish Kumar Singh and H.P. Lal

Clinical diagnostic data of 11 years from 1995 to 2005 were critically examined, retrospectively for epidemiological study of canine morbidities including dermatoses. A total of 8674 cases were examined during the present study. The data was processed to determine the occurrence of disease in different age, sex, month, season, years and incidence of various diseases was also studied. The diagnosis taken up for the epidemiological study comprised of systemic canine

diseases and also different skin involvements in dogs. The study was conducted in Mathura, situated in the north western semi-arid region in India. Endemicity of various diseases remained as digestion disorders 25.0%, skin affections 19.6%, PUO 7.3%, endoparasitic infestations 6.9%, malnutrition 6.3%, respiratory system involvement 2. 0%, gynaecological disorders 1.4%, canine distemper 1.2%, nervous disorders 1.1%, opthalmological 0.7%, urinary system disorders 0.5% and rabies cases 0.3%. A higher incidence of these diseases was encountered during the summer and rainy season when the climate remains hostile due to heat and humidity in this region. Younger dogs between three to nine months of age were found more prone to ailments, although some diseases were more common in aged ones above 5 years of age. Canine dermatoses also occurred more frequently during the rainy season which could be diagnosed as dermatitis (pyoderma, nonspecific dermatitis and fungal dermatitis) 52.4%, acariasis 27.2%, otitis 6.4%, alopecia 5.8%, mange 2.8%, pruritus 2.3%, eczema 1.7% and allergy 1.4%.

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9. Studies on some aspect of clinical and sub-clinical mastitis in cow and buffaloes

Amarpal Singh and H.P. Lal

The present study was carried out on clinical and sub-clinical mastitis in cows and buffaloes, with the object to study the incidence, etiology, and diagnosis with hematological changes in blood, biochemical changes in milk and therapeutic trials. A total of 538 lactating animals from various dairy farms and Kothari Veterinary Hospital Mathura were included in the study. On month wise basis the higher incidence of clinical mastitis was recorded in month of January (11.24%), September (11.17%) and lower incidence in the month of May (6.41%) and June (7.0%). While season wise highest cases were recorded in rainy season 49.12% than winter 27% and only 23.32% in summer season. Species wise incidence of clinical mastitis was 7.84% and 13.05% in buffaloes and cows respectively. A total of 119 isolates were obtained from 108 milk samples belong to 108 lactating bovines positive for mastitis. Among the cultural isolates *Staphylococci* 62.18% (47.89% coagulase +ve and 14.28% coagulase -ve) and *Streptococci* 20.16% were found to be most prevalent organism causing Bovine mastitis. Besides these, the other isolated organisms were E. *coli* (7.56%), *Corynebacterium* (3.36%), *Klebsiella* (1.68%), *Bacillus spp.* and Fungi each (2.52%). *In vitro* antimicrobial sensitivity test revealed that bacterial isolates were highly sensitive to ciprofloxacin (91.37%), chloramphenicol (85.62%) and gentamicin (83.62%), moderately sensitive to amoxicillin (77.50%) and erythromycin, cephadroxil each (79.31%1, and slightly sensitive to cloxacillin and ampicillin. Most of the organism had got resistance against the streptomycin and penicillin. Animals were screened by three indirect tests i.e. Mastrip test, MCMT and MWST for the detection of clinical and sub-clinical mastitis. Among the four diagnostic tests, SCC was found to be the most efficient (100% for +ve cases), followed by MCMT (94.81%), Mastrip test (87.19%) and MWST (80.79%). In the treatment of clinical mastitis better results were obtained by the use of amoxicillin + cloxacillin combination by parenteral route along with intra- mammary infusion Mammitel with highest efficacy 85.71%, followed by amoxicillin + cloxacillin with 83.34/0 efficacy by parenteral and intra- mammary route simultaneously. Lower efficacy reported in case of clinico-therapeutic trial of antimicrobial agents by parenteral route alone without intra- mammary infusion, along with supportive therapy. So it is concluded that when a drug is used by parenteral and intra- mammary route simultaneously gives good results.

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10. Studies on some metabolic profile in buffaloes before and after parturition

Gyanendra Singh and H.P. Lal

The present study was undertaken at local dairy farms of Mathura. The study was carried out in 20 buffaloes, which were between second and fifth lactation and were between 8-13 years of age. In the previous lactation the average milk production of these buffaloes was 2500-3000 liters/lactation of 305 days. Blood samples were collected from these 20 buffaloes on 14th, 7th, 3rd, 2nd, and one day before expected date of parturition and on the day of parturition. Blood samples were also collected on 1st, 2nd, 3rd, 7th, 14th, 21st, 45th, 60th day post parturient. All these blood samples were for various serum metabolic tests such as calcium, phosphorus, glucose, cholesterol, total protein, albumin, A: G ratio and blood urea nitrogen.

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11. Evaluation of some anthelmenthic against GI parasite of sheep and goats

Vinay Kumar Sharma and H.P. Lal

The present study was undertaken with a view to find out the prevalence of gastro-intestinal parasitism in goat and sheep maintained at Department of Physiology unit, Veterinary College, Mathura and at Aurangabad goat farm, Mathura. These animals were kept on indoor feeding system as well as grazing. In all, 230 animals (200 goat and 30 sheep) were examined. For determination of prevalence of gastro-intestinal parasitism, the EPG values were taken as the basis. On the basis of EPG values, the animals were classified as pure and mixed infection cases. Comparison was made between species (Goat and Sheep) and breeds (Jamunapari and Barbari). Haematological and biochemical studies were carried out tin both the species, in sub clinical and clinical cases of infection, using standard techniques. Two anthelmintic preparations available in the market with the trade name-Fenbendazole (Panacur bolus) and Ivermectin (Neomec injection) were tried in clinically infected animals of both species and their efficacy against gastro-intestinal parasites was determined based on post-treatment EPG values and improvement in levels of altered haemato-biochemical profiles.

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12. Studies on calf diarrhea with special reference to hematology, biochemistry, microbiology and therapeutics

Sudheer Kumar and H.P. Lal

In the present study total 54 faecal samples were collected from diarrhoeic calves (below one month of age). During cultural examination of faecal samples from all the 54 diarrhoeic calves, 24 faecal samples were positive for *E. coli.* The prevalence of *E. coli* in diarrhoeic calves was 44.44%. These 24 isolates of *E. coli* were used for *in vitro* drug sensitivity test to seven commercially available antibiotics, and isolates were classified as sensitive, intermediate and resistant based on comparison of zone of inhibition. The antibiogram showed highest sensitivity to ciprofloxacin followed by amoxicillin and ampicillin, but highest resistance to oxytetracycline followed by streptomycin and Penicillin G. The 24 diarrhoeic calves which were positive for *E. coli* were divided into three groups (each group exhibit 8 calves), and given different therapeutic agent to each group. In this study 8 healthy calves were kept as control group. The efficacy of various therapeutic agents were observed by the restoration of various clinical and haemato-biochemical parameters towards normalcy. On the basis of restoration of these parameters towards normalcy, Pefloxacin showed faster recovery followed by Sulphadiazine- Trimethoprim and Amoxicillin.

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13. Studies on post parturient anorexia in buffaloes with special reference to hematology, biochemistry and therapeutics

Chandrabhan Singh and H.P. Lal

In the present study a total of forty buffaloes were selected out of which thirty were detected to be the cases of non- specific anorexia and remaining ten were apparently healthy animals, which served as control. Anorectic animals were selected on the basis of anorexia, its duration, type of feed given, feed pattern, environmental factors and status of the milk yield. The anorectic buffaloes after their complete examination were assigned two treatments, viz. rumbion and appetonic to ameliorate the anorexia in the animal with possibility of metabolic disorders. The studies were performed in apparently healthy, anorectic and after treatment of the anorectic with regard to the changes in their rumen fluid, blood and milk. The clinical parameters, viz. rectal temperature, pulse and respiration rate did not vary among the different groups of buffaloes indicating that these animals were free from the infectious disease. The individual case history from the owners showed that change in feed and feeding poor quality feed were related to digestive disturbances in these anorectic animals. Variations were found in colour, odour and consistency of rumen fluid in clinical cases of anorexia in comparison to normal buffaloes. Protozoa motility was found to be weak to dead protozoa in all the cases of anorexia. Anorexic buffaloes regained their ruminal protozoa motility to the level of vigorous motility after treatment with rumbion and appetonic. The rumen motility was found to be decreased ($P<0.01$) in all the cases of anorexia (1.9±0.233/5min.). Anorexic buffaloes, regained their rumen motility and normal characteristics of rumen fluid when treated with ru.mbion and appetonic separately due to re-set of the rumen ecosystem. The pH of rumen fluid was found to remain unchanged in different groups of animals. The 'SAT, MBRT and CDT increased significantly ($P<0.01$) in all anorectic buffaloes (16.1±1.882 min., 11.32±0.877 min. and 75.0±2.886 hrs., respectively) in comparison to apparently healthy animals (5.1±0.481 mm., 3.99±0.299min. and 35.3±0.5I8hrs., respectively) which returned back to normal level after treatment with rumbion and appetonic. Total volatile fatty acid concentration in rumen of anorectic buffaloes got significantly reduced (65.7±3.24 meq/L), in comparison to apparently healthy buffaloes (75.9±1.378 meq/L. The rumbion considered as having better effect on restoration of rumen ecosystem in comparison to appetonic so far as TVFA concentration is concerned.

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14. Evaluation of Anthelmenthic against anchylostomiasis in dogs

Virendra Kumar Bansal and H.P. Lal

The present study was undertaken with a view to find out the prevalence of ancylostomiasis in dogs. The faecal samples were collected from clinical cases referred to Kothari Veterinary Hospital Mathura and its adjoining areas. Prevalence rate at different age groups, sex, different months and different breeds was studied from December, 2007 to May, 2008. During this period, 256 dogs were examined. Out of which 155 were found positive. For the determination of prevalence of ancylostomiasis the EPG values were taken as the basis. Comparison was made between breeds, sex, age and different months. Haemato-biochemical and enzymatic analysis was carried out in a representative sample of 30 dogs during 21 days at weekly time interval in pre and post treatment periods. Three different anthelmintic were used for treatment. Out of three, two were synthetic (pyrantel pamoate and mebendazole) and one was herbal preparation from seed extract of *Embelia ribes*. These drugs were tried in infected dogs and efficacy was determined on pre and post treatment EPG values. Dogs were screened by direct coproscopy and quantitative examination was done by Mc-Master technique. Haemato-biochemical and enzymatic studies were carried out in dogs, using standard techniques.

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15. Studies on prevalence of *Toxocara canis* infestation in dogs with special reference to hematology, biochemistry and therapeutics

Manoj Kumar and H.P. Lal

The present study was undertaken to find out the prevalence of *Toxocara canis* in dogs in and around Mathura region, the effect on haemato-biochemical profile of clinically infested dogs and comparative efficacy of three anthelmintics (piperazine, ivermectin and levamisole) against disease. During the period from October, 2008 to May, 2009, 121 dogs were screened by direct coproscopy and quantitative examination was done by Mc-Master technique. Out of which 35 were found positive. Prevalence rate was determined on the basis of EPG values which were 28.92% (35/121), infection was found 29.23% in male dogs and 28.57% in female dogs. A high prevalence of *Toxocara canis* was found in non-descript breeds or local breeds (40.68%) than breeded dogs (17.74%). The highest prevalence was recorded in February (41.66%) and lowest prevalence was found in May (9.09%). Age wise prevalence was highest in the age group of 2-3 months (55.55%) and lowest in age group of 8-12 months (3.70%). During the study the most prevalent symptoms were distened abdomen (pot bellied appearance), stunted growth, intermittent diarrhea and noisy breathing. Observations were recorded on the effect of *Toxocara canis* in dogs on various haemato-biochemical parameters. A significant decrease ($p<0.05$) in values of Hb, PCV, TEC, lymphocyte, serum protein and serum glucose and a significant increase in the value of eosinophils, neutrophils, total leucocytes ($p<0.05$) and SGOT, SGOT ($p<0.01$) was observed. However, the results of monocytes were nonsignificant ($p>0.05$). Three anthelmintics- piperazine, ivermectin and levamisole showed a comparative efficacy of 99.89%, 99.01% and 94.56% respectively.

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16. Clinico-therapeutic studies on GI parasites of sheep and goats with special reference to Ivermectin therapy

Vijendra Singh and H.P. Lal

The present study was undertaken to find out the prevalence rate of gastro-intestinal parasitism in sheep and goats in and around Mathura region. Total 240 animals were taken for the present study (190 goat and 50 sheep). The prevalence rate was determined on the basis of EPG results. Subsequently animals were classified as pure and mixed infection cases. In gastro-intestinal parasite infection a significant drop in Hb, PCV and TEC count was observed in the present study which could be due to loss of blood in the gastro-intestinal tract due to active feeding habit of the developing stage of bursate worm. A leucocytosis with neutrophilia, lymphopoenia and eosinophilia occurred in infected animals and values differed significantly in subclinical and clinical stage of parasitism when compared with the normal values. In this study, anthelmintic drug Ivermectin (Neomec) was tested to determine its anthelmintic efficacy at two different doses. Ivermectin at the rate 200 mcg/kg body weight showed higher efficacy 97.38% in goat and 98.42% in sheep, where as in case of 100 mcg/kg body weight its efficacy reduced to 35.64% in goat and 37.95% in sheep.

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17. Studies on Clinico-therepeutic and diagnostic aspect of renal failure in dogs

Ramakant and H.P. Lal

The present study was undertaken for comparative evaluation of ultrasonography, haematology, serum electrolytes, serum biochemistry and urinalysis in renal failure dogs and apparently healthy dogs and to undertake therapeutic evaluation of ACE inhibitors on

management of hypertension in case of renal failure. The serum creatinine (>2 mg/dl) and BUN levels were used as parameters for diagnosis of renal failure in the present study. In present study 100 clinical cases were screened having history of anorexia, vomiting melaena, oliguria. Out of which 24 cases were found positive for renal failure. Ultrasonography was studied to detect morphological changes if any present in the kidneys of dogs suffering from renal failure dogs. Haemato-biochemical and urinalysis observations were recorded in case of renal failure and healthy dogs. Dogs with renal failure showed significantly lower Hb, PCV and TEC in comparison to healthy animals. The value of TLC, Eosinophil and Neutrophil of renal failure dogs were significantly higher in comparison to healthy animal. Lymphocyte values of renal failure dogs were significantly lower than healthy dogs. The values of basophil and monocyte did not differ significantly from healthy animals.Most important center of attention was significantly increased value of serum creatinine and BUN in comparison to healthy animals. The values of total protein and glucose did not differ significantly. Dogs with renal failure showed significantly lower serum sodium than healthy dogs whereas serum potassium values of renal failure dogs were significantly higher than healthy dogs. Urinalysis of dogs suffering renal failure revealed moderate to severe proteinurea, occasionally glucosuria, pyuria, and haematuria. Ketone, bilirubin, bilirubinogen and nitrate were not observed in dogs with renal disorder. Epithelial cells and casts were present in significantly higher concentration in the urine of dogs suffering from renal failure in comparison to healthy dogs. A non-significant difference was observed in urine pH and specific gravity of renal failure and healthy dogs. Arterial hypertension was managed with ACE inhibitors (captopril @ 0.5 to 2.0 mg/dl). After four days of treatment, animal showed significant reduction in hypertension. Serum creatinine level was measured after five days of treatment with captopril. The serum creatinine value increased non-significantly after the treatment.

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18. Studies on gastroenteritis, its therapeutic management with probiotics and role of campylobacter in dogs

Rajesh Kumar and H.P. Lal

The present study was undertaken on dogs exhibiting symptoms of gastroenteritis at teaching veterinary clinical complex. DUVASU, Mathura. During the period under study, 10 clinical cases were treated by the use of intravenous fluid therapy (DNS and Ringer lactate), Ceftriaxone @ 10mg/kg body weight, Botrapase @ 0.5-.1.0 ml (total dose), i/m metoclopramide @ 0.01-0.2 mg/kg body weight i/m, metoclopramide @0.01-0.2 mg/kg body weight i/m, Ranitidine @ 0.5 mg/kg body weight i/m, and Multivitamins infusion@ 1 ml/10 kg body weight i/m, while 10 clinical cases were treated with all the previous mentioned drugs along with probiotics (lactic acid bacillus 60 million spores) @ 120-240 million spores twice a day. All the dogs were physically examined and temperature, pulse rate, respiration rate were recorded along with clinical signs. Blood samples were collected before the treatment and after 3 days of treatment and processed immediately for hematological parameters viz. packed cell volume, haemoglobin, total erythrocyte count, total leukocyte count and differential leukocyte count as per standard techniques. During the present study, a total of 100 rectal swabs were collected from the dogs showing clinical signs of diarrhea with or without vomiting, presented to the teaching veterinary clinical complex, DUVASU, Mathura. The fecal samples were processed for isolation of *Camplylobacter spp.* and identified by colony characteristics, gram staining and biochemical tests. All the isolates were examined for their drug sensitivity pattern.

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19. Some studies on coli form diarrhea in cow and buffalo calves with special reference to antibiotic sensitivity

Subhash Malik and S.D. Sharma

The study was undertaken to find out the prevalence of *E. coli* diarrhea in cattle and buffalo calves in districts of north-western Uttar Pradesh; to compare the incidence rate of colibacillosis in cattle and buffalo calves and to study the haemato-biochemical alterations in positive cases of colibacillosis, isolation and identification of *E. coli* strains associated with calf diarrhea along with their antibiogram. Out of 930 cases recorded, 499 (53.66%) were of calf diarrhea. Out of these 930 cases, 572 (61.50%) were of buffalo calves, which differ significantly ($P<0.05$) in comparison to 358 (38.50%) cattle calves. Incidence rate of calf diarrhea in cattle calves (52.51%) was almost comparable to that in buffalo calves (54.37%). Hematological examination revealed significant increase in PCV, TLC, and neutrophils whereas biochemical examination revealed significant elevation in serum chloride level in diarrheic calves. Out of 109 faecal samples collected from diarrheic calves, 41 were found positive for *E. coli* on isolation. Out of these 41 isolates, 13 were found pathogenic based on hemolysin test. On the basis of ABST, Amikacin (87.80% sensitivity), Aztreonam (73.17% sensitivity) and gentamicin (51.21%) were found effective against the isolated enteropathogenic *E. coli*. Other drugs which were found to be less efficacious were Kanamycin (39.02%), Cefadroxil (19.51%) and Ciprofloxacin (15.63%). The isolates were completely resistant to Ampicillin, Cefdinir, Co trimoxazole, Cloxacillin, Erythromycin, Lincomycin, Norfloxacin, Peflocaxin, Penicillin, Rifampicin, Tetracycline and Vancomycin.

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20. Clinico-therapeutic study on mastitis in lactating bovines in Mathura region

Anurag Sharma and H.P. Lal

The work was carried out on clinical and sub-clinical mastitis in cows and buffaloes, with the object to study the prevalence, etiology and diagnosis with cultural examination, hematological changes in blood and therapeutic trials. A total 681 lactating animals of various organized, unorganized dairy farms and Kothari Veterinary Hospital Mathura were included in the study. In the present study a total of 212 isolates were obtained from the affected 205 cows and buffaloes. From the 212 isolates 128 (62.43%) were *Staphylococcus* spp, 45 (21.954%) were *streptococcus* spp, of which 31(15.12%) were hemolytic type and remaining 14(6.82%) were non-hemolytic type.18 (8.78%) *E. coli.* 10(4.87%) *corrtybacterium,* 4(1.95%) *Klebsiella* spp. 7(3.41%) *Bacillus spp.* In-vitro antimicrobial sensitivity test revealed that bacterial isolates were highly sensitive to enrofloxacin and gentamicin (96.69%) followed by ampicillin (80.18), Chloramphenicol (88.67%), lincomicin (76.41), ceftriaxone, oxytetracycline and penicillin (73.58). Maximum resistance was observed against colistin (53.77%). Comparative study of PCV, Hb and TEC values of mastitic animals with healthy animals revealed no significant difference, but statistically significant alterations were recorded in TLC, Neutrophils and lymphocyte counts between mastitic and healthy animals. In the treatment of mastitis better results were obtained by the use of Ceftak-SB (cefoperazone + sulbactum) @ 6.8mg/kg b.wt in combination with pendistrin-sh (Procaine Penicillin- llakh IU + Streptomycin Sulphate 100mg, Sulphamerazine-500mg,Hydrocortisone-29 Mg/Tube) with highest efficacy 83.33% in group A.lower efficacy reported in group B Ceftak-SB (cefoperazone + sulbactum) + Mammitel(Colistin Sulphate 5 Lac Iu, Cloxacillin Sodium 200 mg/ Syringe (8gm) and Group C Mamal (Erythromycin 300mg, Neomycin 250mg, Bacitracin Zinc 200 Iu/5gm Syringe) +Ceftak-SB (cefoperazone + sulbactum).

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21. Epidemiological and clinical investigation on canine dermatological disorders particularly bacterial pyoderma and its management with immunomodulation

Alok Kumar Chaudhary and H.P. Lal

Overall prevalence rate of canine dermatological disorder was 18.70% (282 cases) out of 1508 clinical cases of dogs. Prevalence rate of Pyoderma 23.04%; Demodectic mange 47(16.66%); Scabies 18(6.38%); Dermatomycosis 29 (10.28%); Allergic dermatitis 28 (9.92%); Ectoparasite infestation 39(13.82%); Mixed infection 52(18.43) and Hypothyroidism (1.47%) was recorded, Prevalence higher in summers season as compared to in rainy season, and winter and the highest prevalence rate was found for dermatological disorder in August 2010 and canine pyoderma respectively in April 2011. Canine dermatological disorders were more common among animal between one to three years of age. Canine dermatological disorders were more common in long hair breed German shepherd. Dominants isolated bacteria was *Staphylococcus spp.* (92.30%), others were *E. coli spp.; Pseudomonas spp. Proteus spp. Klebsella spp. Streptococcus spp.; Gram -ve coccobacillus spp.* In biochemical analysis 87% samples were positive for Staphylococcus *intermedius.* Insignificant difference was recorded in rectal temperature, pulse rate and respiratory rate, routine haematology before and after treatment in Pyoderma cases in dogs among all groups. Isolated strains of *Staphylococcus intermedius* were susceptible to Ampicillin-Clavulanic acid, Cephalexin, Rifampicin, Doxycycline Enrofloxacin, and Oxytetracycline no 100%; 96.15%; 94%86%;86%; 84.61%; and 0% respectively. Pyoderma treated with systemic cephalexin,topically shampoo with immunomodulator levamisole enhance the efficacy (83.33%) were recovered (15 days) while group treated with systemic cephalexin,topically shampoo only efficacy was less (66.6%) in 20 days of duration. Rifampicin, topically shampoo with immunomodulator Ranitidine (83.33%) were recovered (18 days) while group treated with systemic Rifampicin, topically shampoo without immunomodulator was (66.6%) was recovered in 30 days of duration. Systemic Amoxy-clav, topically shampoo with immunomodulator levamisole (100%) to short duration (18 days) while group treated with systemic Amoxy-clav,topically shampoo without immunomodulator was (66.6%) in 23 days of duration. Systemic Doxycycline, topically shampoo with immunomodulator Ranitidine (83.33%) duration (15 days) while group treated with systemic cephalexin, topically shampoo without immunomodulator was (50.0%) in 24days of duration. Systemic Enrofloxacin, topically shampoo with immunomodulator levamisole (83.33%) duration (18 days) while group treated with systemic Enrofloxacin, topically shampoo was (50.0%) in 28days of duration.

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Ph.D.

1. Studies on Some indigenous plant in bursate worm infection in goat

Niddhi Arora and S.D. Sharma

The present study was undertaken with a view to find out the prevalence rate of gastro-intestinal parasitism in sheep and goat under organized farms in semi arid zone. In all 329 animals formed the material for the present study (297 goat and 32 sheep). The prevalence rate of gastro-intestinal parasitism was determined on the basis of EPG results and the animals were classified as pure and mixed infection cases. Comparisons were made between species and breed to know the differences, if any. Hematological and biochemical studies in blood were carried out in both the species in subclinical and clinical stage of infection using standard techniques. Four anthelmintic preparations available in the market with the trade names, Fenbendazole (Fentas)*, Albendazole (Velbezan)*, Morantel tartrate (Banminth)* and Doramectin (Dectomax)* were tried in clinical infected animals and efficacy was determined based on post treatment EPG values and improvement in levels of altered hemato-biochemical profiles.

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2. Studies on status of ketosis in goats and sheep

Vinod Kumar Gupta and S.D. Sharma

The present study was conducted at Central Institute for Research on goats Makhdoom, Mathura, UP. A total of 514 goats (Barbari, Jamunapari breed) and 76 sheep (Muzzafarnagri breed) were screened in advance stage of pregnancy and early stage of lactation for the diagnosis of ketosis cases. No clinical case was found in either goats or sheep based on overt signs of ketosis, but subclinical ketotic cases were identified on the basis of hypoglycemia and ketonemia. The relationship of predisposing factors Viz. type of birth, kid's birth weight, dam's weight, age and breed, lactation number, feeding status, environmental stress etc. were studied. The prevalence rate of subclinical ketosis was found to be 9.32, 20.27 & 14.86% in Barbari, Jamunapari goats and sheep respectively, with overall prevalence rate 10.89%. Sheep were found more susceptible than goats and among the goats Jamunapari breed appeared more vulnerable as compared to Barbari goats. Overall 15.04% goats were found positive for sub-clinical ketosis in pregnancy whereas only 7.64% goats in lactation stage, while in sheep 16.22 & 13.51% detected positive for subclinical ketosis in pregnancy and lactation stages, respectively. The overall prevalence was higher in sheep as compared to goats in both the states of pregnancy & lactation.

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3. Studies on carrier stage of *Theileria annulata* in bovines with special to evaluation of chemotherapy and Immunotherapy

Nidhi Singh and H.P. Lal

In the present study, efficacy of different chemotherapeutic agents was ascertained in experimental calves infected with prevalent *Theileria annulata* strain. Drugs studied were Diminazine aceturate along with oxytetracycline, Buparvaquone, Chloroquine and vaccine Rakshavac-T. The result of the treatment trial in experimental calves indicated that the Buparvaquone was 100% effective under experimental conditions. The single dose therapy was found to be highly effective in eliminating the protozoan parasite from the blood with breakdown of subclinical stages of Theileriosis, whereas the vaccine Rakshavac-T also provided the full protection from *Theileria annulata*. The use of Berenil along with Oxytetracycline was also found beneficial in controlling the disease and animals also recovered but with subclinical state of *Theileria annulata* which needed repetition of the treatment for having negative subclinical stage of Theileria while chloroquine proved totally ineffective against *T. annulata* infection.

Veterinary Microbiology

SNo.	Titles	Name of Scholars	Name of Major Guide	Years	Page No.
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1.	Immunomodulatory role of prebiotic (*Saccharomyces cerevisae* 1026) and herbal performance enhancer in broilers	Dr. Amirul Islam Mallick	Dr. D.P. Singh	2003	129
2.	Studies on the occurrence of *Mycobacterium avium* subspecies paratuberculosis in goat kids with reference to serological and molecular diagnosis by ELISA and PCR	Dr. Pradeep Kumar	Dr. A.K. Bhatia	2004	129
3.	Development of an antigen capture ELISA for detection of bovine viral diarrhoea virus infection and genetic analysis of BVD viruses from sheep and goats	Dr. Rahul Dubey	Dr. C.M. Kulshreshtha	2005	130
4.	Studies on occurrence of mycobacterium subspecies paratuberculosis in bovine with reference to serological and molecular diagnosis	Dr. Pawan Kumar	Dr. A.K. Bhatia	2006	130
5.	Studies on the immunogenicity of three different immunizing motifs against enterotoxigenic *E. coli*	Dr. Anil Kumar	Dr. A.K. Bhatia	2006	131
6.	Studies on immunomodulatory competency of *Ocimum sanctum* with reference to gamma interferon expression at molecular level	Dr. Dilip Kumar Singh	Dr. A.K. Bhatia	2007	131
7.	Evaluation of immunogenecity of the outer membrane protein (omp) of *Pasteurella multocida* (b: 2) identified on TKE basis of multiplex PCR	Dr. Biswanath Chowdhury	Dr. A.K. Bhatia	2007	132
8.	Studies of virullence attributes *E. coli* isolated from diarrhoeic neonatal goat and sheep	Dr. Ramji	Dr. C.N. Kulshreshtha	2007	132
9.	Studies on bovine and human rota virus strains detected by RNA PAGE and ELISA	Dr. Sandeep Kumar Dash	Dr. A.K. Bhatia	2008	133
	Ph.D.				
1.	Studies on virulence attributes of *E. coli* causing diarrhea in neonatal calves	Dr. Archana Sharma	Dr. A.K. Bhatia	2004	134

M.V.Sc.

1. Immunomodulatory role of prebiotic (*Saccharomyces cerevisae* 1026) and herbal performance enhancer in broilers

Amirul Islam Mallick and D.P. Singh

In commercial broilers effect of probiotic (live culture of *Saccharomyces cerevisae* 1026) and polyherbal product (Zigbir) containing two herbs *Andrographs paniculata* and *P. amarus* was studied to see their effect on immunological aspects along with the traditional growth and production criteria. Also the virus used during the whole study was purified and concentrated. As alone vaccination is not sufficient to enhance maternal antibody level in chicks to the desired level. it was found that probiotic in lower dose produced significant increase in body weight with better feed conversion, but with higher dose of performance enhancer and probiotic fed to chicks lead to significant increase in weight of liver, spleen and bursa of fabricus indirectly suggesting better immune system activity. Dietary supplementation of probiotic and performance enhancer influenced significantly the total protein and globulin concentration of serum. The increased antibody titer to the chicks fed with different level of probiotic and performance enhancer was due to homogeneity of virus used and its molecular weight, it was confirmed by SDS-PAGE. The enhancing effect on humoral immune response was seen when in chicks fed with higher level of probiotic plus performance enhancer and also those fed with lower level of probiotic only by consistent and steady increase in HI and ELISA titer against RD virus. ELISA and HI titer were significantly correlated. Results also suggest that probiotic and Zigbir might have primed the lymphocytes and enhanced the cellular immunity of broilers.

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2. Studies on the occurrence of *Mycobacterium avium* subspecies paratuberculosis in goat kids with reference to serological and molecular diagnosis by ELISA and PCR

Pradeep Kumar and A.K. Bhatia

A total of 444 samples (50 faecal 60 tissue samples and 334 serum samples) were screened for studying the prevalence of juvenile, paratuberculosis infection in kids of varying age groups. The faecal and tissue sample were processed for the evidence of presence of MAP by direct microscopy and culture on HEY media containing mycobactin j, in conjunction with genomic analysis of 17 samples (8 culture suggestive of MAP on HEY medium and 9 decontaminated tissue collects) by using IS900PCR for confirming the presence of MAP bacilli. Serum samples were screened for detection of MAP antibodies by PLATE ELISA test using two antigens viz. S5 (bison type) and US (bovine type) reference antigen. In the faecal samples 19 faecal smears showed acid fast bacilli with ZN stain. The faecal sample inoculated on HEY medium 28 showed typical colonies like MAP and 16 in these showed acid fast bacilli. 19 decontaminated tissue smears showed acid fast bacilli with ZN staining. The tissue samples were inoculated on HEY slant and 35 were recovered as typical colonies of MAP. Among 179 serum samples in organized form were 58.6% were found seropositive for S5 antigen and only 2.7% for US antigen by ELISA. Among 155 serum samples collected from the farmers goat herds 35.4% were positive with S5 antigen and nil for US antigen. For genomic analysis PCR technique was conducted. 17 DNA samples were extracted for amplification using IS900PCR. Of these 17 DNA samples 11 samples could be amplified. The 229 bp PCR product was obtained from all the 11DNA samples and corresponding bands were obtained at the same position as in case of MAP (S5) isolate. The sample gave similar band pattern method with the standard DNA ladder (1kb). The sizes of band were compared to the molecular weight of the marker and were estimated to the band of approximately 250 bp.

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3. Development of an antigen capture ELISA for detection of bovine viral diarrhoea virus infection and genetic analysis of BVD viruses from sheep and goats

Rahul Dubey and C.M. Kulshreshtha

Blood samples from 42 sheep and 52 goats were tested for the presence of BVD antigens in leucocytes by employing antigen capture ELISA developed in the study and also by using commercial kit. 10 sheep and 8 goats were found positive for BVDV infection in both the test which showed the efficacy of the test developed in this study. These animals were found to be persistently infected as no antibodies were detected in the serum. However out of 57 animals 7 (6 sheep, 1 goat) were found positive for BVDV antibodies. The serum from the P.I. animals were passed in MDBK (madin darby bovine kidney) cells and BVD virus were isolated. These isolates were of ncp biotype. RT PCR was employed for detection of BVDV genome in leucocytes using primers specific for 5'UTR and Npro regions of the viral genome. All the 18 PACE positive animals generated 288 bp amplicons using 5'UTR primers, while 504 bp product of Npro region could be amplified from 11 animals out of 18 tested. The 5'UTR amplicons (14) were purified by Pst-1 and Ava1.Pst 1 site was found in 8 amplicons of 5'UTR which suggest the prevalence of BVDV 1 type, while Pst1 site was absent in rest of the 6 amplicons suggesting that they are different from BVDV 1 Ava 1 site was found in all amplicons of 5'UTR. 3 selected amplicons of 5'UTR and 4 selected amplicons of Npro were cloned into PGEM-T easy vector and the recombinant plasmid bearing inserts were sequenced and the sequences were assembled. Both 5'UTR and Npro sequences generated during this study were aligned with the reference pestivirus. The sequence pair distance analysis showed highest percent identity between three goats 5'UTR sequences and BDc413 and 890 strains of BVDV2. The phylogenetic analysis clustered these three Indian goat 5'UTR sequences with subgroup of BVDV2. The partial Npro sequences obtained from 3 sheep and 1 goat were also analyzed by aligning with reference pestivirus. The sequence pair distance analysis showed highest percent identity with Indian cattle isolates followed by CP7 which are subgenotypes of b of BVDV1.On the basis of deduced amino acid sequence alignment it showed that they are more similar to Indian cattle isolates sequences. To conclude an antigen culture ELISA was developed for detection of BVDV infection in blood and genetic characterization established the prevalence of BVDV1b in sheep and goats and BVDV2a in goats first time in India.

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4. Studies on occurrence of mycobacterium subspecies paratuberculosis in bovine with reference to serological and molecular diagnosis

Pawan Kumar and A.K. Bhatia

The aim was to diagnose the infected animal and estimated prevalence of paratuberculosis in bovines of Mathura region by direct microscopy culture method on HEY medium with mycobactin j, detection of antibodies against MAP by plate serum ELISA test and isolated culture confirmed by IS900PCR amplification for confirmatory diagnosis. A total 344 samples (120 fecal and 224 sera sample) were screened for studying the prevalence of JD infection. A total of 120 fecal samples were examined for direct microscopy and 37 fecal smears showed morphology and staining reactions of acid fast bacilli with ZN stain. 120 fecal samples were processed for isolation and identification of MAP by culture method and inoculated on HEY medium using three slants with mycobactin j and 1 without mycobactin j and were incubated for 18weeks at 37 degree centigrates.34 fecal samples yielded typical slow growing colonies of MAP. There was a strict dependency on mycobactin j of the typical colonies of MAP on HEY medium as there was no growth on HEY medium without mycobactin j. Primary colonies on HEY medium with

mycobactin j were very small, colourless, flat, translucent, hemispherical, with raised nipple and entire margin, the surfaces were smooth and glistening. In older cultures the colonies became more opaque, mucoid, with straw colour and increased in size. The plate serum ELISA was employed for the detection of antibodies against MAP in the 120 serum samples and 25 samples were seropositive for MAP. All the 34 culture positive samples were subjected to IS900 based PCR amplification. 29 were found genomically positive for MAP. All the positive sample gave similar band pattern with the standard DNA ladder (1kb). The size of band was compared to the molecular weight of marker and estimated to be band 229 bp. Out of 120 fecal and serum samples processed 10 were positive in all the 4 test whereas 22, 11 and 17 samples were common positive in direct microscopy and culture, direct microscopy and ELISA and culture and ELISA, respectively.

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5. Studies on the immunogenicity of three different immunizing motifs against enterotoxigenic *E. coli*

Anil Kumar and A.K. Bhatia

Six *E. coli* strains belonging to O9, O15, O26, O39, O101 O115 was chosen. The presence of k99 colonizing factor and heat labile enterotoxin producing ability and immunizing potential was studied in the experimental rabbit. Enterotoxoid and polyvalent bacterin in combination with enterotoxoid were prepared. To study the immunogenicity of these three immunizing motifs 9 pregnant buffaloes were vaccinated with either of the three vaccine twice subcutaneously at 20 days interval during last 1-2 month of gestation. Serum samples were tested for serum antibody titer by tube agglutination. After first doze of immunization the antibody titer ranges between 1:40 to 1:80 in first and second group while all the three buffaloes of polyvalent enterotoxoid group 1:80.20 days after second doze of vaccination antibody titer raised t 1:160 to 1:640 in first group,1:320 to 1:640 in second group and third group of buffaloes showed 1:640 to 1:1280. Serum titer of first and second group declined while of third group it was remain same as 20 days past second vaccination who. This study shows that formalinized adjuvant polyvalent *E. coli* bacterin with enterotoxoid gave much higher antibody titer which was maintained even at 0 days past second dose of immunization. Colostrums of third group which was vaccinated with polyvalent formanilized adjuvant bacterin contain highest colostral antibody titer and it was protective against diarrhea in neonatal calves.

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6. Studies on immunomodulatory competency of *Ocimum sanctum* with reference to gamma interferon expression at molecular level

Dilip Kumar Singh and A.K. Bhatia

For evaluating non-toxic dose of aqueous leaves extracts of *Ocimum sanctum* (100mg/250 mg/500mg/kg body wt) were fed to three different group of albino rat for 20 days. None of experiment showed sign of ill health and dose of 250 mg/kg body wt was taken as idea land safe dose for studding further biological activities. Haematological parameter PCV, Hb and TLC was increased and a significant rise in leucocyte by 14% was seen. There was increase in T, B lymphocytes and also total lymphocytes. Effect on immune response was studied by determining humoral and cell mediated immune response. There was increase in antibody titer in serum samples of treated rat in compression to control group rats indicated using indirect ELISA test cell mediated immune response was evaluated by skin hypersensitivity reaction using DNCB as antigen. There was significant decrease in skin thickness after 48 hr in control group in compression to *Ocimum sanctum* treated group thickness even after 72 hr. The effect of aqueous extract of *Ocimum sanctum* leaves on spleen cell proliferation and interferon gamma

mycobactin j were very small, colourless, flat, translucent, hemispherical, with raised nipple and entire margin, the surfaces were smooth and glistening. In older cultures the colonies became more opaque, mucoid, with straw colour and increased in size. The plate serum ELISA was employed for the detection of antibodies against MAP in the 120 serum samples and 25 samples were seropositive for MAP. All the 34 culture positive samples were subjected to IS900 based PCR amplification. 29 were found genomically positive for MAP. All the positive sample gave similar band pattern with the standard DNA ladder (1kb). The size of band was compared to the molecular weight of marker and estimated to be band 229 bp. Out of 120 fecal and serum samples processed 10 were positive in all the 4 test whereas 22, 11 and 17 samples were common positive in direct microscopy and culture, direct microscopy and ELISA and culture and ELISA, respectively.

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induction were further studied. For *in vitro* effect the result showed that there was no significant change at the conc. of 125 microgram/ml, 250 microgram/ml and 500 microgram/ml. *In vivo* effect on interferon gamma induction showed marked increase 5754±315 in comparison to control (3525±532). *In vitro* effect was also investigated using different concentration of *Ocimum sanctum* and there was not so remarkable production. Maximum induction of cytokine IFN gamma was observed at 250 microgram/ml. Detection of IFN gamma by mRNA expression using RTPCR confirmed that spleen cells harvested from *Ocimum sanctum* fed rats induced more IFN gamma on agarose gel electrophoresis 460bp indicative of IFN gamma was found denser in treated rats than control. There was much earlier healing in rats fed with *Ocimum sanctum* along with topical application of ointment.

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7. Evaluation of immunogenecity of the outer membrane protein (omp) of *Pasteurella multocida* (b:2) identified on TKE basis of multiplex PCR

Biswanath Chowdhury and A.K. Bhatia

Pasteurella multocida causative agent of haemorrhagic septicemia was isolated from the 10 suspected clinical blood samples of haemorrhagic septicemia (4 of cattle and 7 of buffaloes).Identified on the basis of morphological, staining and biochemical properties. Further confirmation was done by species specific polymerase chain reaction (PM-PCR) using primer pairs KMT1SP6-KMT1T7 and approximately 460 bp amplified product was obtained, thus all the isolated cultures were taken as *P. multocida*. For all these PM-PCR positive *P. multocida* cultures multiplex PCR was done using all primer sets A, B, D and F of *P. multocida*, it revealed that all the isolated *P. multocida* belonged to serogroup B. For studying the antigenecity of outer membrane proteins, a structural component of *P. multocida* OMP was extracted and was characterized by SDS-PAGE in which 9 polypeptide bands with molecular weights 25.6 to 88kDa were obtained, among which 25.6 kDa and 37kDa and 84.5kDa were major polypeptides. To identify antigenic protein of OMP western blot was done in which 5 immunogenic proteins were obtained on SDS-PAGE. Among these 5, 66.8kDa, 44kDa and 27kDa of proteins were major antigenic. To assess the efficacy of OMP as subunit vaccine three vaccines i.e. alum precipitated, whole cell oil adjuvant vaccine, alum precipitated whole cell vaccine and OMP oil adjuvant vaccine were prepared and studied in mouse model. Seromonitoring from '0' day to '35'Th day was done, booster was given 14th day post vaccination. It was found through indirect ELISA that the antibody titer in case of OMP oil adjuvant vaccine and alum precipitated whole cell vaccine were lower compared to whole cell oil adjuvant vaccine. The efficacy of these vaccines was investigated by challenging the vaccinated mice with *P. multocida* live cultures at dose rate 10LD50, 0.2 ml of 0.0001 dilution of 18h broth culture/mouse I/Pat 35th day post vaccination. Highest protection level was found in mice vaccinated with whole cell oil adjuvant vaccine. So, the conclusion is that *Pasteurella multocida* serotype B:2 is main causative agent of HS in this region and outer membrane proteins are effective vaccine candidate to control HS in animals.

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8. Studies of virulence attributes *E. coli* isolated from diarrhoeic neonatal goat and sheep

Ramji and C.N. Kulshreshtha

Study was carried out on the virulence attributes of *E. coli* isolates, from 51 diarrhoeic goat and sheep and 91 non-diarrhoeic goat and sheep apparently healthy from both species maintained at organized farms and under individual domestication. Goat and sheep faecal *E. coli*, 55 bacterial isolates distributed in 13 different 'o' serogroups. Out of 6 serogroups 2

serogroups showed hydrophobicity in ammonium sulphate solution. The hydrophobicity of *E. coli* strains could be linked with the presence of fimbrae on the surface of bacteria.6 *E. coli* serogroups were selected for the mannose sensitive and mannose resistance haemagglutination. Result showed that in absence of 3% D-mannose, only 3 strains haemagglutinated cattle erythrocytes, the strains haemagglutinated horse erythrocyte. A total of 6 serogroups selected for haemolysis pattern study of sheep blood agar (SBA). O158 and O163 produced typical haemolytic zone. While O1, O5, O103 and O146 produces no haemolysis. All the 6 *E. coli* strain O1, O5, O103, O146, O158 and O113 were positive for heat labile (LT) enterotoxin followed by dialization rabbit, neal loop technique. The protein concentration of dialyzed enterotoxins was determined by Lowry method using UV spectrophotometer. Further study of enterotoxin by SDS-PAGE showed that O1, O5, O103, O146, O158 and O113 showed the presence of protein bands migrated uniformly. Protein was of 40 and 20kDalton. Antibiogram study was done for serogroups of *E. coli* sensitivity to ciprofloxacin- 100% 6 strains and 4 strains for chloramphenicol, amoxicillin respectively. Polymerase chain Reaction was made an ideal choice for screening of the pathogenic *E. coli* strains.

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9. Studies on bovine and human rota virus strains detected by RNA PAGE and ELISA

Sandeep Kumar Dash and A.K. Bhatia

To detect Rotavirus in 67 calf faecal samples (diarrhoeic and non-diarrhoeic) and 100 faecal samples of children, RNA PAGE technique was used. After RNA extraction from all the faecal extracts, they were subjected to PAGE, in which samples yielding 11 segments were taken as positive for rotavirus strains. Out of 67 faecal samples 11 were showing reproducing 11 segments by PAGE, the 11 samples were all diarrhoeic and belonged to group rotaviruses. In positive samples majority were males, indicating the higher susceptibility of males for rota virus. In case of children faecal sample out of 100 samples 22 were rota virus positive and all belonged to group A. out of 22, 19 samples were of the children under 12 months of age, and further 14 samples were of males indicating the higher prevalence of rotavirus infection in males. Using MDBK and MA-104 cell lines, virus isolation was done from RNA-PAGE positive extracts. CPE was produced by 2 samples each of bovine and children. The virus was harvested by three cycles of freezing and thawing of cell culture material. A total of 33 rotavirus positive faecal samples based on RNA-PAGE and four cell culture lysate were confirmed by using sandwich ELISA kit. All the positive faecal samples of children were subjected to RT PCR assay for VP4 and VP7 gene. It was found that both long and short pattern of rotaviruses along with different subgroups are prevalent in these area.

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Ph.D.

1. Studies on virulence attributes of *E. coli* causing diarrhea in neonatal calves

Archana Sharma and A.K. Bhatia

The study was carried out on the virulence of *E. coli* isolates from 329 diarrhoeic cow and 71 buffalo calves aging between 1-90 days. Out of 70 *E. coli* strains showed hydrophobicity in ammonium sulphate solution. Hydrophobicity of *E. coli* can be linked with presence of fimbrae. Out of 75 faecal samples 30 were positive for the presence of presence of k99 antigen. 156 *E. coli* strains of 41 serogroups were examined for k99/88 antigen and percent positivity was 41 and 20. Sixty *E. coli* strains belonging to 32 serogroup, 59 and 46 showed MSHA and MRHA respectively. The surface fimbrae of pathogenic *E. coli* O99 0101 and O172 were examined by electron microscopy which confirmed the presence of fimbrae on the bovine ETEC isolates.116 *E. coli* dirrahoeic and nondiarrhoeic strains were evaluated for haemolytic character.11 *E. coli* strains showed complete haemolysis, 8 shows partial haemolysis and rest were negative.8 out of 41 *E. coli* strains were positive for heat labile enterotoxin and majority were positive for heat stable enterotoxin tested by rabbit ligated ileal loop assay. The VT2 primers directed a 478 bp in the region coding VT toxin was used for direction of verotoxin gene in *E. coli* isolates from bovine calves. Twelve *E. coli* strains of bovine faeces showing positivity in one or other virulence test. The phenotypic or genotypic characterization of individual strain of *E. coli* is time consuming, expensive, laborious.PCR for detection of genes would be an ideal choice for screening of pathogenic *E. coli* strains.

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Veterinary Parasitology

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1. Studies on some parasitic infections in different breeds of dog

M. Singhal and R.D. Agrawal

This study was undertaken with a view to find out the prevalence of parasitic infection in different clinical and subclinical cases of dogs in Aligarh district and surrounding areas from October, 2004 to September, 2005. A total of 888 dogs examined of which 616 dogs of eleven breeds were found positive for parasitic infections. The major parasitic diseases were ancylostomiosis, tick infestation and demodecosis and most of the cases were found infected with mixed infections. Sex wise study revealed that prevalence in males was higher (83.27%) as compared to the females (60.62%). Age-wise prevalence was highest in age group of 0-6 months followed by above 12 months and lowest in 6-12 months. Breed-wise study showed maximum infection in Rott wailer with 85.00% and minimum in nondescript dogs with 37.56% infection. The blood profile of the dogs infected with *Ancylostoma* spp. showed drop in Hb, PCV and TEC values. The range of TLC values was 18.63 to 21.68X10^3/uL, Neutrophils 61.16 to 64.33%, Lymphocytes 23.00 to 27.16 and Eosinophil 5.83 to 8.66. In chemotherapeutic studies ivermectin and wormstop were evaluated in naturally infected ancylostomiasis and both the drugs were found highly effective against the paraisite. A total of 413 (46.50%) dogs were found to carry for Rhipicephalus sanguineus. Maximum incidence was recorded in winter season and minimum in summer season. Maximum infestation was found in Non- descript and minimum in Doberman (30.37%) animals. Age wise prevalence of ticks in dogs was found maximum (36.46%) in 6 to 12 months of age group and minimum (28.22%) in 0 to 6 months of age group. In chemotherapeutic studies neomec (ivermectin 1% w/v) and cyprol 100EC (cypermethrin 100mg/ml) were evaluated. Neomec was found more effective than cyprol in controlling ticks in dogs. It also helped in improving the general health status of dogs as compared to untreated. Out of 888 dogs, examined, 203(22.86%) were found positive for Demodex canis. Maximum prevalence was recorded in rainy season (27.05%) and minimum in summer Season (17.67%). Prevalence was found maximum in Neopolitan Mastiff (100.00%), and minimum in Doberman (06.32%). Prevalence was more in female dogs (38.43%) than in male (16.02%). Efficacy of ivermectin 1% w/v and amitraz (12.5% w/v) was evaluated and no live mite was found 4 days after treatment with amitraz and 3 days after treatment with ivermectin.

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2. Studies on the effect of tick on haematology and body weight on cattle and buffalo calves

P.K. Singh and R.D. Agrawal

This study was undertaken to find out the effect of ticks on haematology and body weight in cattle and buffalo calves. Prevalence of ixodid ticks infestation among cattle and buffalo calves of either sex maintained at District Dairy Demonstration farm, Mathura and Damodarpur village was studied from March 2006 to August 2006. 86.5% of cattle and 79.2% of buffalo calves were found positive for tick infestation. Maximum prevalence was 97.0% in August and minimum 50.0% in March in cattle calves. In buffalo calves maximum and minimum prevalence was 93.3%, and 58.5% in August and March, respectively. Under haematological studies there was significant drop in haemoglobin, packed cell volume and total erythrocyte counts was observed. Leucocytosis with neutrophilia, lymphopoenia and eosinophilia was the consistent findings. Efficacy of ivermectin and deltamethrin was evaluated against natural infestation of Boophilus spp. and Hyalomma spp. in cattle and buffalo calves. Both ivermectin @ 0.2 mglkg body weight (s/c) and 0.0025% deltamethrin as body spray was found cent percent effective and safe against the infestation of Boophilus spp. and Hyalomma spp. The body weight of cattle and

buffalo calves after treatment revealed an increase of 32.18 kg and 36.86 kg after 6 months of treatment by ivermectin respectively. Average gain in body weight of treated cattle and buffalo calves was higher by 21.68 kg and 25.72 kg than that of control group, respectively. There is necessity of proper treatment of tick infestation with acaricide for ensuring better returns from livestock, regularly.

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3. Studies on coccidian infection of sheep and effect on body weight

Hari Om and R.D. Agrawal

In this study prevalence of coccidian infection in sheep was made, between the period from October, 2006 to February, 2007, through examination of 596 faecal samples, collected from Damodarpura and Aurangabad villages of district Mathura. Out of 596 faecal samples examined 345 (57.42%) were found positive for parasitic infections. Helminthic infections were found in 224 (38.31%)faecal samples and coccidian infection in 208 (34.77%). Highest coccidian infection was observed in October, 2006 (41.5%) and lowest in December 2006(29.40%). The rate of prevalence varied in different age groups. Maximum infection (42.57%) was detected in the animals below six months of age. In the positive cases, five eimerian species were identified and mixed infections with other species of Eimeria was encountered in most of the samples. The individual species prevalence was: Eimeria ovina (27.68%), E. parva (15.43%), E. ovinoidalis (11.07%), E. intricata (0.11%) and E. faurei (11.24%). In the sporogonic study at different incubation temperatures (25°C, 30°C, 37°C, 40°C and 43°C±0.50°C), the favorable temperature for all the five species ranged between 30°C and 37°C with 37°C as the most optimum temperature. An attempt has been made, to observe the effect of some physical (boiling water), and chemical (pheneol and formalin) agents on the viability of coccidian oocysts of sheep. Amongst them, boiling water and pheneol in concentration of 1% and 2% were found to be 100% lethal to coccidion oocysts but formalin has no lethal effect even at 5% strength. Effects of coccidian infections on the body weight of sheep in naturally infected adult sheep were studied. There was gain in the body weight of treated sheep than that of sheep in the control group with an average increase of 1.21 Kg. Average gain in body weight of treated sheep was higher by 1.29 Kg. than that of untreated sheep. There is necessity of proper control and treatment of coccidiosis with anticoccidial drugs for insuring better returns from sheep, regularly. Effects of coccidian infections on the body weight of naturally infected lambs with mixed infection of Eimeria spp. were studied. There was gain in the body weight of treated lambs than untreated control lambs. The average body weight of lamb after treatment revealed an increase of 0.80 Kg. The body weight in untreated lamb was -0.10 Kg. Average gain in body weight of treated lambs was higher by 0.90 Kg. than that of control (untreated) lambs.

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4. Studies on the levels of drug resistance amongst ticks against flumethrin, amitraz and ivermectin in cattle and buffaloes

Geeta Patel and Daya Shanker

Ticks are economically important ectoparasites of livestock. Epidemiological studies of ticks are essential to evolve a suitable strategic control program. Epidemiology of tick infestations was studied in cattle and buffaloes at various locations of Mathura district from July 2010 to June 2011 to know the prevalence of ticks in relation to months of year, seasons of the year, age of the host, species of ticks involved and site of their attachment. During the study period 3150 animals (2515 cattle and 635 buffaloes) were examined and found that overall prevalence of ticks in cattle and buffaloes was 58.41%, among these 60.07% (1511/2515) cattle and 51.81% (329/635) buffaloes were found positive for ixodid ticks. The present study revealed that the prevalence rate of ticks

is more in cattle (60.07%) as compared to buffaloes (51.81%). Maximum rate of prevalence was reported in month of September (cattle: 75%; buffaloes: 69.09%) and minimum in the month of January (cattle: 46.07%; Buffaloes: 37.74%). Maximum tick infestation was found in rainy season (cattle: 69.46%; buffaloes: 61.14%) followed by in summer season (Cattle: 62.55%; Buffaloes: 50.95%) and minimum in winter season (Cattle: 47.96%; Buffaloes: 43.46%). Overall maximum percentage of tick infestations was noticed in the young ones (Cattle calf: 80.21%; Buffaloes calf: 74.17%) followed by grownups (cattle: 68.48%; buffaloes: 60.93%) and minimum tick infestation was observed in adult cattle (Cattle: 44.85%; Buffaloes: 36.33%). Cattle were mostly infested with *Boophilus species* while buffaloes were mostly infested with *Hyalomma species*. On the basis of morphological studies, four species of ticks were identified namely *Boophilus microplus, Hyalomma anatolicum anatolicum, H. marginatum issaci* and *H. dromedarii*. Among these identified tick species, *H. marginatum issaci* and *H. dromedarii* were collected only from buffaloes. The most common feeding sites for adult ticks were neck, axilla, belly, groin, udder, perineal regions and tail. Present study focusing on the detection of resistance of *Boophilus microplus* ticks against three different acaricides was carried out in Mathura district. Laboratory tests were carried out on 14 days old larvae and fully engorged females *Boophilus microplus* ticks, to determine the levels of drug resistance against flumethrin, amitraz and ivermectin by use of larval packet test (LPT) and adult immersion test (AIT). Ticks collected from Adeeng, susceptible to all the three acaricides was designated as reference susceptible population. Concentration-mortality data were subjected to Probit analysis to generate lethal concentration (LC). Resistance Factor (RF) value of each tick sample was calculated by dividing its LC with that of acaricides susceptible strain. Results of LPT and AIT indicated that tick population collected from all target locations were found resistant against flumethrin with Resistance Factor (RF) value greater than17.00 for tick population of all the locations. LPT bioassays results revealed that tick populations collected from Jay Gurudev dairy farm, Balajipurum and DDD farm were tolerant (low level of resistance) (RF=3-5) and tick population of Damoderpura was found susceptible (RF<3) against amitraz. Results of AIT showed that tick population of all four locations under study was found susceptible against amitraz. The results of both test for ivermectin indicated that the tick population of all target locations were found susceptible. The comparative study of LPT and AIT for the detection of acaricide resistance showed that LC_{50} vales of the three drugs in different regions were found to be more by LPT as compared to the AIT for the same tick population.

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5. Studies on the level of resistance in gastrointestinal nematodes of sheep against different benzimidazole drugs

Pradeep Kumar and Daya Shanker

Present study was done to detect anthelmintic resistance in sheep against Benzimidazole group in four farms (Damodarpura, Balajipuram, Aurangabad and Madhurikund sheep farm). Faecal Egg Count Reduction Test (FECRT), Egg Hatch Test (EHT) and Larva Development Test (LDT) were conducted to detect anthelmintic resistance. The overall prevalence of GI nematodes was found 67.17% in these farms. Coproculture study showed maximum presence of *Haemonchus spp.* followed by *Trichostrongylus spp., Oesophagostomum spp.* and *Strongylus spp.*. Faecal egg count reduction test (*in vivo*) results revealed that parasites were resistant against fenbendazole in all studied farms. Albendazole was found resistant against GI nematodes in all sheep farm except Damodarpura sheep farm where it was found suspected to resistance. Oxfendazole was found susceptible against GI nematodes in all studied sheep farms. Egg hatch test (*in vitro*) results revealed that all sheep farms were resistant against benzimidazole with maximum resistance found in Madhurikund sheep farm with ED_{50} value 0.58 μg TBZ/ml (RF=11.2) followed by Aurangabad farm (0.50 μg TBZ/ml, RF=9.6), Balajipuram (0.48 μg TBZ/ml,

RF=9.3), and Damodarpura (0.39 µg TBZ/ml, RF=7.5) sheep farm. Larva Development Test (*in vitro*) showed that GI nematodes of all studied farms were resistant against benzimidazole. Maximum resistance found in Madhurikund farm ED_{50} value 0.55 µg TBZ/ml (RF= 12) followed by Aurangabad (0.47 µg TBZ/ml, RF=10.2), Balajipuram (0.45 µg TBZ/ml, RF=9.8) and Damodarpura (0.31 µg TBZ/ml, RF=6.8) sheep farm.

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6. Studies on anthelmintic activity of *Chenopodium album* and *Annona squamosa* against gastrointestinal nematodes in goat

Arti Sachan and Daya Shanker

The *in vitro* anthelmintic activity of methanol, ethyl acetate and chloroform extracts of *Annona squamosa* (seeds) and *Chenopodium album* (whole plant) against the GI nematodes in goats were evaluated via egg hatch test (EHT) and larval development test (LDT). Distilled water and albendazole were used as negative and positive control respectively for test. Percentage efficacy and ED_{50} value were evaluated by log probit analysis using SAS 9.2. Methanol, ethyl acetate and chloroform extracts of *A. squamosa* at 25 mg/ml and above concentration had 100% egg hatch inhibition. The ED_{50} values for methanol, ethyl acetate and chloroform extracts of *A. squamosa* were calculated 1.52, 2.48 and 3.02mg/ml. The ethyl acetate extract of *C. album* was found highly effective as inhibited 100% egg hatching at 25 mg/ml and above concentration. In methanol and chloroform extract of *C. album* at 100 mg/ml concentration 100% egg hatch inhibition was recorded. The ED_{50} values for ethyl acetate, methanol and chloroform extracts of *C. album* calculated for ovicidal activity and were found 2.73, 3.86 and 4.41mg/ml respectively. In LDT dose dependent larval development inhibition was reported. The methanolic extract of *A. squamosa* showed 100% larval development inhibition at 25 mg/ml and above concentration. Ethyl acetate extract was secondly effective showing 100% efficacy against larval development at 50 mg/ml concentration. The minimum larvicidal effect found in chloroform extract at 50 mg/ml concentration 96.4%. The ED_{50} values for methanol, ethyl acetate and chloroform extracts of *A. squamosa* were calculated 3, 3.17 and 3.39 mg/ml respectively. Results revealed ethyl acetate extract of *C. album* was most effective against larval development showing 100% larval development inhibition at 25, 50 and 100 mg/ml concentration. Methanol extract at 100 mg/ml concentration exhibited 98.2% and chloroform extract at 100 and 50 mg/ml concentration showed 100% larval development inhibition. The calculated ED_{50} values for ethyl acetate, methanol and chloroform extracts of *C. album* for larval development inhibition were 2.99, 3.87and 4.71mg/ml respectively.

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Veterinary Pathology

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M.V.Sc.

1. Pathobiology of Argemone cake toxicity in buffalo calves- an experimental study

R.K. Tripathi and A.K. Srivastava

The present study was undertaken to elucidate the pathobiology of argemone cake toxicity in buffalo calves. For this study 12 healthy male buffalo calves about 3 months of age were selected and divided into 3 groups comprising 4 animals in each. The animals were fed orally Argemone cacke @ 2gm/kg BW in group 1 and 500mg/kg in group 2 and group 3 served as control. The animals of both toxicity group showed almost similar clinical symptoms. Significant increase in resoiration and pulse rate and anorexia than lacrimation and incordination. Group 2 also showed diarrhoea and swelling of fetlock joint. Hematologically decrease in Hb, PCV, TEC, L, ALC and increase in ESR, MCV, MCHC and N. Lymphocytopenia was observed only in group 2. The serobiochemical profile of both group showed decrease in total protein, albumin, globulin and increase in creatinine as AST. ALT increase only in group 2. Decrease in the CMI and HMI in group 2. Pathomorphologically, liver appeared dark brown and small necrotic foci with enlarged gall bladder. At some places coagulative necrosis were also seen. Kindneys showed degeneration of glomeruli with tubules and desquamation in lining epithelium. Lungs revealed congestion with pneumonia and thickening of interalveolar septa with infiltration of MNC. Brain, liver, heart, spleen showed congestion and degenerative changes. Ultrastructural changes in liver of animals of group 2 were disruption of plasmalemma of hepatocytes with swollen mitochjondria.

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2. Copper toxicity in sheep-clinicopathological and pathomorphological studies

H. Kumar and A.K. Srivastava

The present study was undertaken to investigate clinicopathological, pathomorphological and immunopathological changes in sheep due to copper toxicity. For this study 12 sheep of either sex aged below 1 year were taken and randomly divided into 3 groups, comprising 4 animals in each. The animals were fed orally 1% soln. of $CuSO_4$ @ 80 mg/kg BW and 40 mg/kg BW in group 1 and 2 for 28 and 42 days resp. the group 3rd kept as control. All the animals of group 1 and 2 showed dullness, depression and pale anemic mucous membrane. Hematological observation showed dcrease in Hb, TEC, MCV, MCHC, L, N and PCV and increase in ESR, eosinophil in both groups. Serobiochemical profile showed decrease in total protein and globulin in group 1 and 2 and increase in A/G ratio, total bilirubin, amino transferas and serum urea. Immunopathological observations revealed decrease in CMI and HMI in group 2. PM lesions were almost similar in both toxicity groups as jaundice, congested abomassum, kidney, lungs and heart. Histopathologically liver showed centrilobular necrosis, fatty changes and infiltration of MNC. In kidney, glomerular degeneration, fatty changes in renal tubules. Lungs showed congestion and edema, thickening of alveolar septa. Heart showed congestion and vacuolization in myofibres. Brain showed congestion of BVs spongiosis in white matter and perineuronal edema. Spleen and lymph node showed depletion of lymphoid cells. Abomassum and intestine showed congestion with hemorrhage.

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3. Effects of cow urine on the health of rats

Amita Gupta and A.K. Srivastava

The present study was aimed to analyze different biochemical constituents of the cow's urine and to elucidate the usefulness as antimicrobial agent, effect on body weight gain, heamatological and biochemical profile, immunomodulatory agent and effect on healing of

surgical wounds in experimental rats. Antimicrobial activity of cow's urine (InVitro) against E-coli serotype 026 was assessed by different preparations of urine by disc diffusion method. The sensitivity was found lyophilized urine, protein precipitate of urine and whole urine in descending order. Antimicrobial activity of cow's urine against the same E-coli serotype was also studied following I/P administration of 1 ml broth culture containing $3x10^9$ CFU/ml. All the infected rats showed general clinical symptoms viz diarrhoea, dullness, anorexia and hyperpyrexia in early stage of infection with lateral recumbency in the terminal stages. The overt clinical symptoms and mortality were more severe in rats from control groups than the urine treated rats. Pathomorphological examination of experimental rats succumbed to infection revealed changes of acute non suppurative hepatitis characterized by centrilobular necrosis with infiltration of neutrofils and macrophages. Thickeneing of alveolar septa due to congestion of blood vessels with peribronchial lymphoid cuffing. Congestion myocardial blood vessels and at few places separation of myofibres with degeneration in heart, slightly swollen dark brown kidney showing congested corticomedullary junction with increased cellularity and vacuolization of glomerular loops along with tubular degeneration and thickened intestinal wall with congestion along with presence of cattarhal to heamorrhagic exudate. The above pathomprphlogical changes were more severe in control group rats. The effect on body weight showed highly significant weight gain in urine treated rats indicating anabolic effect of cow's urine. The hematological observation revealed highly significant increased in the value of Hb, PCV and TLC. Serobiochemical profile revealed significant increase in values of total protein, albumin, globulin and albumin globulin ratio in between the groups and in between the periods of experiments. The CMI and HI response was significantly enhanced in urine treated rats. The wound healing effects of cow's urine was better as assessed by macroscopical and histomorphochemical examination.

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4. Pathobiology of Spontaneous and experimental Para tuberculosis in goats with special reference to early lesions

S. Hajra and A.K. Srivastava

The present investigation was attempted to diagnose early cases of paratuberculosis by AFB in faecal and tissue samples, isolation, ELISA test and histopathological lesions in experimentally produced paratuberculosis. For this a total of 142 faecal smears were examined in which 52 showed the presence of typical AFB. On isolation 26 samples revealed presence of typical colonies of *M. avium subsps*. Out of 74 tissue samples isolation of colonies suggestive of AFB was seen in 27 cases with typical colony on Hey medium. A total of 17 animals were considered as positive on the basis of comparative analysis and evaluation of results of isolation from ICJ and MLN. In the experimental studies the observation of different parameters on 30 and 60 dpi were almost similar. Out of 4 sacrificed animals 3 were positive in direct microscopy and 1 case on hey medium. All 4 cases were positive in scraping smear examination and in 3 cases isolation of organism was found from the tissue. In pathomorphological and serological test ELISA all the animals were found negative for paratuberculosis. On 90 dpi, 3 animals were positive in direct microscopy of faecal examination and positive growth on inoculation on hey medium. Sacrificed animal on 90 dpi showed mild granulomatous changes characterized by thickening and folding of intestinal mucosa and in the terminal and ileo-caecal junction. Messentric and abdominal lymph nodes enlarged and edematous. Microscopically the intestinal villi sjowed disruption and desquamation of epithelial lining. Infiltration of large no. of lymphocytes plasma cell and macrophages were seen in lamina propria. The messentric lymphnodes showed infiltration of MNC in cortical areas. No clear acid fast bacilli were seen in macrophages of intestine and MLN. After 4 month of infection (120 dpi). All the 3 animals were

positive in faecal smear examination and 2 cases were isolated in hey medium. Pathomorphologically the lesions of kids sacrificed on 120 dpi showed thickening and corrugation of intestinal mucosa. Macroscopically there is infiltration of lymphocytes macrophages and epitheloid cells leading to distinct morphological changes in the villi and crypt regions. MLN were enlarged and thickened with presence of granuloma. All the 3 animals were positive by plate ELISA test.

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5. Fowl cholera in experimental birds in reference to pathogenesis, pathobiology, molecular diagnosis and vaccine strategy

Rajul Saxenaand and A.K. srivastava

The present study was undertaken to elucidate comperative pathology, pathogenesis of fowl cholera in chicken and quails, caused by *P. multocida* (A: 1) in different groups of the birds. The freeze-dried culture of *P. multocida* (A: 1) in lyophilized form was confirmed on the basis of morphology, biochemical characteristics and cultural characteristics. Passaging for 4 times in 8-week-old chicken and quails enhanced pathogenecity of the organism. The re-isolated organism was further tested for pathogenecity in order to produce the disease in mice, chicken and quails. The LD_{50} was found to be 1ml of 10^{-6} dilution of 18 hours BHI broth culture. The tested pathogenic cultures of *P. multocida* (A: 1) were further used in the entire experiment. In the present study 140 chicken divided in three groups comprising of 60 birds in group I (non immunized and infected), 60 birds in group II (immunized and infected) and 20 birds in group II (control). The different groups were assigned as subgroup IA IB, IIA IIB, IIIA IIIB. On clinical examination, most of the birds showed acute form of the disease with decreased feed and water consumption, pyrexia, depression, increased respiratory rate, foetid diarrhoea and urge of vomition with hock sitting posture along with mucus discharges from nostrils, conjunctivitis, dyspnoea, soiled vent and some degree of lameness. Whereas, cyanosis of comb and wattle accompanied with oedema, hock sitting posture, lameness and nervous manifestation in the form of torticolis were observed in the chicken of group I. The haemato-biochemical observations studied in birds showed decrease in Hb and MCH and increase TLC, PCV and ESR. Sero-biochemical studies revealed significant hypoproteinemia, decrease in serum albumin, increase in the level of globulins, elevated values of ALT, AST and alkaline phosphatase and decrease in ionic calcium in chicken. There was decrease in CMI and increase in HMI. Pathomorphological studies, in various organs viz. liver, lungs, heart, spleen, kidneys, intestine and brain were found to be severe in the birds of nonimmunized and infected group (Group I) as compared to immunized and infected group (Group II). The liver in chicken was firm and larger in size with distended gall bladder with few to numerous and small to large confluent about 1-2 cm diameter grayish white necrotic foci, generalized congestion in the sinusoids and central veins and varying degree of degenerative and necrotic changes in hepatocytes. The portal area revealed moderate to marked degree of cholengitis. Focal areas of regeneration of hepatocytes characterized by karyomegaly, cytomegaly, pleomorphism and mitotic figures were also recorded in the hepatocytes. Grossly, lungs of chicken (Group IIC) were moderately to severely congested, enlarged, heavy, firm, reddish brown with deposition of fibrinous exudates with presence of serofibrinous exudates in the distorted lumen of para-bronchi and inter bronchial septa with infiltration of large number of heterophils and monocytes. The heart of the chicken (Group I) showed hydropericardium, petechial haemorrhages on epicardium and adhered pericardium with the lesions of fibrinous pericarditis along with lesions of acute to subacute myocarditis. The spleen of chicken (Group I) revealed presence of grayish white necrotic spots with varying degree of degeneration to early necrotic changes in germinal centers of the malpighian corpuscles and hypocellularity in the white pulp and depletion of the lymphoid cells

in periarterial lymphatic sheath (PALS). The kidneys of chicken were congested and slightly enlarged with moderate to marked congestion in the region of cortex and medulla. The enlarged glomerular tufts occupying most of the spaces showed proliferation of endothelial cells, podocytes and mesangeal cells. The anterior segments of small intestine of the chicken (Group I) showed congestion and catarrhal to necrotic duodenitis, jejunitis and ileitis with severe degeneration and desquamation of lining epithelium forming naked villi in chicken. The brain of chicken revealed leptomeningeal congestion and softer consistency with degeneration of neurons and perivascular oedema with lesions of demyelination featured by presence of small to large empty spaces. The few birds of (Group I) showed only mild perineuronal and perivascular oedema along with lesions of demyelination.

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6. Fowl cholera in quail with reference to vaccine efficacy and molecular diagnosis

Vivek Srivastava and A.K. Srivastava

Present study was undertaken to develop and evaluate immunogenicity of low value fowl cholera vaccine in comparison with a commercial battering to detect the sensitivity of PCR technique for diagnosis of fowl cholera from stored tissue sample. The freeze dried culture of *P. multocida* (A:1) of quail origin in lyophilized form was procured from IVRI Bareilly. 2 groups comprising of 36 quails with 6 control birds. Both the vaccines 100% protection on 21day post challenge, on 45th day improved fowl cholera vaccine provided 100% protection whereas; commercial vaccine conferred 66% protection against challenge. On 60th day the protection reduced to 80.3% and 33.3% for commercial vaccine. The HMI measured by IHAT was higher in improved vaccine than commercial. The CMI was studied by DNCB showed similar response in both vaccines. On clinical examination most the birds showed acute to sub acute form of disease with decreased feed and water consumption, pyrexia, conjunctivitis, nasal discharge. Grossly liver showed enlargement congestion with necrotic foci and non suppurative hepatitis with infiltration of PMN. In lungs there will be pneumonia with serous exudates in the air vesicles. Depletion of lymphoid cells in the spleen, tubular degeneration and glomerulonephritis in kidneys. For diagnosis of fowl cholera by PCR showed 15 days storage of morbid material at -20^{o}C and in the 10% NBF to produce the satisfactory result.

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7. Studies on immunomodulating role of Gangateri cow urine in endosulfan induced toxicity in rats

Chandrakant Singh and A.K. Shrivastava

The present study was conducted on rats to study the effect of distilled cow urine of Gangateri cattle and endosulfan on various clinicopathological, immunological, pathomorphological and body weight gain.Observations were taken on 40 adult albino wister male rats. Distilled cow urine was given to the treat group @ 5 ml/ rat/ day. The effect of distilled cow urine and endosulfan on the performance of rats for various traits relating to body weight gain, clinicopathological, immunological, and pathomorphological aspects had shown significant results. For biochemical and immunological parameters, various tests were performed on blood serum. Blastogenic capacity of both B- and T-lymphocytes was evaluated in the presence of mitogens, LPS and ConA, respectively. The effect on body weight showed highly significant weight gain in the urine treated rats as compared to control rats indicated anabolic effect of cow urine. The haematological observations revealed highly significant increase in the values of Hb, PCV and TLC in the urine treated rats as compared to that of control group.Serobiochemical profile revealed highly significant increase in values of total protein, albumin, globulin and A/G

ratio in cow urine treated rats as compared to those of the control group.There was significant increase in B-lymphocyte blastogenesis in urine treated rats as compared to that of control group. Similarly the T-lymphocyte response was increased in cow urine treated as compared to that of the control rats, which indicated the enhancement of cell mediated immunity in rat.Histopathological changes comprised of lesions in various organs, being more predominant in kidneys, liver and testes. The most consistent lesion in the kidneys were proximal convoluted tubular degeneration with necrosis. Liver showed mild to moderate degenerative changes. Haemosiderosis was observed in spleen. Testes showed degenerative changes with presence of pinkish homogenous mass in intertubular connective tissue. Some of the tubules revealed loss of spermatogonial cells. Cow urine proved immunomodulating.

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8. Pathology, pathogenesis and molecular diagnosis of fowl cholera in turkey poults with special reference to vaccine strategy

Sachin Kumar Singh and A.K. Srivastava

The present study was undertaken to elucidate the pathology, pathogenesis, molecular diagnosis and vaccine strategy with special reference to development of a laboratory prepared improved fowl cholera vaccine.The *Pasteurellamultocida* (A: 1) of quail origin in lyophilized form was identified on the basis of morphology, biochemical characteristic and cultural characteristic of the organism. The isolated *Pasteurellamultocida* was further tested for pathogenicity in order to produce the disease in mice and turkey poults. The LD50 and mean death time was found to be 1 ml of 10-8 dilution of 18 hours broth culture and 25.88 hours, respectively. The pathogenic cultures of *P. multocida* were further used in the entire experiment.An improved fowl cholera vaccine was prepared by precipitating the antigen in broth culture with alum and was blended with liquid paraffin, vitamin E as immunoenhancer along with lanolin as emulsifier.The immunogenicity of laboratory prepared oil adjuvant vaccine supplemented with vitamin E was tested in comparison with commercially available vaccine in turkey poults. For this study a batch of 75 turkey poults aged 8 weeks was divided in three groups, comprising 25 poults in each, namely group I, II and IV. Turkey poults of group I and II inoculated with 0.5 ml of the laboratory prepared and commercial vaccine, respectively. The group IV poults were kept as control. The sera from all the three groups were subjected to serological test like SAT, AGPT and humoral immunity (IHA) and cellular immunity (2, 4-dinitro chloro benzene) at the intervals of 0, 7th,14th and 21st day post vaccination and 3rd, 5th and 10thpost challenge. All of these tests indicated increasing trends from 7th to 21st days post challenge. In the invasiveness study, the organism was found to be very virulent as all the birds showed septicaemia in twelve hours post inoculation.Variable concentration of the organism in the blood ranging from 102 to 108c.f.u. /ml was detected in different birds at various time intervals.On clinical examination, most of the birds of group III showed acute to sub acute form of disease depression, inappetance,hyperpnea,foetid diarrhoea along with mucus discharge from nostrils, conjunctivitis and soiled vent. Birds of vaccinated group (I and II) were dull and depressed with partial loss of appetite for 24 to 48 hours of post challenge and subsequently all the birds recovered.The birds died during the course of infection/sacrificed were subjected to necropsy for pathomorphological observations.There were enlargements of liver, kidney, spleen, fibrinousperihepatitis, necrotic enteritis, haemorrhages, congestion and coagulativenecrsosis in the liver, kidney and heart. Besides, there were hyperplasia in bursal follicles leptomeningial congestion in birds of group III.The Transmission Electron Microscopic study was carried out in liver and spleen of birds of group III (non vaccinated and infected). Ultrastructrally, the cells showed slightly fragmented and denuded microvilli at free surface of plasmalemma, presence of large number of glycogen appearing as electron dense granules in hepatocytes; scarce and

swollen of mitochondria; indentation and flocculation of heterochromatin on the inner nuclear membrane, eccentricnucleolus and large number of vacuoles in the cytoplasmkupffer cells.The use of PM-PCR and multiplex PCR assay directly from stored tissue samples was very simple and rapid and gave very good results at 15 days storage of the tissue samples.

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9. Experimental pasteurellosis in rats in reference to vaccine strategy and Immunomodulatory effects of residue of cow urine

V.K. Pandey and A.K. Srivastava

The present study was conducted in different group of rats to study the experimental pasteurellosis along with the immunomodulatory effect of residue of cow urine and efficacy of laboratory prepared vaccine on BW, clinicopathological, immunological and pathomorphological parameters. The vaccine was inoculated @ 0.2 ml/ rat IM in group 2 and booster dose @0.05ml/rat IM after 14 days interval and distillation of cow urine was fed to the rats @ 1gm/rat/day for 2 month. The rats of group 1(medicated), group 2 (vaccinated) and group 3 (-ve control). The effect on BW showed significant weight gain in rats treated with residue of cow urine as compared to group 2 and 3. Hematological examination revealed increase in value of HB, PCV and TEC in group 1 at the interval of 15, 30 45 and 60. The CMI response was evaluated by DNCB method which revealed increase in CMI in group 1 and 2 than group 3. The rats of medicated group showed 60% mortality but the rats of vaccinated recovered from the infection and did not show any mortality. The prominent gross lesions observed in group 4 (+ve control, non medicated, non vaccinated and infected). In this *P. multocida* @ 2ml/rats IP inoculated. These rats showed lobular pneumonia with enlarged and consolidated lungs. Lungs of group 1 showed congestion and mild consolidation and group 3 only congestion. Liver of group 4 showed white necrotic foci. Histopathologically there were marked changes observed in group 4 in all the organs as compared to group 1 and 2. There was severe infiltration of PMN, serous exudates and increase in thickening of alveolar swpta. Liver kidney and spleen showed severe congestion and degeneration of the cells.

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10. Arsenic poisoning in guinea pigs-a clinicopathological and pathomorphological study

Dinesh Kumar and A.K. Srivastava

The present experimental study was conducted to elucidate the clinical, clinicopathological, immunological, and pathomorphological changes along with quantitative assay of arsenic in blood, hair and vital organs of guinea pigs exposed to arsenic trioxide by oral gavages. For this study, 30 guinea pigs of either sex, aged 6-8 weeks were randomly divided into three groups comprising 10 animals in each. The animals of Group I was provided @ 10 mg/kg body weight once to produce oral acute toxicity. The guinea pigs of Group II were given @ 1 mg/kg body weight daily for a period of 90 days to produce chronic toxicity through oral gavages as 1% aqueous solution of arsenic trioxide. The animals of Group III were maintained on plain water to serve as control. All the animals of Group I showed abnormal clinical symptoms. The guinea pigs of Group I revealed 80% mortality within 7 days of post exposure where as no mortality was observed in the animals of Group II. There was significant decrease of hemoglobin, PCV and TEC in the guinea pigs of Group II. The TLC revealed significant leucopenia and DLC showed significant lymphocytopenia. Serobiochemical profile showed significant hypoproteinemia and hypoalbuminemia. The cell mediated immune response was significantly reduced in animals of Group II studied by percutaneous application of DNCB as there was decrease in the mean skin thickness. There was significant increase in superoxide dismutase and Catalase activities in

arsenic exposed animals (Group I & II) as compared to the control. Ninety days exposure @ 1 mg/kg body weight daily showed highly significant increase in arsenic concentration in blood, hair, liver, lungs and kidneys with the mean values of 57.186, 33.719, 311.969, 95.80 and 272.95 ppb in Group II as compared to the values of animals in Group III. Gross and histopathological lesions revealed congestion and degenerative changes.

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11. Experimental pasteurellosis in chickens with reference to immunomodulatory effect of *ocimum sanctum* and molecular diagnosis by PCR.

Krishna Kant Tripathi and A.K. Srivastava

The present experimental study was carried out in chickens to study the pathology and molecular diagnosis of fowl cholera with an attempt to ascertain the efficacy of *O. sanctum* in prophylaxis and control of the disease. For these study 90 chickens aged 4 weeks were divided into 3 groups comprising 35 birds in Group I, 35 in Group II and 20 in Group III. The birds of Group I was given aqueous cold extract of *O. sanctum* to orally @ 250mg/Kg b.wt/birds daily for 21 days and then challenged with 0.2 ml of 18 hrs BHI broth culture of *P. multocida* (A: 1). The birds of Group II were non medicated and challenged with 0.2 ml of 18 hrs BHI broth culture of *P. multocida* (A: 1). The birds of Group III were non medicated and non challenged which served as control. The leucocytic and protein profile showed highly significant increase. Intra-dermal application of DNCB revealed highly significant increase in skin thickness in birds of Group I. On clinical examination, some of the birds of Group II showed per acute form of disease. Birds of Group II revealed 100% mortality whereas, the medicated group showed 60% death. PCR assay using different template DNA preparation from liver and spleen tissue showed amplified bands of identical size. Thus, it was concluded that the procured freeze dried culture of *P. multocida* (A: 1) was highly pathogenic to the chicken, *O. sanctum* leaves can be used for the prophylaxis and control of the fowl cholera due to its immunomodulatory property and Fowl cholera diagnosis using PM-PCR and *P. multocida* (A: 1) specific PCR assay from stored tissue samples is very simple, rapid and specific and gave very good sensitivity.

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12. Pathology of lead toxicity in rats- an experimental study

Satish Chandra and A.K. Srivastava

The present study was carried out to elucidate the clinical manifestations and haematological, sero-biochemical and pathomorphological changes along with quantitative assay of lead in the blood, spleen, liver, kidneys and bone of the rats exposed to lead nitrate by oral gavage. For this study, 45 rats of Wistar strain aged about 6 weeks, either sex, weighing between 200-250 gms were divided into three groups comprising 5 animals in Group I (acute toxicity), 30 animals in Group II (chronic toxicity) and 10 animals in Group III (control). The animals of Group I were provided lead nitrate @ 600 mg/kg body weight, single dose to produce acute toxicity and the rats of Group II were administered lead nitrate @ 100 mg/kg body weight for 90 days to produce chronic toxicity by oral gavages as 10 % aqueous solution in double distilled water. The animals of Group III were maintained on plain water to serve as control. All the rats of Group I showed discomfort, weakness and ascending paralysis of hind legs followed by forelegs and death of all the rats within 7 days of experimentation. Similar clinical symptoms except the ascending paralysis of legs were produced by the rats of Group II on 50th days post administration of lead nitrate. There was no mortality observed in the rats of Group II, however, Hemoglobin, PCV, TEC and Erythrocyte indices in the rats of Group II were found to be significantly decreased. It also revealed leucopenia, lymphocytopenia, neutrophilia, hypoproteinemia, hypoalbuminemia

and Uremia. Bilirubin, glucose, cholesterol AST and ALT were also increased. It also revealed highly significant increase in lead concentration in blood, spleen, liver, kidneys and bone. There were lesions of degenerative changes and necrosis in all the visceral organs.

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13. Diclofenac sodium toxicity in quail: Clinicopathological and pathomorphological studies with special reference to renal dysfunction & egg quality

Sunil Singh and A.K. Srivastava

The present experimental work was conducted to elucidate the clinical, clinicopathological, immunological, and pathomorphological studies with special reference to renal dysfunction and egg quality in adult quails treated with Diclofenac sodium. For this study, 120 apparently healthy Japanese quails, of either sex, aged 4-weeks, having 150 to 200 gm body weight, were randomly divided into 2 groups comprising 80 quails in Group-I (Toxicity) and 40 in Group-II (Control). The quails of Group-I were administered per os with 0.5 mg/kg body weight Diclofenac sodium daily for 60 days. The birds of Group-II were maintained on plain water to serve as control. Japanese quails of Group-I revealed observable clinical signs viz- anorexia, emaciation, dehydration, depression, letharginess, feather plucking and swollen and painful joints after a month of daily oral administration of Diclofenac @ 0.5 mg/kg body weight. There was significant decrease of lymphocytes, protein, Hb, PCV and TEC while increase in MCV, heterophil, creatinine and urea. The cell mediated immune response was significantly reduced in birds of Group-I observed by epicutaneous application of DNCB as there was decrease in the mean skin thickness at the site of application on 24, 48 and 72 hours post challenge as compared to the birds of control group. Effect of Diclofenac sodium toxicity on the external and internal egg quality revealed no significant change in the egg quality except shell thickness and pigmentation on the shell of eggs laid by the birds of toxicity group. The eggs of Group-II (control) have multiple brownish black small to large spots which were irregularly distributed on shell surface. The eggs of Group-I (toxicity), the pigments which were present on the shell surface were light in colour and scarcely distributed. Gross and histological examination revealed deposition of crystals of urates over the visceral organs and joints.

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14. Diclofenac sodium toxicity in broilers with special reference to renal dysfunction & therapeutic effect of *boerhaavia diffusa*

Neeraj Singh and A.K. Srivastava

The present experimental work was conducted to investigate the clinical, clinicopathological, and pathomorphological studies with diclofenac sodium toxicity and therapeutic effect of *Boerhaavia diffusa* on renal dysfunction in broiler chicken. For this experiment, 48 broiler chicken aged 4-weeks, having 900 to 1000 gm body weight, were randomly divided into 3 groups. The boilers of Group-I administered orally @ 0.25 mg/kg body weight diclofenac sodium daily for 28 days and the birds of Group-II administered orally diclofenac sodium @ 0.25 mg/kg body weight with extract of *Boerhaavia diffusa* orally @ 125 mg/kg body weight daily for 28 days. The broilers of Group-III were given plain water and served as control. The broilers of Group-I revealed observable clinical signs viz- anorexia, emaciation, dehydration, depression, letharginess, feather plucking and swollen and painful joints on 20th day post medication. The birds of Group-II revealed similar clinical symptoms but of mild intensity. The clinicopathological studies included hematology and serobiochemical parameters carried out at various intervals in the birds of all groups. In the present study there was no significant change in the total leucocyte count in the birds of all the Groups. In the birds of Group-I, the differential leucocyte count

showed significant lymphocytopenia on 21st day and highly significant on 28th day of experiment with relative heterophilia indicated depletion of lymphoid reserve in the body. The birds of Group-I revealed significant hypercretinemia, uremia, hypoproteinemia and albuminemia. The birds of Group-II did not reveal significant increase in the level of above parameters. The mean values of ALT and AST in the toxicity groups of bird did not show any significant variation. The pathomorphological observation in the birds of Group-I showed lesions of visceral gout and articular gout affecting visceral organs and joints. Microscopically, degenerative changes in tubular epithelium, degeneration of glomeruli and incresed periglomerular spaces with or without interstitial nephritis in kidneys; cloudy swelling to periportal fatty changes, presence of focal necrotic areas with or without lesions of hepatits in liver; oedema formation in the parabronchi with or without lesions of pneumonia in lungs; thickening of pericardium with degenrative changes in underlying myofibres in heart; degenration and sesquamation of glandular epithelium with hyperplasia of parietal cells in proventriculus; depletion of keratinized layer and degenration and desquamation of glandular epithelium in gizzard; proliferation of endothelial cells in blood vessels and mild depletion of lymphoid tissue in spleen and perivascular and perineuronal oedem in cerebral cortex, depletion of purkinje cells with seperation of molecular and granular layers in cerebellum and proloferation of endothelial cells of choroid pluxes causing partial obstruction of vascular lumens in brain were observed. The birds of Group-II revealed similar lesions but of mild intensity in kidneys and liver. The other organs did not reveal significant gross and microscopic changes.

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15. *Datura stramonium* seed toxicity in rats- a clinicopathological and pathomorphological study

Santosh Kumar Verma and A.K. Srivastava

For this study, 63 rats of either sex, aged 4 weeks were randomly divided into three groups comprising 21 animals in each. The animals of group I provided distilled water and served as control, group II were provided *Datura stramonium* seed extract @ 1000 mg/ kg body weight and group III were administered *Datura stramonium* seed extract @ 2000 mg/ kg body weight orally for 90 days. All the rats of both groups showed reduced appetite, dilatation of pupils (mydriasis), increased thirst, restlessness, dry mucus membrane, tachycardia and hypothermia, dyspnea, dizziness, nervousness, muscular tremor. The absolute organ weight of liver, kidneys, lungs, spleen, heart and brain showed highly significant decrease in the rats of group-II and group-III at variable intervals. The relative weight of liver was decreased significantly in the rats of group-III on 90th day, kidneys and spleen in group-II and III on 30th post administration of *Datura stramonium* seed extract. Pathomorphologically, the size of the brain of both the toxicity groups (II and III) was comparatively smaller with congestion in meninges and choroid plexus, spongiosis, petechial hemorrhages, perineuronal and perivascular oedema, degenerative changes in neurons with chromatolysis, satellitosis and neuranophagia. In two cases in rats of group-III revealed presence of micro-abscess. The liver of rats of group-II and group-III were slightly enlarged and showing degenerative changes in the hepatocytes, dilated and congested sinusoids, focal area of necrosis. The kidneys of rats of group-II and group-III showed degenerative to necrotic changes in tubules and presence of eosinophilic finely granular excretions retained in the tubular lumens. The lungs of rats of group-II and group-III showed desquamation of bronchial and bronchiolar epithelium. There was a variable sized nodule in the lungs of rats of group-II and III at 90 days of intervals showing presence of large flat squamous pleomorphic cells with hyperchromatic nuclei arranged irregularly in the alveolar lumens, at places degeneration, indicated lesions of pneumocytocarcinoma. Mild to moderate vacuolization and degenerative changes in cardiac myofibres at various intervals were observed. The testes showed

congestion of scrotal plexus, excessive accumulation of watery fluid appearing as pink coloured homogeneous mass in the interstitial.

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16. Chlorpyrifos induced toxicity in broilers with ameliorative effect of selenium

Upendra Kumar and A.K. Srivastava

In the present study entitled "Chlorpyrifos induced toxicity in broilers with ameliorative effect of selenium" was investigated. For this purpose a total of fifty four chicks were randomly divided into three equal groups. The chlorpyrifos was given @ 3 mg/kg body weight daily by oral route in birds of group-II and group-III for 42 days. The feed of the birds of group III was supplemented with sodium selenite @ 0.25 ppm. The birds of the group-I were kept as control. At the intervals of 14, 28 and 42 days post feeding various parameters of study were carried out. Clinical signs of gasping, incordination in movement, stiffness, difficult breathing, muscle twitching, inability to stand, dull, depressed, dry oral mucous membrane with mucous like substances present in the oral cavity, ruffled feathers, reduced appetite, listlessness, diarrhoea etc. were observed after 14 days post feeding in the toxicity group but of milder intensity in the birds of group-III. The body weight gain of the birds of group II & III revealed significant reduction from third week to end of experimentation. The weight of lungs, heart, kidneys, brain and spleen was found to be significantly lower in group-II and group-III. Hematological observations revealed significant decrease in the values of Hb, PCV and TEC. The mean values of TLC revealed significant decrease at all intervals with neutrophilia and monocytosis with significant Lymphocytopenia in toxicity groups. The mean values of AST and ALT was significantly increased in between the groups and in between the intervals. The ALP revealed significant increase in the birds of group-II on day 42 as compared to control. The mean values of LPO depicted significant increase while the mean values of GSH revealed significant decrease and there was significant increase in the level of glucose, urea and creatinine and decrease in total protein level at different time intervals in toxicity groups. The values of various biochemical attributes were less severe and low frequency at different time intervals in birds of group III. Pathomorphological studies of the birds of group-II fed chlorpyrifos @ 3.0 mg/kg body weight showed degenerative to necrotic changes in liver; congestion, edema with or without focal consolidation, extravasation of erythrocytes in the lumen of air vesicles and parabronchi with or without infiltration of heterophils, lymphocytes, macrophages and occasionally giant cells in the lungs; mild to moderate congestion with degenerative changes in the tubular epithelium and endothelial cells of glomerular tufts with or without hypercellularity in kidneys; congestion, extravasation of erythrocytes and degenerative changes / necrosis of myofibres with or without infiltration of heterophils, lymphocytes and macrophages in heart; excessive glandular secretion in the secretary glands and degeneration, desquamation of epithelial lining the mucosa with or without inflammatory cells in proventriculous; mucinous degeneration and necrosis of the tip of the villi in intestine; perivascular and perineuronal edema, sattelitosis and spongiosis in brain; mild depletion of lymphoid cells in malpighian corpuscles of spleen and in follicles of bursa of Fabricius. Similar mild morbid lesions except pneumonia and myocarditis were also recorded in birds of group III administered sodium selenite @ 0.25 ppm in feed as compared to the birds of group II which suggested ameliorative effect of antioxidants on the parenchymatous organs and other body tissues.

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17. Cypermethrin induced toxicity in broilers and its amelioration with Vitamin E

Dharmendra Kumar and A.K. Srivastava

In the present study entitled "Pathology of cypermethrin in broilers with ameliorative effect of vitamin E" was investigated. For this purpose a total of fourty five chicks were randomly divided into three equal groups. The cypermethrin was given @ 800 mg/kg body weight in birds of group-II and group-III and vitamin E @ 150 mg/kg body weight in the birds of group III daily by oral route for 30 days. The birds of the group-I were kept as control. At the intervals of 10, 20 and 30 days post feeding various parameters of study were carried out. Clinical signs of reduced appetite, ruffled feathers, hyperexcitability, dullness and depression, gasping, open mouth breathing, nasal discharge, diarrhea, skin irritation and scratching, emaciation, pale comb, twitching of muscle, stiffness, difficult breathing, inability to stand were observed after 10 days post feeding in the toxicity group but of milder intensity in the birds of group-III. The body weight gain of the birds of group II & III revealed significant reduction from second week to end of experimentation. The weight of liver, lungs, spleen and bursa was found to be significantly lower in group-II. Hematological observations revealed significant decrease in the values of Hb, PCV, TEC and MCHC in group-II. The mean values of TLC revealed significant decrease at 10 and 30 days intervals with significant increase in heterophils and significant decrease in lymphocytes count in group-II. The mean values of AST and ALT was significantly increased in the toxicity groups as compared to control. The ALP revealed significant increase in the birds of group-II on day 20 as compared to control. There was significant increase in the level of creatinine, urea, total protein and glucose level at different time intervals in group-II. The values of various biochemical attributes were less severe and low frequency at different time intervals in birds of group-III. The mean values of SOD and GSH significantly decrease in toxicity groups and LPO and catalase significantly increase in group-II. Pathomorphological studies of the birds of group-II showed degenerative changes ranging from cellular swelling to vacuolization and focal areas of necrosis in hepatocytes along with congestion and lymphoid aggregation in portal areas in liver; presence of varaiable amount of light pink colour oedematous fluid in air vesicles and parabronchi and lymphoid aggregation around bronchi and sedimentation of erythrocytes in the portal vein in the lungs; hypercellularity of glomeruli due to proliferation of endothelial cells, infiltration of mononuclear cells occupying most of the glomerular spaces, degenerative changes in the lining epithelium of renal tubules, infiltration of mononuclear cells particularly lymphocytes in the peritubular spaces and extravasation of erythrocytes in the cortical areas in kidneys; subepicardial hemorrhages and degenerative changes in myofibres especially vacuolization with or without extravasation of erythrocytes and focal area of myocarditis characterized by degeneration and necrosis of myocardium with infiltration of polymorphonuclear cells and mononuclear cells in heart; degeneration and desquamation of epithelial lining of the mucosa with focal area of necrosis forming ulcers in proventriculous; degeneration and desquamation of villous epithelium forming naked villi with necrosis in intestine; central chromatolysis in neurons and perineuronal edema, extravasation of erythrocytes and infiltration of glial cells, proliferation of ependymal cells and separation of molecular and granular layer with depletion of purkinje cells in brain; mild depletion of lymphoid cell in malpighian corpuscles of spleen; degeneration and necrosis of lymphoid follicle in bursa of Fabricius and in the caecal tonsils, hyperplasia of goblet cells were observed. Similar mild morbid lesions except pneumonia and myocarditis were also recorded in birds of group III administered vitamin E @ 150 mg/kg body weight orally as compared to the birds of group II.

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18. Status of pathological lesions vis a vis diagnostic efficacy of different tests for *mycobacterium avium* subspecies *paratuberculosis* (map) infection in adult goats

Shivam Chaturvedi and A.K. Srivastava

Understanding pathogenesis during progressive stages of infection by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and finding suitable methods for its diagnosis is the key to the control of Johne's disease in animals. In this study, the pathological lesions and the diagnosis of Johne's disease in goats is investigated using histopathological (HP) examination, Acid Fast Staining (AFS) of tissue sections, immunofluorescent test, tissue PCR and culture. These tests were carried out on 74 paired tissue samples of intestine and mesenteric lymph nodes. The gross lesions were mainly observed at the terminal ileum especially at ileo-caecal valve and the mesenteric lymph nodes. Histopathological examination of routine stained tissue sections from the 74 ilea and corresponding lymph nodes revealed variable grade of lesions of JD in 37 (50%) ilea and 21(28.40%) mesenteric lymph nodes. In general the affected part of the intestine revealed degeneration and partial to complete denudation of lining epithelial cells forming naked villi. The villi exhibited variable changes that included dilated lacteals, villous distortion and thickening and fusion of villi. At places, the villi were shortened, thin and atrophied. Histologic lesions were classified into four grades from grade 1 (least severe) to grade 4 (most severe) on the basis of types and density of cellular infiltrate (lymphocytes, macrophages and epithelioid cells). In AFB staining, 16 (21.60%) and 10 (13.50%) out of 74 paired cases of intestine and MLN respectively, revealed presence of acid fast pink colour bacilli individually or in clusters in the epithelioid cells indistinguishable from *Mycobacterium avium paratuberculosis.* Thirteen (17.56 %) and 9 (12.16%) out of 74 paired tissue samples of intestine and MLN respectively were found positive in fluorescent antibody test. Eleven (14.86%) and 7 (9.40%) cases of intestine and mesenteric lymph nodes were found positive by PCR (IS900). Culture was positive in 7 (9.50%) and 8 (10.81%) cases out of 74 paired tissue samples of intestine and MLN respectively. In present study, sensitivity of histopathology (H&E staining) was found to be more than the any other test for diagnosis of MAP infection in goats. Sensitivity of ZN staining was 100% in comparison to PCR (69.23%). The strength of agreement the ZN staining and FAT was considered to be very good. Sensitivity of FAT was 90.91% in comparison to ZN staining (76.92%). The strength of agreement between the FAT and PCR was considered to be very good. Sensitivity of FAT was 100% in comparison to PCR (81.82%). Bacterial culture showed poor sensitivity. There was no significant difference between the sensitivity of ZN staining, FAT and PCR in grade II, III and grade IV lesions. In grade I lesions, histopathologic H&E staining based diagnosis was found to be more sensitive followed by ZN staining. Histopathology was found to be a better indicator of paratuberculosis infection in goat.

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19. Pathology of cadmium induced toxicity in rats with ameliorative effect of S. Adenosil methionine

Pratima Singh and A.K. Srivastava

For this purpose a total of 72 rats were randomly divided in to four groups comprising 18 rats in each. Cadmium chloride was given @ 200 mg/L in distilled water in rats of group-II and group-IV and S-adenosyl methionine @ 1 mg/kg body weight in distilled water in rats of group-III and group-IV daily by oral route for 90 days. The rats of group-I were kept as control. At the intervals of 30, 60 and 90 days post study of various parameters were carried out. The rats of group-II fed cadmium @ 200mg/L distilled water grossly revealed pale and occasional pinpoint hemorrhages on the dorsal surface of liver. Lungs revealed congested and edematous with grayish nodules. Kidney and testis showed mild congestion. Microscopically, liver showed cellular swelling to vacuolization and coagulative necrosis. Lungs revealed mild congestion of alveolar capillaries with extravasations of erythrocytes in the lumen, lymphoid aggregation in the alveolar septa and around bronchi and bronchioles with mononuclear cell infiltration. Necrotic changes in tubules, sub acute interstitial nephritis in kidney. Heart showed vacuolization and separation of cardiac myofibres and heamorrhages. Intestinal mucosa revealed hyperplasia of goblet cells, degeneration and necrosis of villous epithelium. Brain showed congestion of blood vessels in piamater and choroid pluxes of ventricles, neuronophagia, spongiosis and encephalomalacia. Testis showed depletion of spermatogonial cells of seminiferous tubules. The lungs of three rats of groups II on day of 90 also revealed the lesion of pneumocyto–carcinoma featured by presence of large, flat, pleomorphic squamous type cells filling the alveolar lumen having hyper chromatic nuclei. Similar but mild morbid lesions except pneumocyto-carcinoma and encephalomalacia were recorded in rats of group-IV treated with S-adinosyl methionine @ 1 mg/kg body weight orally as compared to the rats of group II.

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Ph.D.

1. Comparative Pathobiology of experimentally induced Pasteurellosis in chicken and quails

Rajul Saxena and A.K. Srivastava

The present study was undertaken to elucidate comperative pathology, pathogenesis of fowl cholera in chicken and quails, caused by *P. multocida* (A: 1) in different groups of the birds. The freeze-dried culture of *P. multocida* (A: 1) in lyophilized form was confirmed on the basis of morphology, biochemical characteristics and cultural characteristics. Passaging for 4 times in 8-week-old chicken and quails enhanced pathogenecity of the organism. The re-isolated organism was further tested for pathogenecity in order to produce the disease in mice, chicken and quails. The LD_{50} was found to be 1ml of 10^{-6} dilution of 18 hours BHI broth culture. The tested pathogenic cultures of *P. multocida* (A: 1) were further used in the entire experiment. Antibiogram for *Pasteurella multocida* revealed the sensitivity pattern of commonly used drugs in order to help in selection of suitable antibiotics for effective management, treatment and control of *Pasteurella multocida* infection in birds. The *Pasteurella multocida* organism was found to be highly sensitive against ampicillin, cloxacillin, cefatrioxon and ciprofloxacin. An improved low volume fowl cholera vaccine with high cell concentration was prepared by precipitating the antigen in broth culture with alum and was blended with liquid paraffin along with lanolin as emulsifier. It was found to be safe when tested in chicken, quail and mice. In the present study 90 chicken and 90 quails were equally divided in three groups. The different groups were assigned as Group IC, IIC, IIIC for chicken and Group IQ, IIQ, IIIQ for quails, respectively. Out of which the birds of group IC and IQ were vaccinated with 1 ml of laboratory prepared formalin killed alum precipitated improved fowl cholera oil adjuvant vaccine intramuscularly and after 21 days challenged with 1ml of 10^{-6} dilution of 18 hours BHI broth culture of *Pasteurella multocida* (Immunized and challenged). The birds of group IIC and IIQ were inoculated with 1ml of 10^{-6} dilution of 18 hours BHI broth culture of *Pasteurella multocida* (Non-immunized and challenged). While the birds of group IIIC and IIIQ were kept as negative control and inoculated with sterilized BHI broth (non-immunized and non-challenged). All the experimental birds of Group-IC, IQ, and IIC & IIQ showed varying degrees of clinical symptoms at different intervals of experimentation, which were more severe in group-IIC and IIQ. On clinical examination, most of the birds showed acute form of the disease with decreased feed and water consumption, pyrexia, depression, increased respiratory rate, foetid diarrhoea and urge of vomition with hock sitting posture along with mucus discharges from nostrils, conjunctivitis, dyspnoea, soiled vent and some degree of lameness. Whereas, cyanosis of comb and wattle accompanied with oedema, hock sitting posture, lameness and nervous manifestation in the form of torticolis were observed in the chicken of group IIC, only. The three chicken of group IIC died following inoculation of *P. multocida* in short period of time without manifestation of clinical symptoms indicating peracute course of disease. The haemato-biochemical observations studied in birds of group IIC & IIQ (Non-immunized and challenged) and IIIC & IIIQ (non-immunized and non-challenged) revealed no significant change in TEC, PCV, MCV while significant increase in ESR, TLC, hetrophils and eosinophils in both the species of birds while, significant decrease in values of Hb was observed in chicken but there was no significant change in Hb concentration in quails. Sero-biochemical studies revealed significant hypoproteinemia, decrease in serum albumin, increase in the level of globulins, elevated values of ALT, AST and alkaline phosphatase and decrease in ionic calcium in chicken but decreased serum albumin, increased globulins and decreased ionic calcium was observed in quails leaving other parameters without any significant change. The mortality pattern was 93.24% in the non-immunized infected chicken (Group-IIC), 70% in non-immunized infected quails (Group-IIQ), 10.00% in immunized challenged chicken (Group-IC)

and 6.66% in immunized challenged quails (Group-IQ). Cell mediated immunity response revealed significant reduction in the increase in skin thickness between the vaccinated challenged and non-vaccinated non-challenged groups indicating the immunosuppressive potency of the pathogen in the birds of IC and IQ. There was no significant difference observed in increase in skin thickness between groups of chicken (IC) and quails (IQ) at 21st day post vaccination and at 5th day post challenge. Similarly no significant difference was observed in increase in skin thickness between groups of chicken (IIIC) and quails (IIIQ) at 21st day post vaccination and at 5th day post challenge. Humoral immunity assay by slide agglutination test observed in the serum of vaccinated /challenged group of birds (IC and IQ) and non-vaccinated non-challenged group of birds (IIIC and IIIQ). Agglutination was seen in the serum from all vaccinated chicken and quails except two, one each from group IC and IQ on 7th day post vaccination, with the intensity expressed as one plus. The magnitude of agglutination increased during the subsequent periods of test, being all 2 plus, except one quail in group IQ on 14th day and all 3 plus except one chicken in group IC on 21st day of vaccination. The intensity decreased to 2 plus on 3rd day post challenge and also maintained up to 5th day. However, on 10th day post challenge all the birds of group IC and IQ showed elevated level of agglutination up to 3 plus. For invasiveness studies of *P. Multocida* (A: 1) 9 chicken and 9 quails were used. In all the chicken and quails, the bacterial invasion in the blood was detected, sooner or later after inoculation of *P. Multocida.* In 88.88% and 66.66% cases the blood invasion was detected at 6 hours post inoculation in chicken and quails, respectively. However, at 12 hours post inoculation all the birds showed septicemia except one quail which was found positive at 18 hours post inoculation. At 24 hours post inoculation 88.88% chicken and 44.44% quails died and by the 36 hours post inoculation the case fatality rate was 100%. Pathomorphological studies, in various organs viz. liver, lungs, heart, spleen, kidneys, intestine and brain were found to be severe in the birds of nonimmunized and infected group (Group IIC and IIQ) as compared to immunized and infected group (Group IC and IQ). The gross and microscopic examination of the dead birds of group IIIC and IIIQ did not reveal any noticeable lesions. The three chicken (Group IIC) died immediately after the inoculation of organism in per-acute cases revealed no marked lesions, and the surviving birds succumbed during the later course of experimentation manifested moderate to marked degree of lesions in both the species of birds of group IIC and IIQ. The liver in chicken was firm and larger in size with distended gall bladder with few to numerous and small to large confluent about 1-2 cm diameter grayish white necrotic foci, generalized congestion in the sinusoids and central veins and varying degree of degenerative and necrotic changes in hepatocytes. The portal area revealed moderate to marked degree of cholengitis. While quails of group IIQ manifested enlarged and congested liver appearing black coloured with normal sized gall bladder with few pin point grayish white necrotic patches which microscopically revealed ballooning degeneration and presence of necrotic foci appearing as homogeneous bright red spots in the congested lobules occasionally infiltrated with polymorphonuclear cells. Focal areas of regeneration of hepatocytes characterized by karyomegaly, cytomegaly, pleomorphism and mitotic figures were also recorded in the hepatocytes. Grossly, lungs of chicken (Group IIC) were moderately to severely congested, enlarged, heavy, firm, reddish brown with deposition of fibrinous exudates with presence of serofibrinous exudates in the distorted lumen of para-bronchi and inter bronchial septa with infiltration of large number of heterophils and monocytes. The lungs of quails (Group IIQ) revealed focal to diffusely congesion, reddish brown, firm and consolidated with scanty fibrinous exudates which microscopically revealed congested lungs with engorgement of blood in the capillaries lining the air vesicles and extravasations of erythrocytes in the lumen of air vesicles and para-bronchi. Presence of watery oedematous fluid in the air vesicles and in the lumen of para-bronchi with occasional presence of heterophils and macrophases were seen in some cases. The heart of the chicken (Group IIC) showed

hydropericardium, petechial haemorrhages on epicardium and adhered pericardium with the lesions of fibrinous pericarditis along with lesions of acute to subacute myocarditis. The heart of quails (Group IIQ) had no marked changes except slight increase in the pericardial fluid and mild congestion with degenerative changes characterized by presence of small to large vacuolization in the most of the myofibres. The spleen of chicken (Group IIC) revealed presence of grayish white necrotic spots with varying degree of degeneration to early necrotic changes in germinal centers of the malpighian corpuscles and hypocellularity in the white pulp and depletion of the lymphoid cells in periarterial lymphatic sheath (PALS). The spleen of quails (Group IIQ) showed slight splenomegaly with mild hyperplasia of reticular fibers with decreased density of lymphoid cells in the malpighian corpuscles. The kidneys of chicken and quails both were congested and slightly enlarged with moderate to marked congestion in the region of cortex and medulla but quails (Group IIQ) revealed lesions of mild subacute glomerulonephritis characterized by hyper cellularity in the bowmen's capsule. The enlarged glomerular tufts occupying most of the spaces showed proliferation of endothelial cells, podocytes and mesangeal cells. The anterior segments of small intestine of the chicken (Group IIC) showed congestion and catarrhal to necrotic duodenitis, jejunitis and ileitis with severe degeneration and desquamation of lining epithelium forming naked villi in chicken. Almost similar lesions but in mild nature were observed in the quails (Group IIQ). The brain of chicken revealed leptomeningeal congestion and softer consistency with degeneration of neurons and perivascular oedema with lesions of demyelination featured by presence of small to large empty spaces. The few birds of (Group IIQ) showed only mild perineuronal and perivascular oedema along with lesions of demyelination. For transmission electron microscopy 3 chicken and 3 quails, inoculated with 1ml of 10^{-6} dilution of 18 hours BHI broth culture of *P. multocida* organism was sacrificed at 48 hours of infection and small tissue pieces from liver, lungs and spleen were collected in chilled 3% gluteraldehyde solution and processed for electron microscopy. In the hepatocytes, the mitochondria were swollen, enlarged, and vacuolated with the loss of mitochondrial cristae, some of the hepatocytes revealed indented nuclear membrane marked by preponderance of electron dense heterochromatin at periphery and few other cells revealed nuclear fragmentation with the electron lucent rod shaped microorganisms, causing disruption of the membrane. In the lung the pneumocytes were desquamated and found in alveolar lumen with indented nuclear membrane with disappearance of nucleolus and flocculated chromatin mass along with markedly dilated capillaries containing large number of oval or elongated erythrocytes bearing electron dense nucleus. The sinusoidal cells of spleen revealed presence of flocculants heterochromatin in the nucleus, vacuolated cytoplasm and rounded swollen mitochondria The endothelial cells lining sinusoids were appeared as electron dense mass with disrupted plasmalemma and presence of a few vacuoles. Large number of organisms appeared as electron lucent to dense, oval or elongated, cut in different planes, were present in the sinusoids.The observations of the electron microscopical studies were almost similar in both the species of experimental birds but were of milder nature in the quails.

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2. Pathology of paratuberculosis with reference to vaccine strategy in goats

Ashwani Kumar and A.K. Srivastava

The present study was conducted to compare efficacy of laboratory prepared indigenous vaccine and imported commercial vaccine (Gudair) in protecting the MAP infection in goats. For this study 40 goats was divided in to three groups, comprising 10 goats (Sham-immunized) in group I, comprising 15 goats (Indigenous vaccine) in group II and 15 goats (Gudair vaccine) in group III. All the groups were challenged twice with 3X109 MAP Bison type strain S5 on 50 DPV and with 5X109 MAP Bison type strain S5 on 270 DPV. The goats of group II & III gained higher body weight as compared to sham-immunized goats while, there was no significant difference in body weight gain observed in between the vaccinated groups. The studies of cell mediated immunity revealed the impact of both vaccinated and experimental infection by MAP S5 strain on the proliferation of PBMCs. The CMI response (SI value) increased at 30 DPV and showed down regulation from 90 DPV and onwards in vaccinated goats and control goats. The studies on humoral immune response revealed, at 180 DPV reveled significantly increase in vaccinated goats and maintained till 450 DPV. Microscopic examination of faecal samples showed at 180 DPV, 5 animals of control group started showing positive results, while at 400 DPV one goat of each vaccinated group was found to shed bacilli.Culture of faecal samples shows growth on HEY medium with mycobactin J confirmed the goats as positive for JD. IS 900 PCR applied in the DNA samples of all goats of each group revealed that at 360 DPV, 100% (4/4), 11.9% (1/9) & 11.9% (1/9) were positive for MAP DNA in animals of group I, II & III respectively. The control animals at 200 DPV showed emaciation and depletion of body fat and mild to moderate lesions of focal/diffuse thickening of small intestine with or without corrugations specifically at ileocaecal junction characterized by mild to moderate catarrhal enteritis with infiltration of mononuclear cells and epitheloid cells. The remaining goats sacrificed at 450 DPV showed thickening of small intestine in 5 cases each in group II and III with chronic catarrhal enteritis and shortening, thin, atrophied and ballooned villi with infiltration of mononuclear cells and epitheloid cells, which at places fused to form giant cells. In vaccinated groups there were focal thickening of intestines in 5 cases each at 450 DPV with lesions of chronic catarrhal enteritis adorned with presence of lymphocytic, plasma cells and macrophages with a few epitheloiod cells.MLN collected at 200 DPV sacrificed goat revealed presence of oedematous fluid and focal infiltration of mononuclear cells with scattered presence of epitheloid cells and few giant cells and on 450 DPV showed mosaic like arrangement of epitheloid cells with presence of multinucleated giant cell. In vaccinated groups, the MLN showed infiltration of MNC and a few epitheloid cells. The study of body score at 200 and 450 DPV on the parameters of body conformation, carcass components fat measurements revealed better marks in vaccinated animals (groups II & III) than control (group I). These parameters in both the vaccinated groups did not differ significantly.

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No.	Titles	Name of Scholars	Name of Major Guide	Years	Page No.
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1. Disposition kinetics of ofloxacin in neonatal calves

Ashok Gaur and Satish Kumar Garg

Disposition kinetic behaviour of ofloxacin was studied in neonatal calves following a single intravenous, intramuscular and subcutaneous administration at the dose rate of 5 $mg.kg^{-1}$ body weight. In vitro plasma protein binding of the drug was also investigated using equilibrium dialysis technique. Blood plasma concentrations of the test drug were determined by microbiological assay method using *E. coli* (ATCC 25922) and minimum sensitivity of the assay method was found to be 0.1 $\mu g.ml^{-1}$. The mean *in vitro* plasma protein binding of ofloxacin in neonatal calves in the plasma concentration range of 0.16 to 5.0 $\mu g.ml^{-1}$ was 24.40±3.72%. Based on the disposition kinetic data of ofloxacin following a single intravenous, intramuscular and subcutaneous injection, it was evident that the drug could be administered by any of these three routes. However, peak plasma concentration to minimum inhibitory concentration ratio (C_{max}/MIC) values of more than eight and area under the curve to minimum inhibitory concentration ratio (AUC/MIC) above one hundred were observed only in case of intravenous administration at the dose rate of 5 $mg.kg^{-1}$ when the MIC value was considered to be 0.25 $\mu g.ml^{-1}$. However, for microorganisms requiring the MIC values of >0.1 $\mu g.ml^{-1}$, the above pharmacokinetic-pharmacodynamic variables favour all the three routes (IV, IM and SC) for administering ofloxacin @ 5 $mg.kg^{-1}$ body weight in neonatal calves. Suitable dosage regimens of ofloxacin, lbased on compartment-model derived pharmacokinetic parameters following different routes of drug administration (IV, IM and SC) have been computed for neonatal calves and summarized. Based on the present studies in neonatal calves, it may be inferred that ofloxacin may be administered by any of the three parenteral routes, namely intravenous intramuscular and subcutaneous injections in neonatal calves at the dose rate of 5 $mg.kg^{-1}$ body weight. Ofloxacin is rapidly absorbed following intramuscular and subcutaneous injections, however, absorption of the drug is rapid and almost complete after subcutaneous administration. Ofloxacin seems to be very well distributed to different body tissues and fluids and thus appears to be suitable for treating various systemic infections including the deep seated ones. Neonatal calves appear to eliminate ofloxacin at a slower rate compared to calves aging more than three months. In vitro plasma protein binding of ofloxacin is lower in neonatal calves and almost comparable to that in humans and pigs. Model-independent method of pharmacokinetic analysis is almost equally good for determining pharmacokinetic variables as the classical compartmental method. At the dose rate of 5 $mg.kg^{-1}$ body weight, intravenous administration proved to be the route of choice. But, it may be used only in emergencies in severe cases where higher blood levels are required to kill the bacteria. Secondly, subcutaneous route should be used taking into consideration the ease of administration and bioavailability. Intramuscular route should not be advocated at the same dose rate due to pharmacokinetic pharmacodynamic variables and comparatively lower bioavailability values and thus, the dose need to be increased.

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2. Plasma levels and noncompartment based pharmacokinetic behaviour of ofloxacin in female *Bubalus bubalis* calves

Pratishtha Sharma and Satish Kumar Garg

Disposition kinetic studies on ofloxacin were conducted in buffalo calves following a single intravenous, intramuscular and subcutaneous administration at the dose rate of 5 $mg.kg^{-1}$ body weight. Blood plasma concentrations of ofloxacin were determined by microbiological assay method using *E. coli* (ATCC 25922) as the test organism and spectrofluorimetric assay method. Plasma concentrations versus time data generated using both the assay procedures was

subjected to both, the compartmental and noncompartmental pharmacokinetic analysis method.After IV administration of ofloxacin at the dose of 5mg.kg^{-1}, mean plasma concentration of the drug using microbiological assay method was 13.60±0.43 μg.ml^{-1} at 0.04 h. Drug could be detected in plasma of buffalo calves upto 36 h in a constant declining fashion, however, traces of the drug could be detected even upto 120 h. But concentrations above the MIC value of >0.25 μg.ml^{-1} were maintained upto 12 h only. Based on the noncompartmental method, the values of AUC_{0-36}, $AUMC_{0-36}$, MRT and F were determined to be 18.53±0.50 μg.ml^{-1} h, 203.18±9.74 μg.ml^{-1} h^2, 33.34±1.99 h and 95.75±4.72 per cent. The mean predicted steady state concentration of ofloxacin in buffalo calves at 24 h dosing interval is expected to be 1.56±0.05 μg.ml^{-1}. Compared to intramuscular administration, significantly higher ($P<0.05$) values of area under the curve, mean residence time and time for the peak plasma concentration after subcutaneous administration suggested that SC route can be used more effectively and conveniently for administration of ofloxacin in buffalo calves. Based on the results of pharmacokinetic studies in buffalo calves, it may be concluded that ofloxacin can be administered by any of the three parenteral routes, namely- intravenous, intramuscular and subcutaneous injections in buffalo calves at the dose rate of 5 mg.kg^{-1} body weight. Ofloxacin seems to be very well distributed to different body tissues and fluids and thus appears to be suitable for treating various systemic infections including the deep seated ones. Following extravascular administration, absorption of ofloxacin is rapid and almost complete in buffalo calves. However, subcutaneous route merits consideration over intramuscular route due to ease of administration and being less injurious and painful. Noncompartmental method of pharmacokinetic analysis is almost equally good for determining pharmacokinetic variables as the classical compartmental methods.

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3. Evaluation of some indigenous medicinal plants for anthelmintic activity

Vipin Kumar Rathi and H.S. Panwar

Cold (aqueous, alcoholic) and hot (aqueous, alcoholic and ether) extracts of *M. azedarach* bark, *V. anthelmintica* and *B. variegata* bark were found to react positive for the presence of alkaloid, glycosides and sterols. Whereas cold (aqueous, alcoholic) and hot (aqueous, alcoholic and ether) extracts of *B. variegata* leaves were found to react positive for the presence of glycosides. The hot (aqueous, alcoholic and ether) and cold alcoholic extracts of *M. azedarach* produced maximum adverse effect on motility after 3 h at 10% concentration. Whereas the hot (aqueous, alcoholic and ether) and cold alcoholic extracts of *B. variegata* produced maximum reduction in motility after 2 hr at 10% concentration. While the extracts of *B. variegata* bark and *V. anthelmintica* did not produce any marked effect. Cold aqueous extracts of *M. azedarach* bark showed a marginal larvicidal activity of 10.78% at concentration of 8000 μg/ml after 40 hr incubation. While cold alcoholic extract produces 20% and 30% activity at concentration of 6000 μg/ml and 8000 μg/ml, respectively after 40 hr incubation. The hot aqueous extract produces 4.75% lethal activity at 8000 μg/ml concentration after 12 hr incubation and 4.83%, 9.65% at concentration of 6000 μg/ml and 8000 μg/ml respectively after 40 hr incubation; hot alcoholic extract produces 2% and 21% larvicidal effect at concentration of 6000 μg/ml and 8000 μg/ml respectively after 12 hr incubation and 22%, 29% at concentration of 6000 μg/ml and 8000 μg/ml, respectively after 40 hr incubation. The hot ether extracts of *M. azedarach* show larvicidal activity of 8% at concentration of 8000 μg/ml 12 hr incubation and in 40 hr incubation 26% mortality at 8000 μg/ml concentration. Whereas cold aqueous extracts of *B. variegata* leaves produces larvicidal activity of 3% and 10% at concentration 6000 μg/ml and 8000 μg/ml, respectively after 12 hr while 5% and 12% at concentration 6000 μg/ml and 8000 μg/ml, respectively after 40 hr incubation. Cold alcoholic extract of *B. variegata* leaves produces larvicidal effect of 18%, 24.68%,

and 34.88% at concentration of 4000 µg/ml, 6000 µg/ml and 8000 µg/ml, respectively and 12 hr incubation while 16%, 30.78%, and 42% at concentration 4000 µg/ml, 6000 µg/ml and 8000 µg/ml, respectively after 40 hr incubation. Hot aqueous extract of *B. variegata* leaves shows larvicidal activity of 13% and 20% concentration 6000 µg/ml and 8000 µg/ml, respectively after 12 hr incubation while 19% and 22% at concentration 6000 µg/ml and 8000 µg/ml, respectively after 40 hr incubation. However hot alcoholic extract of B. variegate leaves have potent larvicidal effect of 20, 27.75 and 37.86% at concentration 4000 µg/ml, 6000 µg/ml and 8000 µg/ml, respectively after 12 hr incubation and after 40 h incubation it produced 16.9, 37.85 and 46.82% at concentration 6000 µg/ml and 8000 µg/ml, respectively. Hot ether extract of B. variegate leaves produces mortality of 17.73% and 31.82% at concentration 6000 µg/ml and 8000 µg/ml, respectively after 12 hr incubation; 20% and 39.70% at concentration 6000 µg/ml and 8000 µg/ml, respectively after 40 hr incubation. While Cold aqueous and hot ether extracts of *B. variegata* bark at any concentration did not exert any effect. However cold alcoholic extract of *B. variegata* bark at 6000 µg/ml and 8000 µg/ml exerted larvicidal activity of 17.69% and 26.20% after 40 hr. Hot aqueous extract of *B. variegata* bark produced activity of 11.17% at concentration of 8000 µg/ml after 40 hr incubation and hot alcoholic extract of the bark produced larvicidal activity of 18.68%, 29.10% at concentration 6000 µg/ml and 8000 µg/ml, respectively after 40 h incubation. In case of *V. anthelmintica*, only hot alcoholic extract showed 4.3% larvicidal activity at a concentration of 8000 µg/ml after 40 h incubation. In vitro incubation of *H. contortus* infective larvae in fenbendazole produced 18.50, 29.50 and 100% larvicidal effect at 250, 500 and 1000 µg/ml. Distilled water and 10% gum acacia did not show any larvicidal effect on infective larvae of *H. contortus*. Hence from the present investigation of different Indigenous plant for their anthelmintic activity against *H. contortus*, it may be concluded that *B. variegata* leaves and *M. azedarach* bark possess promising results for the use as anthelmintic.

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4. Pharmacological screening of some indigenous plants for their acaricidal activity along with phytochemistry

Mrituyanjay Kumar Chaturvedi and Rajendra Singh

Infestations of ticks in dairy animals causing production losses by way of blood sucking and continuous annoyance results in reduction milk production, stunted growth, loss of body weight gain, hide damage and more importantly, as a transmitting agent of many pathogens. Calotropis procera leaves, Jatropha curcas seeds, Chrysanthemum spp. leaves and flowers of Catharanthus roseus were selected for the assessment of their acaricidal properties by both *in vitro* and *in vivo* studies. For both the studies, crude extracts were prepared by Soxhlet extraction using methanol and distilled water. Subsequently, different solvents fractions of methanolic extract of crude extracts were also prepared. Four crude extracts and their fractions were tested at concentrations 250 mg/ml, 500 mg/ml and 1000 mg/ml for *in vitro* studies and at the 500mg/ml tested for *in vivo* studies. In laboratory Catharanthus roseus leaves methanolic extract and its various fractions showed very little activity as is evidenced by much higher values of LC_{50} even after 24 hrs in 1000mg/ml concentration. Among fractionated extracts, ethyl acetate fraction showed a little bit activity of 55% at 1000mg/ml concentration at 24hr. Lowest activity was seen in n-butanolic fraction (25% mortality). Ethyl acetate fraction had the lowest LC_{50} (1051.69) 24 hr. Rest of fractions of the plant leaves showed higher LC_{50} values. Since we know lower the value of LC_{50}, better the extract, hence ethyl acetate fraction was having better acaricidal effect than other fractions of this plant's leaves extracts. The maximum activity improvement factor (2.90) was noticed in ethyl acetate fraction at 2 hrs and 1.19 after 24 hrs in the same fraction. Almost similar activity improvement was also observed in solvent ether (1.95) and aqueous remnant (1.88). The AIF in solvent ether was greater than 1 at one and four hour but it was less than 1 at 24 hr

and/or K^{+}_{Ca} cannot be ruled out. Over all, *Moringa oleifera* flowers should be exploited in drug-development programme for evolving natural and effective oxytocics or abortifacients for use in human being and animals.

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8. Evaluation of bioenhancer potential of *Moringa oleifera* flowers and its efficacy in ameliorating lead-induced toxicity in broiler chicks

Atul Kumar Baranwal and Satish Kumar Garg

Present investigation was undertaken to evaluate the bioenhancer potential of hot aqueous extract of *Moringa oleifera* flowers (MOFE), its safety and also the ameliorative potential against low dose lead-induced toxicity in broiler chicks, including its effect on production performance of broilers, haemato-biochemical parameters, oxidative stress indicators and histo-cellular architecture of vital body organs. Results indicated that weekly body weight gain in chicks of group II and III did not differ significantly from control group (I) up to 4th week. During 5th week, weekly weight gain in group III chicks (467.50±23.36 gm) was significantly higher compared to group I, however, during 7th week, group II chicks exhibited significantly lower body weight gain compared to group I and III. Weekly body weight gain data of chicks of group V after 4th week and 6th week was significantly more and less, respectively. MOFE did not have any significant effect on absolute or relative weights of liver, kidneys, heart, bursa or spleen, however, in high dose MOFE treatment group (500 mg/kg), higher relative weight of kidneys was observed.. Blood haemoglobin, total leucocytes count and packed cell volume values in groups II (MOFE @ 250 mg/kg) and III (MOFE @ 500 mg/kg) were significantly higher compared to the control group, while the TEC value was significantly lower in group II and markedly lower in group III. Differential leukocyte count values in group II and III were almost similar to those in control group. Blood glucose data (mg/dl) of chicks of group I, II and III did not differ significantly from one another, but MOFE at lower dose produced marginal hypoglycemic effect. Plasma alkaline phosphatase activity of MOFE treated groups did not differ significantly from control group but aspartate aminotransferase (AST) activity in chicks of group II and group III were significantly lower, exposure of chicks to lead (250 ppm) for 21 days produced significant increase in blood glucose, total proteins and globulin levels, while on long term exposure (42 days), no such effect was observed on blood glucose and less conspicuous but significant effect on total proteins and globulins while plasma uric acid and bilirubin levels in chicks of lead-exposure groups (IV and V) did not differ significantly from those in control group. However, plasma creatinine levels in group IV and group V was significantly lower than in control group. Further, chicks, which were exposed to lead for 21 days had significantly, lower plasma creatinine values than those exposed to lead for 42 days. Plasma cholesterol values in chicks of group IV and V did not differ significantly from each other. Malonaldehyde concentration in chicks of group II and III was markedly higher than in control group while SOD activity in MOFE treated groups was conspicuously lower whereas, glutathione peroxidase (GPx) activity did not differ significantly from control group, however, glutathione reductase activity in MOFE treated groups was significantly higher compared to that in control group. Glutathione-S-transferase activity values revealed just the reverse pattern. Lead exposure of chicks @ 250 ppm for 21 or 42 days did not have any significant effect on malonaldehyde production or SOD activity. In view of some of the promising potentials enumerated above, further detailed investigation need to be taken up on a large sample size with particular reference to titration of dose including other extracts before it is recommended for inclusion in poultry feed as a constituent of poultry ration or feed supplement.

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9. Evaluation of bioenhancer potential of *M. oleifera* leaves and its efficacy in ameliorating arsenic-induced toxicity in broiler chicks

Kapilesh M. Varshney and Satish Kumar Garg

Present investigation was envisaged to evaluate the bioenhancer potential of hot aqueous extract of *Moringa oleifera* leaves (MOLE), its safety and also the ameliorative potential against low dose arsenic-induced toxicity in broiler chicks, including its effect on production performance of broilers, haemato-biochemical parameters, oxidative stress indicators and histo-cellular architecture of vital body organs. Results on production performance, haemato-biochemical parameters, antistress markers and histo-pathological studies suggest that hot aqueous extract of *Moringa oleifera* leaves may have bioenhancer potential as it improved body weight of broiler chicks. *Moringa oleifera* leaves extract possesess haematinic property as it increased blood haemoglobin and packed cell volume. It possesses hypoglycemic activity and thus may be further investigated and exploited for future antidiabetic drug development. It seems to hold promising hepatoprotective activity. It possesses immunomodulatory potential. It seems to possess ameliorative potential against arsenic-induced toxicity. In view of the promising potentials enumerated above, further detailed investigation need to be taken up with particular reference to titration of dose including other extracts before it is recommended for inclusion in poultry feed as a constituent of poultry ration or as feed supplement.

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10. Pharmacological studies on involvement of potassium channels and second messengers in mediating salbutamol-induced tocolysis and molecular characterization of Bk_{ca} channels in buffalo myometrium

Soumen Choudhury and Satish Kumar Garg

The objective of the present study was to unravel the possible signaling pathways of salbutamol-induced tocolysis, particularly the involvement of different types of potassium channels and second messengers- cAMP and cGMP and molecular characterization of calcium activated large conductance potassium channels (BK_{Ca}) in buffalo myometrium collected from the cyclic buffaloes during the diestrous stage of the cycle and the isometric tension in the myometrial strips was recorded. Salbutamol-induced tocolysis was subjected to detailed pharmacological investigations employing specific drugs/ chemicals for modulating different potassium channels, NO, sGC, Gs proteins pathway etc. The levels of cAMP and its modulation by different agents were also studied. RT-PCR technique was employed to characterize the alpha subunit of BK_{Ca} channels. With the help of molecular biology techniques employing RT-PCR, α subunit of the BK_{Ca} channel present in buffalo myometrium was characterized and the gene for α-subunit was amplified. The PCR product of 1097 bp was generated cloned and was sent for gene sequencing. Results of present study suggest that salbutamol-induced myometrial relaxation is mediated mainly through K_{ATP} channels, however, the involvement of BK_{Ca} and K_V channels cannot be fully ruled out. Salbutamol-induced myometrial relaxation seems to be NO-independent. Salbutamol-induced myometrial relaxation does not seem to be sGC-dependent, either it is not involved or the level of cGMP is not sufficient to initiate the relaxant effect. However, further detailed studies are indicated using specific Gs activators. cAMP level increased in tissues following treatment with salbutamol but the levels may not be sufficient to induce myometrial relaxation by itself alone. Therefore, the possibility of cAMP-dependent effect of salbutamol cannot be fully ruled out. The gene responsible for coding the α subunit of the BK_{Ca} channel in buffalo myometrium was characterized, amplified and the PCR product was found to be of 1097 bp thus suggesting the possible presence of α-subunit of the BK_{Ca} channel; cloning and sequencing of the amplified gene is under further investigations.

11. Pharmacological screening of some indigenous plants for their antimicrobial activity

Santosh Kumar Jaiswal and Rajendra Singh

The study was conducted for exploration of the antibacterial activity of *calotropis procera* (flower), *Nyctanthes arbortristis* (leaf), *Eucalyptus globulus* (leaf), *Moringa oleifera* (leaf), *Azadirachta indica* (leaf & bark) and *Ocimum sanctum* (leaf) in their aqueous and methanolic extracts against *E. coli* and S. *aureus.* The effective plant extracts were further screened for their efficacy against these organisms in terms of their MICs. These efficacies were also compared with the standard antibiotics. The antibacterial activity was correlated with the presence of various active principles found in these plants under the phytochemical analysis. The antibacterial studies showed that the methanolic extracts only were effective against these organisms and the aqueous extracts failed to show any response. The antibacterial activity in decreasing order against *E. coli* was revealed as the *Eucalyptus* leaf, *Ocimum* leaf, *Moringa* leaf, *Azadirachta* leaf, *Azadirachta* bark and *Calotropis* flower and *Nyctanthes* leaf. Similar activity against S.aureus in the similar manner was found in *Eucalyptus* leaf, *Ocimum* leaf, *Moringa* leaf, *Azadirachta* leaf, *Azadirachta* bark, *Calotropis* flower and *Nyctanthes* leaf. The results suggested that the *Eucalyptus* and *Ocimum* leaves are highly efficacious against *E. coli* and/ *Staph. aureus,*organIsms placing them at the first and second place of their antibacterial efficacy. Under the studies of sensitivity of these plant extracts against these organisms and their comparison with the standard antibiotics, it was revealed that all the methanolic extracts were well effective against these organisms, whereas, the standard antibiotics like Erythromycin, Oxacillin, Penicillin-G and Cloxacillin failed to show any response. It is suggestive of a superior therapeutic utility of these plants. The antibacterial activity was correlated with various active principles present in these plants by their phytochemical analysis. It was revealed therein that there was an invariable presence of alkaloids, tannins and flavonoids in these plants, whereas, the glycosides, terpenoids and saponins were variably present. It suggested that the antibacterial efficacy could well be due to the invariably present component, however, the other component· can also not be ignored wherever those plants are showing antibacterial activity. Nevertheless, it requires further studies. All these studies, however, need further exploration to undertake a wide variety of organisms for establishing their wide therapeutic applicability.

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12. Evaluation of certain pharmacological activities of *Moringa oleifera* leaves extract with particular reference to immunomodulation

M. Jayanthi and Satish Kumar Garg

The present study was undertaken to assess pharmacological activities of *Moringa oleifera* leaves extract. Per cent yield of hydro-alcoholic (methanol: water-50:50) extract of *Moringa oleifera* leaves was found to be 26.51% and it was much less compared to the pure methanolic (52.87%) or hot aqueous extracts (45.2%). Based on the results of present study on MOLE it may not be unreasonable to infer that MOLE possesses haematopoietic potential including its possible use in thrompocytopenia and anaemic. It possesses weak central but strong peripheral analgesic activity. The moderate anti-inflammatory effect of MOLE seems to be of delayed type. It also possesses potent CNS depressant activity and possibly pentobarbitone-induced hypnosis seems to mediate through modulation of central neurotransmitters. Kidney and testes seems to be most adversely affected by MOLE, however, other organs are not damaged to an extent of concern. Limited immunomodulation studies suggested that *Moringa* potentiates humoral immune response in experimental animal, but further, comprehensive study on modulation and expression of cytokine involved in the regulation of immune systems are required to be taken.

13. Evaluation of certain pharmacological activities of *Nyctanthus arbortristis* flower extract with particular reference to immunomodulation

Chandrabhan Kumar Bharshiv and Satish Kumar Garg

Present study was undertaken with the objective of evaluating different pharmacological activity including immuno-modulation in *Nyctanthes arbortristis* flowers extract (NAFE). The yield of hot aqueous extract of flowers was 85.83 per cent and phytochemical analysis of extract revealed the presence of alkaloids, glycosides, saponins, tannins, sterols, reducing sugars, resins and terpenes. NAFE @ 400, 800 mg/kg b.wt daily given to rats and mice for 21 days did not reveal any apparent signs of toxicity or adverse effects.NAFE produced anabolic, haematinic, central and peripheral analgesic, antipyretic and anti-inflammatory activity in experimental animals. Total COX-activity in NAFE treated group (800mg/kg) was significantly ($P<0.05$) lower compared to the control group and the inhibition was even more than that by etoricoxib a selective COX-2 inhibitor. Evaluation of spontaneous locomotor activity of NAFE at 400, 800 and 1600 mg/kg revealed that NAFE potentiated the effect of diazepam. It also potentiated pentobarbitone-induced hypnosis and the duration of sleep increased form 32.83±3.34min in control group to 55.17±3.30, 62.33±4.64, 72.33±4.29 and 85.33±3.43 minutes with NAFE at 200, 400, 800 and 1600mg/kg. NAFE not only facilitated induction of ether anaesthesia but also the duration of ether anaesthesia was significantly ($P<0.001$) increased. NAFE at higher doses also produced local anaesthetic activity in addition to hepato-microsomal proteins inductions but did not show any anticonvulsant activity. Compared to control group, no significant change in relative weights of kidneys, lungs, heart and testis was observed, however, slight to marked and significant increase in absolute and relative weight of lungs and adrenals was observed in NAFE treated groups, but liver and spleen weights were moderate to markedly decreased. Spleenocytes proliferation test revealed that NAFE at 400 and NAFE 800 mg/kg resulted in significant increase in OD and stimulation index and the effect of NAFE was concentration-dependent. *Ex-vivo* studies on IL-2 and IL-6 cytokines assay in spleenocytes revealed that NAFE @ 400mg/kg and 800mg/kg significantly ($P<0.001$) augmented the IL-2 and IL-6 levels. Similarly, on *in vitro* exposure of spleenocytes to NAFE at (50, 100, 200, 400, 800 and 1600µg/well) also, significantly increase in IL-2 and IL-6 levels was observed at NAFE concentration from 50-400 µg/well treated compared to that in control group. But with further increase in the concentration of NAFE to 800 or 1600µg/well, no further increase in IL-2 and IL-6 levels was observed. Thus, these observations suggest that NAFE possesses immuno-modulatory potential. Based on the result of present study it may be inferred that NAFE possesses, anabolic, haematinic, analgesic, anti-inflammatory, antipyretic, CNS depressant, tranquilizing, muscle relaxant activity and also potentiates pentobarbitone and ether-induced hypnosis. In addition, it seems to possess promising immuno-modulatory potential.

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14. Biomonitoring of metallic pollutants and their impact on macro and micro-minerals and reproductive hormonal profiles in cows and buffaloes of Mathura

Baburam Nigam and Satish Kumar Garg

Keeping in view the increasing menace of environmental pollution due to metallic pollutants, present study was carried out to determine the levels of the metallic pollutants, namely-arsenic, lead, cadmium and mercury, micro-minerals (copper, iron, zinc and selenium) and macrominerals (calcium, magnesium and phosphorous) in blood of cows and buffaloes, different body organs/tissues and fluids of buffaloes and soil, fodder and water samples collected from different locations. Blood levels of all the minerals and metallic pollutants were determined

using Atomic Absorption Spectrophotometer methods while the reproductive hormones (progesterone and oestradiol) with the help of ELISA method. Buffaloes from the village in close vicinity of Mathura Refinery had lowest arsenic blood level (25.00±1.76 ppb), followed by buffaloes with dystocia (103.02±3.68 ppb) and University dairy farm cows (124.20±5.50 ppb). Blood levels of cadmium, cobalt and molybdenum were significantly higher in dairy farm cows in comparison to dystocia buffaloes. Blood zinc (2.36±0.04 ppm) and selenium (968.00±46.09 ppb) in Refinery village buffaloes were higher compared to the zinc in abattoir buffaloes (0.43±0.01 ppm) and selenium in dairy farm cows (178.48±5.77 ppb). Buffaloes with dystocia interestingly revealed hypercalcaemia (206.08±6.42 ppm) while Refinery buffaloes were hypocalcaemic (45.26±2.07 ppm). The levels of copper, iron, zinc and selenium in liver and kidney samples were not high. But the copper,iron and zinc in liver were higher than in kidneys whereas selenium was higher in kidneys than in liver and the difference was statistically significant. Mercury was found to be lowest concentration in water but highest in fodder samples. Similarly, copper, zinc, calcium and phosphorous were also found to be significantly higher in fodder. Soil samples also revealed the presence of highest concentration of iron and magnesium, but selenium level between soil and fodder did not differ significantly. Estimation of female reproductive hormones in serum samples of University dairy farm cows and buffaloes from the Mathura Refinery village revealed that the level of progesterone was significantly higher in dairy farm cows in comparison to the refinery village buffaloes, while oestradiol level was significantly higher in refinery village buffaloes. Based on the results of present study, it may be concluded that less toxic metals may be altering the levels of certain macro and micro minerals in the body which in turn affecting the reproductive status of cows and buffaloes.

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15. Comparative disposition kinetics and interaction studies of ofloxacin and meloxicam in goats

Raj Kumar Singh Yadav and Satish Kumar Garg

Disposition kinetic studies on ofloxacin (10 $mg.kg^{-1}$) and meloxicam (0.5 $mg.kg^{-1}$) in Barbari goats were conducted following a single intravenous, intramuscular and subcutaneous administrations for determining the comparative plasma levels and pharmacokinetic behavior and to compute suitable dosage regimens of these drugs for goats including interaction study of these drugs after IV administration. Blood samples were collected at different predetermined time intervals and blood plasma concentrations of ofloxacin and meloxicam were determined using the HPLC assay methods. These methods were found to be linear and reproducible with the correlation coefficient value of 0.999; the intra-day and inter-day coefficient of variance was less than 10 per cent and the mean recovery was more than 90 per cent. Plasma concentrations versus time data were subjected to compartmental pharmacokinetic analysis using "PHARMKIT" software. Comparison of the plasma levels of ofloxacin after IM and SC administration revealed that plasma levels after SC administration at 120, 180, 240, 360, 600 and 720 min were significantly higher compared to the corresponding values after IM administration. The values of pharmacokinetic determinants, namely-AUC, AUMC, MRT, MAT and F were significantly higher following SC administration compared to IM route. Therefore SC route seemed to be better in comparison to the IM route. Mean plasma concentration of meloxicam following concurrent intravenous administration of meloxicam (0.5 $mg.kg^{-1}$) and ofloxacin (10 $mg.kg^{-1}$) was found to be 3.35±0.12 $\mu g.ml^{-1}$ at 2.5 min and the drug was detected in plasma (0.20±0.02 $\mu g.ml^{-1}$) up to 12 h. The value for $t_{½\beta}$, AUC, $Vd_{(area)}$, and Cl_B were 215.72±9.21 min, 568.47±35.11 $\mu g.ml^{-1}min$, 0.27±0.011 $L.kg^{-1}$ and 0.0009±00 $L.min^{-1}kg^{-1}$, respectively. Statistical analysis of meloxicam levels and comparative pharmacokinetic parameters after single IV and meloxicam (0.5 $mg.kg^{-1}$) and ofloxacin (10 $mg.kg^{-1}$) concurrently revealed that these indices did

not differ significantly from each other except plasma concentration at 15 min and pharmacokinetic parameter K_{21}. On the basis of PK-PD indices and by considering post-antibiotic effect of ofloxacin, it may be suggested that a bolus dose of 10 mg.kg^{-1} at 12 h interval by any of the parentral routes is likely to be sufficient to treat bacterial infections while the loading and maintenance doses of meloxicam were calculated to be 1.77 and 1.56 mg.kg^{-1} body weight and repeated at 12 h interval.

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16. Pharmacological characterization of ATP-dependent potassium channels and signaling pathways of terbutaline and forskolin-induced myometrial relaxation in pregnant buffaloes

Suresh Kumar Pal and Satish Kumar Garg

Objective of the present pharmacological study was to unravel the presence of KATP channels and possible signaling pathways of terbutaline and forskolin-induced tocolysis of buffalo myometrium particularly to elucidate the involvement of different types of potassium channels and second messengers-cAMP and cGMP. The study was conducted on the uteri of pregnant buffaloes and isometric tension of isolated uterine strips was recorded. Terbutaline, cromakalim, pinacidil and forskolin were used as agonists to induce tocolysis and specific potassium channel blockers and other antagonists were used for studying involvement of different modulators/pathways in mediating the effect of different agonists. Based on the results of present study KATP channels are present in buffalo myometrium, stimulation of which induces tocolytic effect. KATP channels are functionally involved in mediating agonists-induced tocolysis; NO-pathway does not seem to be involved in mediating terbutaline-induced myometrial relaxation; In addition to the involvement of KATP channels, AC-cAMP pathway seems to modulate forskolin-induced tocolysis; Kv and BKCa channels seem to up-regulate the AC-cAMP pathway possibly as negative feed-back regulator of $[Ca^{2+}]_i$ and/or through sGC-cGMP. Direct involvement of NO-pathway in mediating cAMP-induced myometrial relaxation cannot be ruled out.

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17. Studies on disposition kinetics of levofloxacin in buffalo calves

Ram Raghvendra and Satish Kumar Garg

Disposition kinetic studies of levofloxacin (10mg.kg^{-1}) in buffalo calves following a single intravenous, intramuscular and subcutaneous administration were conducted in six animals. Blood plasma concentrations of levofloxacin were determined using the HPLC assay method. The method was found to be linear in the conecentration range of 0.05 to 6.4 μg.ml^{-1} and reproducible with the correlation coefficient value of 0.999, and the intra-day and inter-day coefficient of variance was found to be 7.8% and 10%, respectively. Recovery of levofloxacin ranged from 49 to 80 percent and the overall recovery was calculated to be 85 per cent. Plasma concentrations versus time data were subjected to compartmental pharmacokinetic analysis using "PHARMKIT" software. Based on the results of pharmacokinetic studies it may be inferred of levofloxacin in buffalo calves, and pharmacokinetic: pharmacodynamic predictors of efficacy i.e. AUC/MIC and C_{max}: MIC derived from the generated data, that levofloxacin should be administered to buffalo calves at the dose level of 10 mg.kg^{-1} by either of the routes IV, IM, SC and repeated at 24 h intervals, But almost 50% bioavailability of levofloxacin following SC administration does not favour its preferential recommendation over the intramuscular route.

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18. Disposition kinetics studies of levofloxacin in cattle calves

Arvind Kumar and Anu Rahal

Disposition kinetics of levofloxacin was studied in cattle calves following a single intravenous, intramuscular and subcutaneous administration at a dose rate of 10mg/kg. Plasma concentrations of levofloxacin were determined using the HPLC assay method. This method was very consistent and reproducible with the correlation coefficient value of 0.999; the intra-day and inter-day coefficient of variance was less than 10 per cent and the mean recovery was 86 per cent. Plasma concentrations versus time data were subjected to compartmental pharmacokinetic analysis using the computer software "PHARMKIT". Following intravenous administration of levofloxacin in cattle calves (10 $mg.kg^{-1}$), mean plasma concentration of the drug at 0.04 h was 24.00±3.67 $\mu g.mL^{-1}$ and the drug could be detected in plasma (0.10±0.02$\mu g.mL^{-1}$) for up 24 h. The plasma concentrations time data of levofloxacin was best described by two-compartment open model. Based on plasma concentrations of levofloxacin, the mean values for $t_{½\alpha}$ and $t_{½\beta}$ were 0.05±0.01 h and 2.12±0.21 h, respectively. The values of AUC, $Vd_{(area)}$ and Cl_B were found to be 29.32±1.19 $\mu g.mL^{-1}h$, 1.05±0.10 $L.kg^{-1}$ and 0.34±0.01 $L.h^{-1}.kg^{-1}$, respectively while the ratio of drug concentrations between the tissues and plasma (T/P) was 4.47±02.09. The overall mean residence time of levofloxacin was calculated to be 2.87±0.31 h. Following IM administration of levofloxacin (10 $mg.kg^{-1}$), an appreciable and clinically effective concentration of 1.04±0.34 $\mu g.mL^{-1}$ could be detected in plasma within 0.04 h and the peak plasma concentration of 3.08±0.33 $\mu g.mL^{-1}$ was observed at 1 h. Levofloxacin was detected in plasma (0.21±0.05 $\mu g.mL^{-1}$) up to 18 h and the plasma concentration time data of levofloxacin could be best fitted to one-compartment open model with first order absorption rate constant. The mean (±SE) values of $t_{1/2Ka}$, $t_{½\beta}$, AUC, MAT, MRT and F were found to be 0.51±0.09 h, 2.76±0.36 h, 18.43±2.15 $\mu g.mL^{-1}h$, 1.85±0.46 h, 4.72±0.72 h and 62.65±5.99 per cent respectively. After SC administration of levofloxacin (10 $mg.kg^{-1}$), clinically satisfactory concentration of 1.18±0.48 $\mu g.mL^{-1}$ could be detected in plasma within 0.04 h and the peak levofloxacin level of 3.03±0.36 $\mu g.ml^{-1}$ was observed at 1 h. The drug could be detected in plasma of calves up to 12 h when the level was 0.28±0.07 $\mu g.mL^{-1}$. Plasma levels versus time data was best fitted to one- compartment open model and the values of $t_{1/2Ka}$, $t_{½\beta}$, AUC, MAT and MRT were calculated to be 0.75±0.18 h, 2.57±0.29 h, 28.61±6.40 $\mu g.mL^{-1}h$, 0.59±0.62 h and 3.46±0.39 h. The bioavailability of levofloxacin following subcutaneous administration in cattle calves was found to be almost 100% (97.50±19.66%).

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19. Pharmacological studies on nitric oxide pathway and its signaling mechanism(s) in buffalo myometrium

Suchendra Kumar Singh and Satish Kumar Garg

Main objective of our study was to investigate the involvement of NO-NOS-sGC pathway in regulating myometrial contractility in pregnant buffaloes. To demonstrate the involvement of NO, isometric tensions in myometrial strips were recorded after exposure to NO precursor (L-arginine) and NO donor (SNP) in spontaneously contracting and oxytocin-precontracted tissues. To unravel the established pathway of NO i.e. NOS-sGC, L-NAME, O.D.Q. and 1400W compounds were used as antagonists. In addition, antagonistic effect of glybenclamide on L-arginine-induced myometrial relaxation was also studied. Results of present study suggest that nitric oxide exerts relaxant effect on pregnant buffaloes myometrium which not only seems to be NOS but sGC-independent also as both L-NAME and O.D.Q. potentiated the relaxant effects of L-arginine. Therefore, the possibility of involvement of some other signaling pathway(s) mediating L-arginine-induced relaxant effect including through K_{ATP} channels cannot be ruled

out. The eNOS seems to play but not significant role in NO-NOS-sGC pathway. Further, our results also reveal the functional presence of PDE4 and its regulatory role through cyclic nucleotides in pregnant buffaloes myometrium.

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20. Studies on toxicities of binary mixture of arsenic and deltamethrin and its amelioration by aqueous extract of *Withania somnifera* roots and *Moringa oleifera* leaves in rats

Sunil Kumar and Satish Kumar Garg

Present research work was carried out to study toxicity of binary mixture of arsenic and deltamethrin and the ameliorative effects of aqueous extracts *Moringa oleifera* leaves and *Withania somnifera* roots in male Wistar rats, if any. Study included effects on body weight, haemato-biochemical and oxidative stress biomarkers, AChE activity and histocellular architecture of the vital body organs. From the results of present study in rats it may be inferred that sodium arsenite (@ 40 ppm) in general was more toxic than DLM (@ 1.9 mg/kg). Reduced weight gain in As-treated group (21.32) compared to (29.40) was reversed both by MOLE or WRE. Both As and DLM alone or their binary mixture did not adversely affect the haematological parameters expect moderate but insignificant effect. On per cent count of lymphocytes which was reversed by MOLE and WRE. Hepatic and other tissues damage was more with As than DLM and binary mixture did not have any pronounced effect and MOLE on concurrent treatment with xenobiotics prevented such damage. Both As and DLM resulted in renal damage with was not countered by any treatment. But As-induced hypercholesteremia and hyperbilirubinemia were checked by both, MOLE and WRE. WRE was more effective in preventing DLM-induced decrease in total proteins and plasma protein levels. Lipid peroxidation and other oxidative stress biomarkersrevealed that both As and DLM-induced markered to significant accelarations were revesed by both- MOLE and WRE. AChE activity was not significantly affected by any of the xenobiotics. Absolute and relative weights of organs also revealed protective effect of MOLE and WRE. Hepato-protective effect of MOLE was more pronounced compared to other organs.

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21. Evaluation of oxytoxic, anti-inflammatory and antibacterial activities of *Adhatoda vasica* and *Moringa oleifera* leaves extracts

Kamlesh Chandra Prakash and Satish Kumar Garg

Present study was undertaken to evaluate the oxytocic, anti-inflammatory and antibacterial activities of *Adhatoda vasica* and *Moringa oleifera* leaves extracts. The oxytocic effect was studied on isolated uterine strips of pregnant buffalo anti-inflammatory in wistar rats and antibacterial activity was evaluated using standard bacterial cultures. Phytochemical analysis of the hot aqueous, cold aqueous, hot alcoholic and cold alcoholic extracts of *A. vasica* and *M. oleifera* leaves revealed the presence of alkaloids, resins and reducing sugars while proteins, amino acids, nitrates and nitrites were absent. Flavanoids and saponins were present in aqueous extracts but absent in alcoholic extracts and tannins were present in alcoholic extracts but absent in aqueous extracts. Both the hot and cold alcoholic and aqueous extracts of *A. vasica* leaves produced uterotonic effect and the rank order potency of these extracts was: hot aqueous > cold aqueous > cold alcoholic > hot alcoholic while for *M. oleifera* leaves extracts, the rank order of potency was: hot alcoholic > cold alcoholic > cold aqueous > hot aqueous. Cold methanolic extract of *A. vasica* leaves (CMEAVL)-induced uterine contractions were concentration-dependent and 1000μg/ml concentration produced the maximal uterotonic effect. Tissue tension data suggested that atropine (0.1μM) failed to antagonize the effect of CMEAVL but one of the

representative tissues revealed that atropine antagonized the effect of CMEAVL. Thus, warrants further detailed investigations. Tissue tension data revealed the antagonistic effect of phentolamine on CMEAVL-induced uterine contractions; thus suggesting the involvement of excitatory ⊚-adrenergic receptors in CMEAVL-induced uterotonic effect. Mepyramine (1μM) and ketanserin antagonized the effect of CMEAVL involvement of histamine H_1-receptors and 5-HT receptors in mediating uterotonic effect. Complete/partial antagonism by atropine sulphate (0.1μM) and phentolamine suggest involvement of excitatory muscarinic receptors, ⊚-adrenergic receptors. Competitive antagonism by mepyramine (1μM) and ketanserine (1μM) of the uterotonic effect of extract further suggested the involvement of excitatory H_1-histaminergic and 5-HT receptors, however, it requires further studies. Anti-inflammatory studies suggested mild to moderate but delayed activity of *A. vasica* leaves extracts following seven days continuous oral feeding at @ 400 mg/kg but *M. oleifera* leaves failed to produce any such effect. Hot alcoholic extract of *A. vasica* leaves at 250 and 500 mg/ml was effective against *Staphylococcus, E. coli* and *Klebsiella* sp while hot alcoholic extract of *M. oleifera* leaves revealed marked antibacterial activity against *Staphylococcus, Bacillus* and *E. coli* at 500mg/ml concentration of the extract. Thus, based on the results of present study, it may be inferred that both these plants possess promising pharmacological activities worth further investigation for drug development.

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22. Pharmacological screening of *Cimicifuga racemosa* and *Mimosa pudica* for their oxytocic, anti-inflammatory and antibacterial activities

Richa Rathore and Anu Rahal

Present study was undertaken to evaluate the oxytocic/tocolytic, anti-inflammatory and antibacterial activities of *Cimicifuga racemosa* roots and *Mimosa pudica* seeds extracts. The oxytocic/tocolytic effect was studied on isolated uterine strips of pregnant buffalo, anti-inflammatory in wistar rats and antibacterial activity was evaluated using standard bacterial cultures of *Klebsiella* and *Bacillus* species. *Cimicifuga* roots methanolic extract was found to exert a myometrial relaxant effect which was potentiated after inhibition of excitatory muscarinic, *alpha*, adrenergic, H_1-histaminergic and 5HT receptors and β-receptors. *M. pudica* seeds extract also produced concentration-dependent inhibitory effect on buffalo myometrium which seemed to be mediated through inhibitory β receptors. Calcium channels did not seem to regulate tocolytic effect of *Mimosa pudica* seeds extract. Both the plants lacked promising anti-inflammatory and antimicrobial activities against *Klebsiella* and *Bacillus* species. Further studies are indicated on mechanistic aspects of tocolytic effect of extracts of both the plants particularly to elucidate the involvement of Ca^{2+} and K^+ channels, NO and other signaling mechanisms including second messengers.

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23. Pharmacological studies on *Trachyspermum ammi* and *Raphanus sativa* with particular reference to their uterotonic, anti-inflammatory and antibacterial activities

Md. Sarfaraz Alam and Satish Kumar Garg

Present study was undertaken to study the phytochemistry and evaluate the oxytocic, anti-inflammatory and antibacterial activities of *Trachyspermum ammi* seeds and *Raphanus sativa* roots extracts. The oxytocic effect was studied on isolated uterine strips of pregnant buffaloes, anti-inflammatory activity in wistar rats and antibacterial activity was evaluated using standard bacterial cultures. Both the hot and cold methanolic and aqueous extract of *Trachyspermum ammi* seeds produced uterotonic effect and the rank order of potency of these extracts was: CME>

CAQ> HAQ> HME while for *Raphanus sativa* roots extracts, the rank order of potency was: hot aqueous> hot methanolic > cold aqueous > cold methanolic. Cold methanolic extracts of *Trachyspermum ammi* seeds-induced uterine contraction was concentration-dependent. Tissue tension data suggested that atropine (1μM) failed to antagonize the effect of CMTASE but one of the representative tissues revealed that atropine antagonized the effect of CMTASE. Therefore, possible involvement of muscarinic receptors cannot be ruled out. CMTASE- induced uterine contraction was found to be be mediated through excitatory α-adrenergic receptors and 5 HT receptors but possible H_1 receptors are not involved in mediating CMTASE-induced uterotonic effects. Antiinflammatory studies suggested mild to moderate but delayed activity of *Trachyspermum ammi* seeds and *Raphanus sativa* roots extracts following a single oral feeding at @ 100mg/kg. Cold methanolic extract of *Trachyspermum ammi* seeds at 10 and 20 mg/disc was effective against *Staphylococcus aureus, E. coli, Bacillus* and *Kliebsiella* while hot methanolic extract of *Raphanus sativa* roots revealed marked antibacterial activity only against *Staphylococcus aureus*. Thus, based on the results of present study, it may be inferred that both these plants possess promising pharmacological activities worth further investigation for drug- development.

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24. Reparative potential of ascorbic acid against lead and cypermethrin induced oxidative damage and alterations in drug metabolizing enzymes

Ajay Kumar and Anu Rahal

Present study evaluated reparative potential of ascorbic acid (100 ppm) in sub-acute toxicity of lead (Pb, 100 ppm) and/or cypermethrin (CPM, 50 ppm) in fifty four female Wistar rats (divided in to nine groups of six each) on the basis of physical and biochemical parameters. Both the toxicants failed to produce any clinically apparent toxicity. Physical attributes i.e. body wt and organ wt showed non-significant alterations on Pb, CPM as well as Pb+CPM treatment except for the early period of study. CPM hindered the absorption of Pb as well as its accumulation in bone but did not alter the protection offered by ascorbic acid while Pb promoted absorption of CPM and also reduced the protection offered by ascorbic acid. With an exception to liver, no significant change in protein content of RBC, kidney, spleen with a change in exposure to metal and/or pesticide. The biochemical profile was differentially affected. Oxidative parameters revealed an antagonistic toxicodynamic profile of Pb and CPM. CYP-450, ANDM, APH & GST were found to play an important role in detoxification of heavy metals and pesticides. Co-exposure to Pb and CPM on xenobiotic metabolizing systems appeared to be almost parallel to those produced by the individual toxicant. Cytochrome b_5, UGT activity and microsomal protein content were unaffected by the treatment. Pb, CPM were found to produce marked histopathological alterations in vital organs as compared to Pb+CPM treatment in rats. Thus induction of phase I and phase II hepatics xenobiotic metabolizing enzymes and improved antioxidant status of erythrocytes, liver, kidney and spleen by ascorbic acid provides strong evidence for considering ascorbic acid as a promising tool for chemoprevention against heavy metal and/or pesticide toxicity in humans and animals as it is consumed on a regular basis globally. Furthermore studies are required to unravel the molecular mechanistic of toxicological implications of the metalloid-pesticide binary mixture toxicity on the physical and biochemical parameters.

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25. Modulatory effect of ascorbic acid against arsenic and chlorpyrifos- induced oxidative stress and alterations in drug metabolizing enzymes

Dinesh Singh Rajpoot and Atul Prakash

Present study evaluated ameliorating effect of ascorbic acid (100 mg/kg body weight) in sub-acute toxicity of arsenic (40 ppm) and/or chlorpyrifos (5 mg/kg body weight) in fifty four male Wistar rats (divided in to nine groups of six each), consecutively for 28 days. Both the toxicants failed to produce any clinically apparent toxicity or mortality in rats. Physical attributes i.e. body wt showed non-significant alterations on As, CPF as well as As + CPF treatment while organ wt showed significant alterations. As and CPF may be disturbed their own metabolism, absorption and/or excretion but did not alter the protection offered by ascorbic acid. LPO was the main parameter significantly affected by exposure in all the tissues as well as erythrocytes examined, except in spleen was insignificantly affected. GSH, SOD, CAT, GST and GPx were significantly affected in all the tissues as well as in erythrocytes with some exceptions. CAT was insignificantly affected by toxicant treatments in erythrocytes. SOD showed most marked changes in liver and spleen. Ascorbic acid exhibited considerable reparative or protective effect against arsenic, chlorpyrifos alone or in combination as revealed by significant restoration of the values of LPO, GSH, SOD, CAT, GST and GPx. Co-exposure to As and CPF on xenobiotic metabolizing systems appeared to be almost parallel to those produced by the individual toxicant. Arsenic, chlorpyrifos and their co-exposure caused significant variation in the levels of microsomal protein. The enzymatic activities of cytochrome P_{450}, cytochrome b_5, APH, ANDM and UGT are significantly decreased. The enzymatic activity of GST was unaffected by the treatment. As, CPF were found to produce marked histopathological alterations in vital organs as compared to As + CPF treatment in rats. Thus induction of phase I and phase II hepatics xenobiotic metabolizing enzymes and improved antioxidant status of erythrocytes, liver, kidney, spleen and brain by ascorbic acid provides strong evidence for considering ascorbic acid as a promising tool for chemoprevention against heavy metal and/or pesticide toxicity in humans and animals as it is consumed on a regular basis globally. Furthermore studies are required to unravel the molecular mechanistic of toxicological implications of the metalloid-pesticide binary mixture toxicity on the physical and biochemical parameters.

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26. Studies on mechanistics pathway(s) of certain uterotonics in buffalo myometrium with particular reference to calcium signaling cascade

Abhishek Sharma and Satish Kumar Garg

Present study was undertaken to unravel the underlying calcium signalling mechanisms responsible for spasmogens (histamine, $PGF_{2\alpha}$, oxytocin and 5-HT)-induced myometrial contractions in non-pregnant (diestrous) and different stages of pregnant buffalo uteri collected from local slaughter house of Mathura. Isometric tension in myometrial strips was recorded under the resting tension of 2±0.5 g following mounting the tissue in Ringers -locke solution. Following an equilibration period of about 2 hr, myometrial strips exhibited a consistent and rhythmic pattern of spontaneity irrespective of the stages of pregnancy, albeit, the nature of myogenic spontaneity varied between different stages of pregnancy which was characterized by an increase in spikes height and decrease in frequency as the pregnancy advanced. Histamine was found to be most effective compared to $PGF_{2\alpha}$ and oxytocin in inducing myometrial contractions in nonpregnant (NP), early pregnant (EP) and mid pregnant (MP) uteri while oxytocin produced maximal relaxation in the late pregnant (LP) uteri. Nifedipine, a L-type voltage dependent Ca^{2+} channels blocker (VDCC), completely abolished the myogenic

spontaneity of uteri from all stages of pregnancy suggesting its contribution in generating spontaneous rhythmic contractions. In the presence of nifedipine, the DRC of histamine and $PGF_{2\alpha}$ was shifted towards right with decrease in maximal efficacy in NP (3.42±0.13 g vs 1.14±0.11 g and 1.29±0.07 g vs 0.85±0.08 g, respectively), EP (2.01±0.15 g vs 0.70±0.08 g and 2.02±0.13 g vs 1.76±0.15 g, respectively) and MP (1.58±0.06 g vs 1.33±0.06 g and 0.53±0.06 g vs 0.46±0.07 g, respectively) uteri. With the exception in MP, VDCC blockade also inhibited oxytocin-induced contractions in NP (1.99±0.16 g vs 1.18±0.14 g) and EP (2.16±0.18 g vs 2.07±0.15 g) uteri. Nifedipine elicited biphasic effect (initial rightward shift followed by leftward shift) with increase in efficacy of oxytocin (0.95±0.06 g vs 3.16±0.73 g) in MP uteri. Inhibition of Ca^{2+} release from sarcoplasmic reticulum by ruthenium red (RuR) apparently but non-significantly inhibited the contractile effects of these spasmogens. Blockade of both VDCC-dependent Ca^{2+} entry and SR Ca^{2+} release either restored or augmented the contractile effects of these agonists. Therefore it may not be unreasonable to inform that following blockade of VDCC and Ca^{2+} release from SR, activation of some other alternative excitatory pathways may be involved in either restoring or augmenting the spasmogens-induced contractile effect on buffalo myometrium. Inhibition of PLC-PI signalling pathways by U-73122 significantly shifted the DRC towards right with decrease in efficacy (1.29±0.07 g vs 0.63±0.10 g) in non-pregnant uteri. Interestingly, all the agonists produced relaxant effects in the myometrial strips from late pregnant uteri. In contrast to the findings on NP, EP and MP uteri, following inhibition of both VDCC-dependent Ca^{2+} entry and SR Ca^{2+} release, the maximal contraction elicited by these agonists was observed to be far below than the average amplitude of spontaneous contraction. Based on findings of the present study it may be conclude that VDCC plays major role in myometrial contractility induced by histamine (NP, EP, MP), $PGF_{2\alpha}$ (NP, EP, MP) and oxytocin (NP, EP) whereas intracellular source of Ca^{2+} seems to be involved but not the major pathways in regulating myometrial spontaneity or contractile responses to the exogenously used spasmogens. PL-C appears to play important role in $PGF_{2\alpha}$ -induced excitation in non-pregnant uteri. Down-regulations of the respective contractile receptors and up-regulation of inhibitory signalling pathways in mediating the relaxant effect of all these agonists in late stage of pregnancy needs further investigation.

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27. Lead-induced adrenoceptors modulation in rat and buffaloes myometrium

Udayraj P. Nakade and Satish Kumar Garg

Present study was aimed at biomonitoring blood lead levels in cows and buffaloes suffering with different reproductive disorders, effect of *in vitro* exposure of non-pregnant buffaloes and rats myometrium to lead, modulation of effect of *alpha* and *beta* adrenoceptor agonists, calcium chloride and high potassium chloride responses by lead and effect of *in vivo* exposure of rats to lead on myometrial responsiveness to *alpha* and *beta* adrenoceptor agonists, calcium chloride and high potassium chloride. Lead acetate (0.1 nM to 0.1 mM) produced concentrations-dependent contractile effect on buffalo myometrium and pD2 and E_{max} values of lead were 4.87±0.39 and 0.79±0.18 g, respectively. Calcium chloride also produced dose-dependent (1μM to 10mM) contraction and pD2 and E_{max} values were 1.88±0.5 and 3.94±0.22 g, respectively. Lead (1nM and 3μM) significantly ($P<0.05$) shifted the DRC of $CaCl_2$ towards right only at lower concentration (1nM) without change in potency. Phenylephrine (0.01nM to 10μM) produced concentration-dependent contractile effect on buffalo myometrium and its pD2 and E_{max} values were 6.00±0.25 and 1.78±0.27g, respectively. In the presence of prazosin (1μM), phenylephrine produced relaxation. In the presence of lead acetate (1nM or 3μM) also, phenylephrine produced dose-dependent contractile effect and its DRC was significantly ($p<0.05$) shifted towards right

without significant change in potency. Salbutamol (0.01 nM to 3 μM) produced concentration-dependent inhibitory effect on myometrium and its pD_2 and R_{max} values were found to be 7.48±0.35 and -0.89±0.49 g, respectively. Dose response curve of salbutamol was shifted towards right n the presence of lead (1nM or 3μM) without significant change in potency but the efficacy was reduced by 28-30 per cent. Lead acetate (10 nM to 0.1 mM) produced concentrations-dependent relaxant effect on rat myometrium and its pD_2 and R_{max} values were 6.15±0.77 and 0.16±0.04 g, respectively. In the presence of lead (1nM or 3μM), DRC of calcium chloride was shifted towards right with decrease in efficacy, but the change in potency and efficacy were not significant. Salbutamol (0.01 nM to 3 μM) also produced inhibitory effect on rat myometrium and pD_2 and R_{max} values were calculated to be 8.60±0.28 and 0.00±0.00 g, respectively and lead (1nM or 3μM) shifted the DRC of salbutamol towards right without significant change in potency and efficacy. Pre-treatment of rats with lead (30, 100, 300 ppm) resulted in changes in amplitude, mean integral tension or frequency of myometrial spontaneity but not statistically significant, except in 300 ppm group there was significant increase in frequency. Dose response curve of $CaCl_2$ was shifted towards left in all the lead-treated groups but nonsignificantly. Lead failed to alter the 80 mM KCl-induced myometrial contraction. Phenylephrine produced concentration-dependent relaxant effect on uteri of all the four treatment groups. The DRC's of phenylephrine in control and lead-treated groups almost superimposed with no significant change in pD_2 and R_{max} values. Similarly, salbutamol DRCs in control and lead-treated groups also superimposed over each other with no significant alteration in pD_2 and R_{max} values. Animals of different treatment groups did not exhibit any apparent signs of toxicity and there was no significant effect on weight gain as a result of lead treatment except in 100 ppm group wherein there was significant ($P<0.05$) decrease in body weight gain after 28days of exposure. Compared to control animals, there was 27.02% decrease in relative uterine weight in rats of groups treated with 30 and 100 ppm of lead acetate but it was not statistically significant. Gradual and linear increase in blood and bones lead levels in 30 and 100 ppm lead-treated groups was observed but in 300 ppm group, there was no further increase in lead levels after 14 days post-treatment, which possibly may be due to enhanced excretion from 14 days onward. Plasma progesterone levels did not differ significantly between different lead treatment groups but plasma progesterone levels in 30 and 100 ppm treated groups (8.78±2.52 ng/ml and 7.91±1.40 ng/ml, respectively) were markedly lower compared to control group (10.03±3.93 ng/ml). In all the lead acetate treated groups, dose-dependent inflammatory and degenerative changes in myometrium were observed.

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28. Sub-acute co-exposure toxicity studies of environmentally relevant heavy metals on urogenital system in male rats

Shaikh Mohammed Zoheb Mohammed Ahmed and Atul Prakash

The present study was undertaken to evaluate the renal and reproductive toxicity of mixture heavy metals (lead, arsenic, cadmium, mercury, iron and copper) and its possible amelioration with curcumin in male rats. The study was conducted in two phases- field survey (estimation of level of heavy metals and essential macro-minerals in different samples of soil, water, feed/fodder) and phase II (*In vivo* trial in rats at 10x and 100x dose of mixture of different metals at the levels obtained from phase-I study). Feed and water intake, body weight gain, absolute and relative organ weight, haemato-biochemical profile, oxidative stress, nephrotoxicity, reproductive toxicity and residual concentration of metals in kidney and testes were estimated. Based on the survey findings, a mixture of lead, arsenic, cadmium, mercury, iron and copper was formulated. In phase II study, forty two rats were divided into seven groups-control, vehicle control, 10x, 100x, curcumin, 10x plus curcumin and 100x plus curcumin. The

metal mixture resulted in a significant and progressive decrease in the feed and water intake resulting in dose dependent reduction in body weight gain at the two dose levels indicative of systemic toxicity which was substantially improved by co-administration of curcumin. The creatinine and urea levels in blood alongwith biomarkers of testes further testify the testicular damage while the rise in total and direct bilirubin are indicative of hepatic damage in mixture alone treated groups though ALT and AST were altered non-significantly. Dose-dependent increase in platelet count, mean corpuscular haemoglobin and haemotocrit and decrease in PCV and TLC was also observed when compared to control and vehicle control groups. A significant ($p<0.05$) dose dependent increase in the renal and testicular concentration of lead, cadmium and arsenic in 10x and 100x mixture group was observed as compared to control group. Pronounced oxidative stress was recorded in kidney as a significant rise in LPO and a non-significant decrease in SOD at 100x level. In testes, 10x and 100x exposure resulted in a significant ($p<0.05$) increase the level of lipid peroxidation and decreased the enzymatic activity of catalase,. Co-administration of curcumin with 10x and 100x metal mixture improves the level of GSH and enzymatic activities of SOD and CAT as compared to 10x and 100x alone intoxicated groups. A slight increase in the activity of SOD was recorded in the groups receiving curcumin with 10x/100x mixture. The dose dependent rise in the activity of a testicular enzyme, acid phosphatase was reverted by curcumin. No significant change in the activity of SDH and LDH was recorded. The GGT activity was further increased significantly ($p<0.05$) in 100x as well as 100x plus curcumin group treated group as compared to control and 100x alone group. Histopathology revealed a dose dependent damage of renal glomeruli and tubules. Marked degenerative to necrotic changes at pericapsular area with loss of cellular debris with increase in the periglomerular space were evident at higher dose of intoxication. In 10x/ 100x plus curcumin treated groups, only mild to moderate degree change in tubules with slight increase in periglomerualr spaces were recorded, suggesting reparative potential of curcumin in these intoxicated groups. Testes of control and curcumin group rats showed normal histological structure of germ cells and leydig cells in seminiferous tubules. In 10x treated group, there was mild to moderate degenerative changes in spermatogonial cells of seminiferous tubules and also there was degeneration of outer capsules of seminiferous tubules which intensified in 100x group. The histology was significantly restored by co-administration of curcumin. The antioxidant prophylactic potential of curcumin was well proven by the reduction in tissue accumulation of heavy metals leading to decline in oxidative stress level and better histological architecture and enzymatic activity in renal and testicular tissues.

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29. Binary mixture toxicity of Arsenic and Deltamethrin in Broiler chick and its amelioration with phytobiotics formulation

Vikram and Satish Kumar Garg

Present study was undertaken on 48 day old broiler chicks to evaluate the toxicity of binary mixture of arsenic and deltamethrin and ameliorative potential of Superliv concentrate, a phytobiotic. Chicks were randomly divided into eight groups (I-VIII) of six chicks each on 7th day. Chicks of different groups were offered different experimental feeds; Group I (control) basal feed, Group II- basal feed + Superliv concentrate (250ppm), Group III- basal feed + arsenic trioxide (50 ppm), Group IV-basal feed + deltamethrin (25 ppm ppm), Group V- basal feed + arsenic trioxide (50 ppm) + deltamethrin (25 ppm), Group VI- basal feed + arsenic trioxide (50 ppm) + Superliv concentrate (250ppm), Group VII- basal feed + deltamethrin (25 ppm) + Superliv concentrate (250ppm) and Group VIII - basal feed + arsenic trioxide (50 ppm) + deltamethrin (25 ppm) + Superliv concentrate (250ppm) for 42 days starting from day 8. Weekly body weights were recorded and weekly body weight gains were determined along with FCR. After 42 days of

experimental trial, blood samples were collected from birds of all the treatment groups for determination of different haemato-biochemical parameters. Thereafter, birds were sacrificed for collection of certain vital body organs, their absolute and relative weights were determined and tissues of these organs were also collected for histopathological examination. Arsenic and deltamethrin alone or both in combination markedly to significantly reduced body weights of chicks and this effect was counteracted by Superliv concentrate. Weekly FCR in chicks of different treatment groups ranged from 1.26 to 3.86 and the overall FCR in different treatment group varied between 2.10±0.07 and 2.84±0.06. Blood haemoglobin (mg/dl) level and PCV in chicks of different treatment groups did not differ significantly from those in control group but TEC $10^6/mm^3$) in group V was significantly lower while TLC ($10^3/mm^3$) was significantly higher in group III compared to control group. Lymphocytes count (%) was significantly lower in group III chicks but there was no effect on per cent count of other leucocytes in any of the groups. Plasma AKP activities were significantly higher in chicks of group III, V and VI compared to control group. Histopathological examination of the liver, kidneys, testes and spleen of chicks revealed different xenobiotics-induced varying degree of tissue insults in different treatment groups compared to those in control. Concurrent treatment of chicks with Superliv concentrate and arsenic, deltamethrin or both in combination revealed protective effects of Superliv concentrate against arsenic, deltamethrin and binary mixture of these both. Therefore, based on the findings of present study, it may be inferred that the phyobiotic used, Superliv concentrate, has bioenhancer, haematinic, hepatoprotective and immunomodulatory potential, however, further investigations are warranted using specific organ-specific biomarkers of toxicity.

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Ph.D.

1. Pharmacological evaluation of *Withania somnifera* (Ashwangandha), in normal as well as in pesticide toxicity in poultry

Rachna Verma and H.S. Panwar

Endosulfan (an organochlorine), Chlorpyrifos (an organophosphate), Deltamethrin and fenvalerate (synthetic Pyrithriod), insecticides and ashwagandha *(Withania somnifera)* were selected for this study. All pesticides (at their sub lethal dose), ashwagandha (at their therapeutic dose) and pesticide + ashwagandha (at same dose rate) were administered in respective groups for 24 week in cockerels. All related parameters of clinical, haematobiochemical, hepatic microsomal metabolising enzyme, immunological and residual analysis in various viseral organs were recorded at 12 and 24 weeks intervals. 'Cholinergic clinical signs such as mild salivation, lacrimation, diarrhea and mild hypothermia and a significant ($P<0.01$) reduction in body weight gain compared to untreated control were observed in all pesticide treated cockerels. Haematological studies revealed significant ($P<0.01$) decrease in TEC, TLC, lymphocyte, PCV and Hb in all pesticide treated cockerels. No alteration in TEC, TLC and PCV values was observed in pesticide + ashwagandha treated cockerels. A significantly ($P<0.01$) higher TEC, TLC and Hb values were observed in ashwagarfdha medicated cockerels after 24 weeks of medication as compared to pesticide treated and untreated cockerels. Total serum protein concentration significantly declined both at 12 and 24 weeks in all pesticide treated cockerels as compared to other groups. No change in the albumin was observed in this study. Level of serum globulins was significantly lower ($P<0.01$) in pesticide intoxicated cockerels both at 12 and 24 weeks intervals. A significant ($P<0.01$) depreciation~ in serum glucose level of pesticide treated groups as compared to other groups was observed at 12 and 24 weeks. Pesticide. significantly ($P<0.01$) increased the level of bilirubin, serum urea, serum creatinine, serum cholesterol after 12 and 24 weeks feeding. The serum calcium and serum potassium values are also significantly ($P<0.01$) increased in all pesticide intoxicated and pesticide + ashwagandha medicated groups. However, there is a significant ($P<0.05$) drop in serum sodium level in all pesticide treated as well as pesticide + ashwagandha treated groups at the same time levels. AChE activity was significantly depressed in all pesticide treated groups (more in chlorpyrifos intoxicated cockerels) in comparison to control and ashwagandha medicated groups. Aminotransferases AST and ALT revealed a significant ($P<0.01$) elevation in pesticide intoxicated group both at 12 and 24 weeks in comparison to control and ashwagandha treated groups. There was no change in the activity of AL T and AST in cockerels fed on Pesticide + ashwagandha even after 24 weeks feeding trial. A significant appreciation in alkaline phosphatase activity was reported in group pesticide fed groups in comparison to other groups. A significant ($P<0.01$) reduction in activity of hepatic microsomal enzymes, aniline hydroxylase, aminopyrene-N-demethylase and glutathione-s-transferase in PMS was observed in pesticide treated cockerels. However, there was no alteration in total protein content of PMS in this investigation. Activity of Cyt P 450 and b_5 was augmented significantly ($P<O.01$) in cockerels fed on pesticide and pesticide + ashwagandha medicated diet for 24 weeks. Significantly ($P<O.01$) lower values of HA titre, DTH response and LST in pestiqide treated cockerels revealed a immunosuppressive effect of the insecticide on humoral and cellular immunity of the cockerels. Ashwagandha treated cockerels, however, revealed significantly ($P<O.01$) higher HA titre, DTH response and LST in cockerels during 24 weeks trial. A significantly higher level of pesticide residues was determined in pesticide treated than pesticide + ashwagandha treated cockerels after 24 weeks in this investigation.

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2. Comparative pharmacokinetics and interaction studies of ofloxacin and meloxicam in yaks and cattle

F.A. Ahmad and Satish Kumar Garg

Disposition kinetic studies on ofloxacin (7.5mg.kg^{-1}) and meloxicam (0.5 mg.kg^{-1}) in yaks and cattle were conducted following a single intravenous, intramuscular and subcutaneous administration, while the interaction studies were undertaken after concurrent administration of both these drugs by intravenous and subcutaneous routes. Blood plasma concentrations of ofloxacin and meloxicam were determined using the well validated HPLC assay methods which were found to be linear and reproducible with the correlation coefficient value of >0.99 and the inter-day coefficient of variance of less than 10 per cent and the mean recovery of more than 90 per cent. Plasma concentrations versus time data were subjected to compartmental pharmacokinetic analysis using "PHARMKIT" software. Following IV administration of ofloxacin in yaks (7.5 mg.kg^{-1}), the values for $t_{½\alpha}$, $t_{½\beta}$, $t_{½\pi}$, $Vd_{(area)}$ and Cl_B were 0.14±0.01h, 1.08±0.03h, 3.82±0.60 h, 1.29±0.05 L.kg^{-1} and 0.83±0.01 L.h^{-1}.kg^{-1},respectively, while after IM administration the values of $t_{1/2Ka}$, $t_{½\beta}$, $t_{½\pi}$, $Vd_{(area)}$ and F were found to be 0.34±0.05 h, 1.82±0.18 h, 31.29±10.50h, 1.36±0.25 L.kg^{-1} and 97.05±13.45 per cent, respectively. After SC administration of ofloxacin in yaks, the values for $t_{1/2Ka}$, $t_{½\beta}$, $Vd_{(area)}$ and F were calculated to be 0.31±0.01 h, 1.02±0.14 h, 0.85±0.28 L.kg^{-1} and 65.71±0.06%, respectively; thus suggesting that IM route should be preferred over SC route. Following IV administration of ofloxacin alone or in combination with meloxicam, plasma levels (mean±SE) were significantly higher in yaks and cattle on concurrent administration compared to ofloxacin alone. Elimination half life ($t_{1/2\pi}$) of ofloxacin was found to be significantly lower in yaks than in cattle thus, suggesting faster elimination from yaks. $Vd_{(area)}$ value was significantly higher in yaks (1.29 L.Kg^{-1}) than in cattle (0.84L.Kg^{-1}) thereby suggesting better penetration of ofloxacin in yaks than cattle. Following meloxicam administration either alone or meloxicam+ofloxacin concurrently by intravenous route in yaks and cattle, plasma meloxicam levels and majority of the pharmacokinetic variables did not differ significantly between yaks and cattle. Pharmacokinetic determinants of ofloxacin in yaks and cattle following IM administration did not differ significantly from each other. But, elimination half life of meloxicam was significantly lower in yaks thus, indicating faster elimination of the drug from yaks. Ofloxacin should be administered to both yaks and cattle @ 7.5 mg/kg body weight and repeated at 24 h interval by IV or IM route. The loading and maintenance doses of meloxicam by intravenous administration to yaks were calculated to be 1.05 and 0.88 mg.kg^{-1} body weight, respectively repeated at 12 h interval, while for cattle, the loading and maintenance IV doses were calculated to be 0.97 and 0.79 mg.kg^{-1} body weight, respectively repeated at 12 h interval. For intramuscular administration of meloxicam in yak, the loading dose of 0.50 mg.kg^{-1} and maintenance dose of 0.36 mg.kg^{-1} at 12 h interval and in cattle a loading dose of 0.72 mg.kg^{-1} and maintenance dose of 0.62 mg.kg^{-1} is recommended for administration at 12h interval. Pharmacokinetic interaction between ofloxacin and meloxicam in yaks and cattle after IV or SC administration was not very substantive or significant; therefore, adjustment in the dosage regimens is not of much concern.

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Veterinary Physiology

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1. A study on epididymal and ejaculated buck semen

Devendra Kumar Singh and Sarvajeet Yadav

During this study ejaculated semen was collected from four healthy Barbari bucks of 2 to 4 years of age from the flock maintained under the traditional farm practice by the department. For epididymal spermatozoa study, the testes were brought from the local abattoir soon after slaughter and the semen extracted within two hours of collection. Non significant differences in volume, gross motility sperm concentration per cent live, between acrosomal damage, GOT enzyme release total protein in seminal plasma between bucks and within buck were recorded. The sperm concentration and per cent progressive motility in cauda semen was significantly higher than caput, isthmus and vas deferens semen. The per cent live spermatozoa after cold shock in caput spermatozoa were significantly higher than isthmus, cauda and vas deferens spermatozoa. A non-significant difference for percent live spermatozoa, percent acrosomal damage, per cent abnormal spermatozoa among caput, isthmus, cauda and vas deferens. The per cent total cytoplasmic droplets in caput spermatozoa were significantly higher than those of isthmus, cauda and vas deferens spermatozoa. The sperm concentration in cauda semen was significantly less than ejaculated neat semen. Live percentage after cold shock was highly significant ($P<0.01$) lower in cauda region, whereas live percentage in caput and isthmus region was high and statistically similar. The per cent acrosomal damage (total) in ejaculated neat semen were significantly less than cauda semen. GOT release in ejaculated neat semen was significantly less than cauda semen. Total protein in ejaculated neat semen was significantly less than cauda semen. The Tris (Salamon) diluent gave better motility than Tris (Hann) diluent. The progressive motility, per cent live spermatozoa, per cent acrosomal damage in cauda epididymis was significantly higher than other component of epdidymis and the vas deferens.A non-significant difference between ejaculated diluted semen and caput diluted semen at 0 hr. whereas at 72 hrs there was significant difference. The per cent live spermatozoa and per cent acrosomal damage in ejaculated diluted semen were significantly less than caput-diluted semen at 0 hr and 72 hrs. Isthmus diluted semen was inferior to ejaculated diluted semen was significantly less than ejaculated diluted semen at 72 hrs. A non-significant difference in per cent progressive motility per cent acrosomal damage and GOT release was noted between ejaculated diluted semen and cauda diluted semen at 0 hr and 72 hrs. The per cent live spermatozoa in ejaculated diluted semen were significantly less than cauda diluted semen. The per cent progressive motility in vas deferens diluted semen was significantly less than ejaculated diluted semen. The per cent live spermatozoa in ejaculated diluted semen were significantly less than vas deferens diluted semen.

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2. Assessment of different serum supplementation on *in vitro* maturation and fertilization of prepubertal goat oocytes

Eeshwari Narain Yadav and D.K. Johari

Present study was carried out to record the effect of different techniques viz slicing, aspiration, puncture and isolation on oocyte recovery, in which 350 prepubertal goat ovaries were processed. Biometry of the individual ovary was taken separately in each technique used for recovery of oocytes. There were significant differences ($p<0.05$) among the large, medium and small follicles recorded between different techniques. The average recovery rate of slicing and puncture techniques differ significantly ($p<0.05$) from that of aspiration and isolation. The investigation of relation between type of follicle and quality of oocyte revealed that oocytes of superior quality recovered at higher rate from medium followed by small and large follicles.

Results showed that EGS, ESS, and FBS were better than control medium (TCM-199 supplemented with sodium pyruvate, gentamicin and L- glutamine) and did not differ significantly among themselves ($p>0.05$) in their ability to induced maturation of prepubertal goat oocytes. The *in vitro* matured oocytes, mature in maturation media supplemented different sera, were fertilize in the TALP medium enriched with 20% EGS and 10 μg/ml heparin. At 18 h post insemination oocytes separated from sperm and transfer on pre-incubated granulosa cell monolayer. The cleavage rate in EGS and FBS did not differ significantly. However, there is significant difference in cleavage rate of EGS and FBS from that of ESS and control. In conclusion, the puncture technique of oocyte recovery and use of 20% EGS in maturation medium were found to be the best for oocyte recovery and *in vitro* maturation.

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3. Evaluation of pineal peptie profile and anti-oxidant activity of melatonin administration in cyclic adult goats

Arti Pathak and M.P. Agrawal

The present work was carried out on twelve healthy female goats of 2-3 years of age. The goats were divided randomly into two groups viz. control and experimental group. The experimental group animals were given melatonin @ 2 mg/ animal/ day subcutaneous upto 60 days. The blood samples were collected at weekly interval upto 60 days. The exogenous melatonin administration resulted in significantly ($P<0.01$) lower values of haemoglobin, total erythrocyte count, total leucocyte count and packed cell volume in the experimental group animals in comparison to control group animal. Where as no significant effect of melatonin administration could be observed on the erythrocytic indices (MCV, MCH and MCHC) between the two groups of animals. The activity of enzymes alkaline phosphatase and transaminase (AST and ALT) and serum potassium were significantly ($P<0.01$) whereas the level of T_4 was significantly ($P<0.05$) found to be lower in the experimental group animals as compared to control group animals.. The level of serum glucose total protein, albumin and A:G ratio were significantly ($P<0.01$) higher in the experimental group in comparison to control group animals. There was no significant difference in the blood level of cholestrol, serum globulin, sodium, blood urea nitrogen and T_3 between the two groups due to exogenous melatonin administration. The blood level of oestrogen was significantly ($P<0.01$) lower in the experimental group whereas the level of progesterone was significantly ($P<0.05$) higher in the experimental group as compared to control group A decrease in the weight gain (%) was observed in the melatonin treated group in comparison to control group animals. The melatonin administration resulted into significantly lower values of lipid peroxides in experimental goats. The glutathione-S-transferase level was significantly increased in melatonin treated group as compared to control group. The sample of pineal gland showed 7 to 9 protein/peptide bands. However, most of the samples showed 8 bands. The samples showed three clear bands above 97 KDa molecular weight, 3 bands between 97 and 66 KDa molecular weight, single band between 66 and 43 KDa molecular weight and one band between 43 and 29 KDa molecular weight. Whereas no bands could be observed between 29 and 14 KDa Molecular weight.

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4. Quality production and freezabiliy of Bhadawari spermatozoa

Vasundhara and Sarvajeet Yadav

The comparative efficacy of three widely used extenders based on changes in micro morphology, viability, and fertilizing capacity of spermatozoa during cryomanipulation was investigated. The three basic diluents were EYT, EYT+G and EYT+G+M. the spermatozoa used for study were of five Bhadawri buffalo bulls of different ages. For deep freezing of semen the

concentration of spermatozoa in diluted semen was adjusted to125x10^6/ml. The final concentration of cryoprotactant (Glycerol) was maintained at 7 percent. An equilibration period of 3 hours was provided before subjecting the samples to cryopreservation. It was observed that Percent motility and Percent live spermatozoa values were significantly differ in different bulls ($P<0.01$), in different diluents ($P<0.01$), and in different stages of freezing and post thaw. Acrosomal damage during cryomanipulation were non-significant between bulls ($P<0.01$), between diluters ($P<0.01$), between stages of freezing ($P<0.01$).the value of critical differential for different diluters was 0.57 which revealed that EYT+G gives least damage to acrosome during cryomanipulation. Hypo osmotic swelling test showed that there was a significant different between bulls ($P<0.05$), and highly significant difference between diluents ($P<0.01$), and between solutions of different osmolarity ($P<0.01$). Results revealed that there was significant difference in microbial load between bulls ($P<0.01$), between diluents ($P<0.01$), between dilutions ($P<0.01$), and between stages of freezing ($P<0.01$).

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5. Comparative study on some physiologically important biochemical parameters and leucocytic pictures in cerebrospinal fluid and blood of Barbari goats

Vaibhav Misra and D.K.Johari

The present investigation was carried out on ten healthy Barbari goats. Goats were sexually divided into two groups i.e. group one having five bucks and another group comprised of five does. The cerebrospinal fluid (CSF) and blood were collected four times from each group at fortnightly interval. The collected samples were analysed for total leucocyte count, differential leucocyte count, protein profile, urea nitrogen, cholesterol, uric acid, creatinine, aspartate amino transferase (AST), aspartate alanine transferase (ALT), alkaline phosphatase, acid phosphatase, sodium, potassium, calcium, phosphorus and chloride. Results of present studies in goats revealed that total leucocyte count, lymphocyte count, neutrophil count, monocyte count, level of total protein, albumin, globulin, cholesterol, aspartate amino transferase (AST), alkaline phosphatase, acid phosphatase, potassium, calcium and phosphorus was lower in CSF as compared to blood of normal healthy goats.Concentration of CSF uric acid was higher in male as compared to blood but lower in female. Whereas, CSF sodium level was higher in male as compared to blood whereas no such variation was observed in female goats. Urea nitrogen level, activity of aspartate alanine transferase (ALT) and A: G ratio were not differ between CSF and blood of normal healthy. Eosinophil and basophil were not observed in CSF compared to blood of normal healthy goats.

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6. Effect of dietary supplementation of zinc and selenium on haemato-biochemical profile and antioxidative enzymes on Barbari goats

Agresh Kumar and Jitendra Kumar

The investigation was conducted in 12 Barbari goats (6 Males and 6 Females) aged between 2 to 4 years, weighing 25-35 kg reared at experimental shed of Department of Physiology. All the animals were screened for zinc and selenium level in blood serum as well as the zinc and selenium were estimated in the soil and fodder. The experimental animals were divided in to control and treated/ experimental group containing six animals (3 Males and 3 Females each). The animals of the control group were not supplemented whereas the treated group was supplemented with zinc and selenium in inorganic form with 150-ppm Zinc Sulfate and 0.50-ppm Sodium Selenate respectively for two months. The study revealed that most of the

haematological and blood biochemical parameters did not show significant change; whereas the antioxidative enzymes of the blood showed significant increase. The level of MDA production decreased significantly indicating a gradual decrease in the reduction of stress. Further studies are required to find out the mechanisms by which the content of antioxidative enzymes in the blood increases and how they are associated with reduction in free radicals. The beneficial effects of Zinc and Selenium can be scientifically used to promote growth and production in the animals

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7. A study on effects of ageing and season on cryopreservability of Barbari buck semen

Arvind Kumar and Sarvajeet Yadav

The effects of ageing and different seasons were evaluated on the cryopreservability of Barbari buck semen. The present study was designed to study the effects of ageing and season on the freezability of semen of the Barbari breed of goats. Eighteen normal, healthy adult Barbari bucks aged between 8 months to 8 years reared at the experimental goat sheds of the Department were used as experimental animals. The result reveled that S2 (winter) was found to be unsuitable both for semen collection and semen preservation. The S1 and S3 were found differentially suitable for semen collection and cryopreservation. The group 2 (G2) (adult) was found to be the best group of animal for all the seminal attributes. The G3 showed impaired quality of sperm in terms of DNA damage. This may lead to the poor outcome of sperm survival and negative AI result. G1 cannot be taken as choice of animal for semen collection and preservation as they are sexually not mature enough to qualify for cryopreservation and long term storage.

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8. Studies on semen quality, antioxidative enzymes in semen and reproductive hormone of Barbari bucks after dietary supplementation of zinc and selenium

Pankaj Kumar and Sarvajeet Yadav

The study was conducted on 8 bucks aged between 2 to 4 years, weighing 25-35 kg reared at experimental shed of the Department. All the animals were screened for zinc and selenium level in blood serum. The experimental animals were divided in control and test groups containing four animals each. The animals of the control group were not given any supplementation whereas animals of test group were given supplementation of zinc and selenium in inorganic form with 150-ppm Zinc Sulfate and 0.50-ppm Sodium Selenate for entire duration of the experiment. Zinc and selenium was dissolved in triple distilled water and administered orally. Zinc and selenium supplementation improved the hormone status (Testosterone, T_3 and T_4) required for growth and development of testes, interstitial cells, secretory activity of accessory sex glands and development of secondary sexual characteristics and spermatogenesis. Zinc and selenium supplementation increased antioxidative status of semen which is essential for preventing free radical damage of spermatozoal membrane and nuclear DNA. Thus improvement in hormonal status in serum and antioxidative status in seminal plasma as well as in spermatozoa has helped in improving all seminal parameters.

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9. Studies on semen quality, antioxidative enzymes in semen and reproductive hormone of Barbari bucks after dietary supplementation of zinc and selenium

Tribhuvan Kumar and Sarvajeet Yadav

Present study was conducted on 8 bucks aged between 2 to 4 years, weighing 25-35 kg which were reared at experimental shed of the Department. All the animals were screened for zinc and selenium level in blood serum 15 days earlier to the experiment to know the status of the Zinc and Selenium level in the blood. The experimental animals were divided in to control and test group containing four animal each. The animals of the control group were not given any supplementation where as test group were given supplementation of zinc and selenium in inorganic form with 150-ppm Zinc Sulfate and 0.50-ppm Sodium Selenate for 105 days. The day of supplementation was regarded as the 0 day. The study concluded that- Zinc and selenium supplementation markedly improved antioxidative status of cryopreserved sperm which is essential for preventing free radical damage of spermatozoal membrane and nuclear DNA. The improvement in the anti oxidant system in terms of the sperm anti oxidant enzymes reduced the generation of the free radicals simultaneously the scavenging activity of the antioxidant enzymes protected the sperm damage from free radicals. The supplementation of Zinc and selenium can be carried out to improve the qualitative aspects of the semen to get better post thaw quality for the optimization of the AI programme. Similar study can also be recommended in case of cow and buffalo bulls where screening of micro minerals in blood serum and seminal plasma can be done and accordingly supplementation strategy can be adopted to improve their semen quality to achieve the second white revolution.

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10. Characterization of segment specific epididymal spermatozoa in bucks

Prashant Kumar Swarnkar and Jitendra Kumar

The present study was designed to characterize the sperms in different segments of epididymis. The study was carried out in 15 pairs of testes. The study revealed that, the protein content increased significantly ($p<0.01$) in tissue homogenate from caput to cauda epididymis where as protein content of epididymal fluid gradually decreased from caput to cauda epididymis. Sperm motility was increased significantly ($p<0.01$) from caput to cauda epididymis where as sperm livability exhibited no significant difference between the three parts of the epididymis. Significant difference was found for sperms having proximal droplet between all segments where as sperms having distal droplets were found to be significantly different between three segments. Sperms having no head were found to be significantly different between caput, corpus and cauda epididymis. Acrosomal integrity was found to be significantly different between three segments. Acrosomal integrity in terms of intactness increased from caput to cauda. Hypoosmotic swollen positive spermatozoa showed a significant difference between caput, corpus and cauda parts of epididymis and HOST positive spermatozoa was increased from caput to cauda epididymis. Comet assay was not done for the caput and corpus epididymal sperms due to low sperm count as required for the Comet assay while comet assay was done for the cauda epididymal sperm. The results revealed 92-93% of sperms with compact and intact DNA. SDS-PAGE revealed caput epididymal fluid showed 12 proteins; corpus exhibited 10 proteins and cauda exhibited 11 protein bands. SDS -PAGE showed caput epididymal tissue homogenate had 7 proteins corpus 8 proteins and cauda exhibited 11 proteins. Proteins of mol. Wt 90, 35, 6.5 and 3 KDa were purely secretary. Proteins having molecular weight 215 KDa, 205 KDa, 195 KDa, 45 KDa, 18 KDa, 15 KDa and 12 KDa were found to be both structural and secretary. Proteins of mol. Wt. 100 KDa, 95 KDa, 75 KDa and 70 KDa were purely

structural. Further studies are required to validate the roles of the epididymal proteins in the process of sperm maturation and function.

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11. A study on effect of dietary vitamin-E on semen quality and cryopreservabilty of Hariana bull

Santosh Singh and Sarvajeet Yadav

The study was conducted on 8 Hariana bulls aged between 3.5 to 4.5 years, weighing between 350-450 kg, reared at DDD farm. The animals selected for the experiment were divided into two groups viz. control and test. Each group comprising of 4 animals. The animals of control group were not supplemented with vitamin E while the test group were supplemented with vitamin E @ 600 IU per animal per day, as Evion, Tocopherol acetate (Merck) making laddu of gur/ concentrate ration for a period of 70 day. The semen samples were collected at regular intervals both before and after Vitamin E supplementation. Collected sample was divided into two equal fractions. One fraction was cryopreserved and another was utilized to analyze quality of neat semen. Parameters analyzed in neat semen were- volume, mass motility, percent live, concentration, abnormal sperms, acrosomal integrity and HOST, Later cryopreserved semen samples were evaluated for progressive motility, percent live, concentration, abnormal sperms, acrosomal integrity and HOST. All the results obtained were within normal range. Results obtained during the study were compared and analyzed statistically both between and within the groups to study the effect of Vitamin E supplementation on quality of semen in Hariana bull. Analysis revealed a positive effect of Vitamin E supplementation on the parameters evaluated to analyze the quality both in neat and cryopreserved semen. Hence it can be concluded that Vitamin E supplementation improves the semen quality of breeding bull in neat and cryopreserved semen and could be a tool to enhance the success rate of Artificial Insemination.

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12. Studies on effect of antioxidants on cryogenic manipulation in Bhadawari bull semen

Pawan Kumar Mittal and A.K.Madan

This study was conducted to determine the effects of vitamin E, Vitamin C and combination of vitamin E+C on standard semen parameters i.e. motality, percent live sperms, concentration, percent abnormal sperms, HOST and acrosomal integrity of Bhadavari bull semen after the freeze-thawing process. Ejaculates collected from four Bhadavari bulls were evaluated and pooled at 37 °C. Semen samples, which were diluted with a Tris-based extender containing the antioxidants vitamin E (5 mM) and vitamin C (5 mM), and an Vitamin E+C combination (13%), and an extender containing no antioxidants (control), were cooled to 5 °C and frozen in 0.25-ml French straws in liquid nitrogen. Frozen straws were thawed individually at 37 °C for 20 s in a water bath for evaluation. Semen extender supplementation with vitamin E (5 mM) and vitamin C (5 mM), and an Vitamin E+C combination (13%) caused significant ($P<0.05$) increases in all sperm attributes while significant ($P<0.05$) decreases was observed in total abnormality rates in comparison to the control group indicating that the supplementation of antioxidant in form of vitamin and their combination improves the quality of post thaw semen A Significantly higher values of semen parameters were observed in the T-3 followed by T-1 and T-2 indicating that the combination of vitamin E+C has most profound role in protecting sperms against ROS production and cold shock when compared to Vitamin E and vitamin C supplemented alone in the extender for semen dilution.

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13. Capacitation like changes in the spermatozoa during the process of cryopreservation of Barbari buck semen

Gunjan Baghel and Sarvajeet Yadav

This experiment was designed to compare the capacitation like changes in spermatozoa of Barbari buck during different stages of processing by using fluorescence patterns observed in chlortetracycline (CTC) staining. For this purpose ejaculates were collected from five Barbari bucks using artificial vagina at biweekly intervals. Freshly collected semen was divided into two equal fractions, one fraction of collected semen was examined for different seminal attributes related to semen quality and rest was diluted (200 x 106 motile spermatozoa/ml) in Tris extender and used for processing for cryopreservation. Seminal attributes in freshly ejaculated spermatozoa were compared with that in extended, equilibrated and frozen thawed semen. No significant difference ($P<0.05$) was found in seminal attributes of freshly ejaculated semen. All the results obtained were will within normal range. A significant difference ($P<0.01$) was observed in various parameters during different stages. The results suggested that diluting, cooling and freeze thawing of goat semen cause an increase in capacitation and acrosome reaction status. The most remarkable effects were after the freeze thawing stage which could be due to the cumulative effects of extending and cooling. The study concluded that the phenomenon-of cryopreservation induce cryocapacitation or premature capacitation of buck spermatozoa which can render sperm membranes to fuse prematurely reducing their fertile life. Promotion of capacitation, before reaching the site of fertilization may result in the reduced fertility of cryopreserved spermatozoa and poor results in artificial insemination (A.I.).

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1. Seasonal effect of exogenous melatonin administration on some haemato-biochemical parameters, reproductive and thyroid status in sheep

Jitender Kumar and M.P. Agrawal

Effect of subcutaneous exogenous melatonin administration at two dose levels (1 and 2 mg/animal) for sixty days was studied in twelve rams and twelve ewes aging between 2 to 2.5 years weighing 25-30 kg. The animals were divided into three groups of four male and four female each. Seasonal effect on heamato-biochemical parameters, reproductive and thyroid status macro and trace element and wool quantity and quality was studied. The haemogram suggested generalized anaemia as suggested by haematological stress syndrome observed after melatonin administration. Melatonin produced significant hyperglycemia and blood urea nitrogen increases and the increase was dose dependent. Triglycerides levels and serum cholesterol level decreased showed overall decrease in at both the dose levels. Total protein concentration, albumin, globulin contents and A:G ratio increased significantly in melatonin treated animals as compared to control group animals. Serum sodium and potassium levels revealed opposing trend. Significant hypernatremia, hyperchloremia and hypokalemia was observed at both the dose levels along with hypercalcemia and hyperphosphatemia, but there was no effect on Ca:P ratio. The mean plasma tri-iodo-thyronine and thyroxine levels in melatonin treated sheep did not change significantly. Melatonin administration resulted into significantly higher values of ejaculated semen volume, motility, sperm concentration and percent live sperm, whereas the sperm abnormalities and acrosomal abnormalities were less as compared to those in control groups. All the above-mentioned variations were directly proportional to the dose of melatonin. No appreciable change was observed between the colours of semen of animals in different groups. As a result of melatonin treatment, seminal plasma GOT and GPT activities significant decreased while the activity of acid phosphates and LDH increased. Exogenous melatonin administration to sheep at different dose levels did not significantly affect the wool yield, number of crimps, diameter sheep revealed that as a result of melatonin administration, the onset of oestrous was advanced and the duration of oestrous was more as compared to those of control animals. Trace elements, namely nickel, manganese, iron, zinc and copper concentration did not show any variations as a result of melatonin administration. But the cobalt concentration was significantly reduced.

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2. Influence of exogenous melatonin on certain hormonal, enzymatic, biochemical and immunological profile in cycling female Barbari goats

Jayant and M.P. Agrawal

Melatonin has been heralded as everything from snake-oil to miracle cure-all. Melatonin is the principal hormone of the pineal gland with a power to control many biological functions, particularly those that are controlled by the photo period and darkness. The pineal gland acts as a transducer of neuro-endocrine signals receiving visual information from the retina, transmitting it to the hypothalamus via retino-hypothalamic tract (RHT) and ultimately to the pineal gland via superior cervical ganglion, resulting into modulation of melatonin secretion into the humoral system. Some cells and body fluids contain exceptionally high levels of melatonin. Melatonin may be used to stimulate the immune response during viral and bacterial infections in order to strengthen the immune reactivity under prophylactic procedure. Besides other biochemicals, melatonin is also fast coming up as a potent antioxidant. The present study was conducted during the months of May to July and December to February showing an average ambient temperature of 32.9°C and 17.3°C, respectively. Twenty-four female Barbari nannies of the age group between 2 to 3 years and weighing between 17 and 24 kg were grouped into four groups- two groups for studying haematology, biochemical and hormone profile; 1) control group given treatment for 10% ethanol as placebo and 2) melatonin treated group given subcutaneous injection of Melatonin @ 300μg per kg body weight per animal per day for 60 days at 16.00 hours. Other two groups for studying immunomodulatory effect of melatonin as 1)control receiving antigen with placebo and 2) experimental group treated with antigen and Melatonin as given for treated group above. With the results obtained from the present study it may be concluded from the present study that Tiaprost can be suitably applied for Heatsync in goats through subcutaneous injection 11 days apart, as an alternative to prostaglandin $F_{2\alpha}$ in goats. The duration of onset of estrus is shorter when treatment is meted in November than in April. Melatonin help to ameliorate heat stress through compromising the energy metabolism adjustment demanded by the body at different environmental conditions. Melatonin did not alter the enzyme activity much, it may also be concluded that this hormone, melatonin did not have any pathological effect on the liver at the dose rate of 300μg/kg BW. The biochemical, hormonal and haematological process of the animal showed seasonal variations, influenced either by photoperiod, maintaining a steady correlation with circulating levels of melatonin. Prolactin level is increased by melatonin treatment, thus causing anestrous in the animals. This can be suitably tailored for estrous synchronization programmes. Progesterone value recorded in the winter season reveals clearly the extreme function of luteal tissue. In the season the luteal phase appears to be prolonged under the influence of melatonin which leads to suggest that animals mated or inseminated artificially in the evening hour's stands a better probability of sustaining pregnancy and reduce embryonic mortality. Melatonin enhances the cellular immunity, but not humoral immunity in goats as recorded in this study.

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Veterinary Public Health

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M.Sc.

1. Studies on bacterial profile of goat, pig and poultry meat and its public health significance

Himanshu Kumar and Basanti Bist

A total number of 123 samples comprising of 25 each from chevon pork and poultry meat, 24 from butcher's hands and butcher's knife each were processed to assess the bacteriological status of above mentioned samples. Quantitative examination of samples was performed by SPC, Coliform count; Staphylococcus count and MPN while qualitative examination by isolation of bacterial pathogens. For chevon, pork and poultry SPC (cfu/gm) was 7.8, 8.1and 6.9 while coliform count (cfu/gm) was 4.24, 4.34 and 4.1 respectively. For chevon, pork and poultry the mean value for staphylococcus count (cfu/gm) was 5.7, 5.6 and 4.9 while MPN/gm was recorded as 7.3, 6.8 and 6.5 respectively. In this study highest contamination of SPC was found for pork and chevon was most contaminated for staphylococcus followed by pork and poultry meat. The MPN values for chevon were recorded highest and lowest for poultry meat. In the qualitative examination 180 isolates of bacteria were procured from 123 meat and swabs samples out of which *E. coli* (40), staph *spp* (31), *Salmonella spp* (20), *Klebsiella spp* (23), *Bacillus cereus* 14, Corynebacterium spp 6, streptococcus 27, proteus vulgaris 11, and pseudomonas aeruginosa 8 were found. A total 112 isolates were subjected to antibiogram, higher range of sensitive were recorded for ciprofloxacin (100%), pefloxacin (100%), Gentamycin (80%), chloramphenicol (71.4%), Amoxicillin (67.8%), tetracycline (60.7%), streptomycin (58%) and kanamicin (53.2%).

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2. Seroprevalence of brucellosis in cattle and buffaloes in certain areas of Gorakhpur district ant its public health significance

Vikas Kumar and Basanti Bist

Blood serum samples were collected from 579 cattle, 407 buffaloes and 210 human beings and serologically tested using RBPT, SAT and Indirect ELISA. These tests were very effective in accurate diagnosis in brucellosis. Over all Seroprevalence of brucellosis in cattle and buffaloes in 17 areas of Gorakhpur was 1.72% and 1.11% animals were doubtful reactors. Out of 210 human sera samples only one human serum sample for brucella antibody (0.48%) and one serum gave doubtful reaction (0.48%). Overall Seroprevalence of brucellosis in cattle was 1.55% (9/579) in 17 areas of Gorakhpur in UP and 1.03 animals were doubtful reactors (6/579). In buffaloes, Seroprevalence was recorded as 1.96% (8/407) and 5 buffaloes were doubtful reactors. 14 strains of bacteria were isolates from vaginal swabs of cattle and buffaloes. The isolates were identified on the basis of cultural microscopic and biochemical reactions. These strains were identified as *Brucella abortus* (2), *Staphylococcus aureus* (5), *Corynebacterium pyogenes* (2), *Listeria monocytogenes* (2), *E. coli* (2), and *Nessesia gonorrhoeae* (1). The isolates were highly sensitive to ciprofloxacin, Norfloxacin, tetracycline andstreptomycin antibiotics. These may be given as drug of choice for genital tract infections of cattle and buffaloes.

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3. Comparison of ELISA and PCR vis-à-vis culture methods for detecting *Aeromonas* spp in foods of animal origin

Sushrut Arora and Basanti Bist

Aeromonads are facultative anaerobic, Gram-negative bacilli residing in family Aeromonadaceae. These are ubiquitous organisms found in aquatic environments; soil; food items, including meat and meat products, fish, eggs, milk and milk products and vegetables; and animal and human faeces. Conventional cultural methods to isolate and identify

aeromonads have proved to be time and labour intensive. These facts and the increased interest of workers all around the world In this emerging pathogens has led to the development of newer methodologies for its efficient and rapid detection. Öut of a total 50 milk samples screen for the presence of Aeromonas by the three methods vis. Indirect ELISA, duplex PCR and cultural method only 1 (2.00%) were found positive by all the three methods. Similarly 50 samples of chicken were tested by all three methods. Three samples turned out to be positive. Thus all the three methods compared in the study showed a good correlation amongst them in detecting naturally contaminated food samples.

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4. Studies of bacterial profile of goat meat and its public health significance

Vijay Kumar Bhaskar and Basanti Bist

A total of 123 samples comprising 75 goat meat, 24 butcher's hands and 24 butchers knife were processed for bacteriological analysis from retail outlets of Mathura. Quantitative examination was performed by Standard Plate Count, Coliform Count and Staphylococcus Count and Qualitative examination by isolation of bacterial pathogens. The mean value of standard plate count for chevon, butcher hands and butchers knife was 7.841, 7.814 and 7.829 respectively. The mean value of coliform count for chevon, butcher hands and butcher knife was 4.313, 4.32 and4.27 respectively. The mean value of Staphylococcus count was recorded as 2.481, 2.756 and 2.751 for chevon, meat butchers hand and butchers knife respectively. A total 212 isolates of bacteria were procured from 75 meat samples and swabs. The percent isolation of pathogens was *E. coli* 16.98 (36/212), *salmonella spp.* 5.66 (121/212), *Staphylococcus aureus* 20.28 (43/212), *klebsiella spp* 9.91 (21/212), *bacillus cereus* 6.13(13/212), *Corynebacterium spp.* 3.30 (7/212), streptococcus faecalis 8.96 (29/212), *Proteus vulgaris* 1.89 (4/212) and *Pseudomonas aeruginosa* 6.13 (13/212), *Streptococcus pyogenes* 4.72 (10/212) and unidentified cocci 7.55(16/212) unidentified rods 8.49(18/212).

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5. Studies on bacterial contamination of fish meat with special reference to *Salmonella* and *E. coli* and its public health significance

L.N. Gupta and Basanti Bist

A total of 110 fish meat samples were collected from different localities of Mathura city. The samples were analysed for bacterial load by Standard Plate Count, Coliform count, and Staphylococcus count for quantitative examination and for qualitative examination only *E. coli* and Salmonella organism were isolated. The mean value SPC, Coliform count and Staphylococcus count from fish meat were 7.99, 3.41 and 4.46 respectively. A total 11 isolates of *E. coli* and 5 isolates of salmonella from fish meat samples were isolated. The serotype of salmonella belongs to *S.Typhimurium* (1) and S.Heidelberg. *E. coli* isolates show higher range of sensitivity to ciprofloxacin, Ampicillin, Norfloxacin, Gentamycin, and Erythromycin and whereas Salmonella isolates were highly sensitive to Ciprofloxacin, Erythromycin and Ampicillin.

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6. Bacterial contamination in meat of poultry and poultry eggs with special reference to *E. coli* and *Salmonella* species and its public health significance

Vinod Kumar Yadav and Basanti Bist

In the present study a total no. of 380 samples comprising of 120 meat samples, 200 egg samples and 30 swabs samples each from butchers hands and knife were taken to assess the bacterial load of poultry meat and egg. In the quantitative examination of samples, the mean value of SPC (cfu/gm) for meat was recorded as 6.092, for egg surface 7.6413 and for egg yolk 6.5505 respectively. Coliform count for meat samples (102) was recorded as 5.2469 while staphylococcus count recorded as 5.60. From the n=380 samples total of 24 salmonella spp and 60 *E. coli* were isolated. The prevalence of salmonella and *E. coli* were reported as 10.9% and 26.17% respectively. A total of 32 *E. coli* and 14 salmonella isolates were subjected to antibiogram. The isolates showing higher range of sensitivity for salmonella were recorded as ciprofloxacin, Norfloxacin and streptomycin while for *E. coli* by amoxicillin and streptomycin.

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7. Sero-prevalence of brucellosis in cattle and buffaloes in certain organized dairy farm and rural areas of Uttar Pradesh state and its public health significance

Vijay Kumar Rathore and Basanti Bist

In this study a total number of 342 cattle, 626 buffaloes, and 40 human samples were tested by STAT and RBPT for detection of brucellosis in the different areas of UP state. In the Agra district total 8.62% (20/ 232), cows 8.8% (11/125) and buffaloes 8.41% (9/107) found to be positive. In the DDD dairy farm total 11.5% (23/200) out of which cows 13.75% (22/160) and buffaloes 2.5% (1/40) found to be positive, while in the rural areas 2.12% (1/47) buffaloes found positive for brucellosis in the Mathura district. In district Lakhimpur Khiri all 200 buffalo's sera samples were negative and in Distt. Bulandshahar 12.24% (6/49) sera samples were found doubtful for brucellosis. In the Distt. Aligarh total 8.33% (5/60) buffaloes were positive while 6.66% (4/60) were doubtful. In the Etah Distt. total 8.33% (15/180) out of which 1.7% (1/57) cattle and 11.38% (14/123) buffaloes found positive in the same district 35% (7/20) cattle and 65%(13/20) buffaloes were found doubtful for brucellosis with the history of abortion and infertility. In the present study 2.5% (1/40) human sera samples found positive for brucellosis.

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8. Studies on prevalence of Echinococosis in buffaloes and goat

Vipin Kumar Gupta and Basanti Bist

The study was initiated in view of the zoonotic effect and economic losses of hydadidosis in ruminants of Mathura and Agra regions. An overall prevalence of hydadidosis in sheep, goat, and buffaloes was observed as 4.52%, 2.78% and 9.87% respectively. The infection rate of hydadidosis in rams and ewes was recorded as 4.34% and 6.25% respectively while the infections rate of hydadidosis in bucks and does was 1.26% and 6.81% respectively and for buffalo bull and she buffaloes the infection rate was 10.71% and 12.29%, respectively. The adult sheep above one year had the incidence as 5.13% and in lambs it was 2.63%, while the adult goats had 3.25% and kids had 1.75%. The present investigation indicated that the most common site of infection was the lungs. In sheep 57.14% of lungs, 42.85% of liver were found to be affected with hydatid cyst. The infection in goats were found to be 80.00% and 20.00% in lungs and liver, respectively and for buffaloes 78.15% and 21.87% lung and liver were infected, respectively.

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9. Prevalence of *Clostridium perfringens* in foods of Braj region

Ankur Priyadarshi and Basanti Bist

A total of samples comprising 60 chicken meat, 60 buffalo meat, 60 pig meat, 60 goat meat, 50 fish meat, 20 cooked chickens meat, 20 badam-milk and 20 ice-creams were collected in and around the Mathura city and analyzed for the level of contamination of *Clostridium perfringens*. The overall occurrence of *Clostridium perfringens* was 48.57% in different food with the highest prevalence in poultry (66.67%) followed by buffalo (61.67%), fish (60%), pig (53.33%) and goat (48.33%). The meat samples of cooked chicken had 10% of *Clostridium perfringens*. In the milk products samples, badam-milk and ice-cream had no *Clostridium perfringens* reported. The presence of *Clostridium perfringens* in meat samples showed the inadequate sanitary and hygienic condition in and around the products leading to cross contamination of samples. CMM showed higher level of the *Clostridium perfringens* during enrichment than other two media (IMM and ATM). A total of 102 isolates of *Clostridium perfringens* were screened to observe lecithinase activity, out of which 91(89.2%) were reported to be positive. In meat samples, poultry (95%), goat (90%), buffalo (90%), pig (85%) and fish (85%) isolates showed lecithinase activity. In cooked meat isolates, 100% lecithinase activity was displayed. For hemolytic activity, 77 strains of *Clostridium perfringens* were observed, 65 strains (84.42%) had shown hemolysis on blood agar plates. The hemolytic activity of the isolates of *Clostridium perfringens* of goat (93.33%), pig (86.67%), poultry (86.67%), fish (80%), and buffalo (73.33%) were found. In cooked chicken hemolytic activity was observed to be 100%. Total 25 raw meat samples isolates and 2 cooked meat samples isolates were screened by PCR to detect the presence of alpha toxin (*cpa*) and enterotoxin gene (*cpe*) in *Clostridium perfringens* strains. The samples of buffalo meat strains (80%) followed by fish (60%), poultry (60%) pig (60%), and goat (40%) had alpha toxin (*cpa*) gene of *Clostridium perfringens*. The two cooked meat samples showed 100% alpha toxin (*cpa*) gene. None of the samples found to be positive for enterotoxin gene (*cpe*) among the samples tested. The antibiotic sensitive/resistant pattern of *Clostridium perfringens* against 16 antibiotics revealed that Piperacillin, Chloramphenicol, Ceftriaxone, and Amikacin were highly sensitive (80-100%), followed by Cephoxitim, Cephradine, Cefuroxime sensitive (50-70%). Gentamycin was found to be resistant around 46%. The drugs like Penicillin G, Tetracycline, Erythromycin and Ampicillin had displayed resistance between (50-70%), while the drugs like Lincomycin, Co-trimoxazole, Cloaxacillin and Ceftazidime showed resistance from 80 to 95%.

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10. Prevalence of *Bacillus cereus* in different foods of Mathura and Vrindavan and its antibiogram studies

Sonia Gupta and Basanti Bist

The 205 samples of food and food products from local and standard shops of Mathura and Vrindavan region were tested out of which 71 samples revealed contamination with Bacillus cereus and the percent positivity was 34.63%. Higher percent of contamination was found in local shops 39.39% then in standard shops 26.47%. However, statistical analysis revealed that the result was not significant. Sensitivity pattern of 70 isolates against 12 antibiotics from food and food products were seen. The all isolates were found sensitive to ciprofloxacin, chloramphenicol, doxycycline and ofloxacin whereas resistant to penicillin and ampicillin. Isolates presented 100% sensitivity to ciprofloxacin, chloramphenicol, doxycycline and ofloxacin but the sensitivity was only 97.1, 94.2, 82.2, 74.2, and 57.1% for norfloxacin, gentamycin, amikacin, streptomycin and tetracycline respectively.

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11. Prevalence of verotoxic *Escherichia coli* in meat, meat products and water from different sources in certain areas of Uttar Pradesh

Seema Singh and Basanti Bist

The objective of this study was to determine the prevalence of verotoxic *Escherichia coli* (VTEC) in meat, meat products and water samples from different sources in certain areas of UP, to characterize them by serological and molecular methods and to investigate for haemolytic activity, Congo red dye binding activity and multiple drug resistance. A total of 372 samples comprising of 192 meat samples (40 carabeef, 30 chevon, 30 mutton, 30chicken, 32 fish, 30 pork), 50 meat products (5 samples each of pork patties, Fish pakoda, ch. curry, ch. patties, ch. nugget, ch. chat, fried chicken, ch. burger and 10 samples of carabeef kabab) and 130 water (40 Borewell, 40 Community supply, 30 River, 20 packed/mineral) samples were collected and screened for the presence of virulence genes stx1 and stx2 by PCR. Overall prevalence of VTEC in meat samples was found to be 18.23%. The highest prevalence of VTEC was detected in mutton(30%) fallowed by carabeef(25%), chevon (20%), chicken(13.33%), pork(13.33%) and fish(9.38%). The serotypes of VTEC reported in meat samples were O108(5), O97(4), 2 strains each of O2, O112, O119, O41, O43.Out of 35 VTEC strains isolated from meat samples 7 strains harboured vt1, while 27 harboured vt2, and 1 strain was positive for both the genes. The overall prevalence of VTEC in meat products was recorded as 6%. It is interesting to note that pork patties (2/3) and chicken curry (1/3) was found positive for VTEC. All the 3 strains of VTEC in meat products carried vt2 gene and belonged to serotype O102. An overall Prevalence of VTEC in water samples was 4.62%(6/130). The highest % positivity of VTEC strains were detected in river water 6.67%(2/30) fallowed by borewell water 5%(2/40) and community supply water 5%(2/40) and they belonged to 3 different serotypes. Serotype O168 (3) was frequently detected fallowed by O102 (2) and O11.Out of 6 VTEC 5 carried vt2 gene and one carried both vt1 & vt2 gene. For the study of other virulence marker of VTEC, haemolytic activity and Congo red binding activity were examined. In the present study overall, 77.27% VTEC produced enterohaemolysin on washed sheep blood agar supplemented with cacl2, interestingly majority of VTEC produced E-hyl except 10 VTEC strains of which 4 were α haemolytic and 6 were non-haemolytic. The positive correlation between VTEC and enterohaemolysin was also observed to be 77.27%, 66.67%, 83.38% for meat, meat products and water respectively. Overall 93.18% (41/44) VTEC strains were positive for congored binding activity. The VTEC isolates were tested for 20 antimicrobial agents, VTEC strains exhibited highest sensitivity to Norfloxacin (93.18%) fallowed by ofloxacin (90.19%), chloramphenicol (88.64%), ciprofloxacin (88.64%), Gentamycin (86.36%). The VTEC isolates were 100% resistance to novobiocin, penicillin G, fusidic acid, Ticracillin, Methicillin.

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12. Prevalence of VTEC in faeces of healthy cattle and diarrhoeic calves, milk and milk products in certain parts of U.P.

Preeti Pandey and Basanti Bist

The objective of this study was to determine the prevalence of Verotoxigenic *Escherichia coli* (VTEC) in faeces, water and milk sampled in dairy farms in certain areas of U.P, to characterize them by serological and molecular methods and to investigate haemolytic activity, congo red dye binding ability and multiple drug resistance. A total of 252 faecal samples (177 of healthy cattle, 75 of diarrhoeic calves), 87 milk samples (57 of raw milk and 30 of pasteurized milk) and 120 milk product samples (30 each of curd, rasgulla, peda, paneer and milk powder) were collected and assessed for the presence of the virulence genes $Stx_1$and Stx_2 by single gene polymerase chain reaction (PCR). In faecal samples, higher prevalence of VTEC was detected in healthy cattle (19.77%) than diarrhoeic calves (14.66%). Prevalence of VTEC was 7.01% and 2.00%

in raw milk and milk products samples, respectively. Out of 53 VTEC isolates, 52 carried Stx_2 gene except one VTEC isolate which harboured Stx_1 gene and was isolated from healthy cattle faeces. 46 VTEC isolated from faeces belonged to 18 different sero groups with O84 (13.04%) being the most frequently isolated serotype followed by O22 (8.69%) and O20, O168 (6.52% each). While most common of the 4 different serogroups identified in 7 milk and milk product VTEC isolates were O20 (42.85%), O55 (28.57%) and O22 and O102 (14.28%). 22 of 53 (41.50%) VTEC isolates were found positive for haemolysis when tested on sheep blood agar. Among the 53 VTEC isolates screened for congo red dye binding ability 50 (94.34%) were found positive. Further, the isolates were tested against for 13 antimicrobial agents. VTEC isolates exhibited highest sensitivity to Gentamicin (79.24%) and lowest sensitivity to novobiocin (100%). In conclusion, the high VTEC prevalence detected in cattle evidences that bovine faeces might play an important role as a contamination source in the regions of U.P. Since VTEC was also detected from milk and milk products, they may act as vehicle for VTEC infection and can pose serious threat.

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13. Prevalence of *E. coli* with special reference to VTEC in faeces of dairy cattle, milk and milk products in Mathura and Vrindavan region

Shashi Kumari and Basanti Bist

A study was undertaken to assess the prevalence, serotypes, and virulence genes (stx_1 and stx_2 through PCR) of *E. coli.* A total of 405 samples comprising of 155 faecal samples, 100 milk samples and 150 milk product samples were screened for *E. coli.* Out of 405 samples processed, 147 *E. coli* isolates were obtained. The highest occurrence was observed in faecal sample (60%) followed by milk (22%) and milk products (21.33%). Serotyping results showed that out of 147 *E. coli* isolates, 18 isolates were rough, 19 isolates were untypable and 110 isolates belonged to 24 different 'O' serogroups. Serogroups O55 and O60 were obtained from all the three sources. A total of 110 *E. coli* isolates (faeces-70, milk-20, milk products-20) were screened by PCR to detect virulence genes *stx1* and *stx2* in *E. coli* strain. Out of 110 samples tested, 3 isolates were found to be positive for *stx* gene One isolate (O55) from faeces of diarrhoeic cow was positive for *stx1* gene whereas, 1 isolate(O60) from faeces of non- diarrhoeic cow revealed the presence of stx_2 gene, while 1 isolate (O172) from faeces of diarrhoeic calf was found to be positive for both stx_1 and stx_2 gene. None of the samples from milk and milk products were found to be positive for the above mentioned virulent genes Thus on the basis of PCR the prevalence of VTEC in faecal sample was found to be 4.28% (3 out of 70). However, no VTEC was detected from milk and milk products. Thus the overall occurrence of VTEC in the present study was found to be 2.72% (3 out of 110 isolates). Therefore, it can be concluded that the prevalence of VTEC in the faeces of domesticated cattle as revealed by this study presents a serious threat. *E. coli* being major zoonotic organism, transmission of these organisms to human through food chain as well as direct contact is obvious.

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14. Evaluation of Bacterial Quality and Isolation of *Escherichia coli* (O157:H7) from different meat samples procured from retail meat shops and local slaughter houses of Agra Region

V.K. Singh and Udit Jain

A total of 120 meat samples,30 each from cara beef, chevon, pork and poultry evaluated for bacterial load i.e. Standard plate count (SPC), Coliform count (CC) and Staphylococcal count (SC) and 240 meat samples comprising 60 each of cara beef, chevon, pork and poultry were

evaluated for presence of *Escherichia coli* (O157 H7). Mean values of SPC (log_{10}cfu/g) were found to be 7.03±0.07 for cara beef, 6.96±0.78 for chevon, 6.86±0.02 for pork and 6.75±0.04 for poultry meat. Mean values of Coliform count (CC) (log_{10}cfu/g) were found to be 3.93±0.14 for chevon, 3.82±0.12 for poultry, 3.40±0.10 for pork and 3.04±0.08 for cara beef.Mean values of Staphylococcus count (SC) (log_{10}cfu/g) were found to be 3.90±0.12 for cara beef, 3.84±0.12 for chevon, 3.35±0.10 for poultry and 2.81±0.11 for pork. Of 74 *Escherichia coli* (non-O157:H7) isolates 18(30%), 16(25%), 15 (26.7%) and 25(41.67%) isolates were obtained from cara beef, chevon, pork and poultry meat respectively. Overall percent prevalence of *E. coli* (non-O157:H7) in meat samples in different areas of Agra region was found to be 30.83%. Isolation of *E. coli* from the meat samples is associated with various diseases in man and animals which is of public health significance. The study revealed an urgent need to improve the hygienic condition at all level of production and retailing of meat.

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15. Prevalence of VTEC in faecal samples of diarrhoeic calves, healthy cows and water from certain areas of Agra and Mathura districts

Suman and Basanti Bist

The objective of this study was to determine the prevalence of Verotoxigenic *Escherichia coli* (VTEC) in faeces, water samples in certain areas of, Agra and Mathura districts to characterize them by molecular methods and to investigate haemolytic activity, congo red dye binding ability and multiple drug resistance. A total of 600 faecal samples comprising of 300 from diarrhoeic calves and 300 from healthy cows were collected and processed. A total no. of 532 *E. coli* isolates were isolated, of which 250 *E. coli* isolates (from 286 samples) were processed for molecular characterization for stx1 and stx2 genes. The overall percent positivity of VTEC in faeces is 15.03% (43/286). A total of 150 water samples were collected and processed for molecular characterization for stx1 and stx2 genes. An overall prevalence of VTEC in different sources of water collected from Agra and Mathura districts, was found to be 4.00%(6/150). 22 of 49 (44.89%) VTEC isolates were found positive for haemolysis when tested on sheep blood agar. Among the 49 VTEC isolates screened for congo red dye binding ability 41 (83.67%) were found positive. Further, the isolates were tested against for 6 antimicrobial agents. VTEC isolates exhibited highest sensitivity to Gentamicin (79.59%) and resistance to Ampicillin (55.10%). In conclusion, the high VTEC prevalence detected in cattle evidences that bovine faeces might play an important role as a contamination source in the Agra and Mathura districts. Since VTEC was also detected from water, indicates faecal contamination thus can pose serious threat.

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16. Prevalence of Verotoxic *E. coli* in meat and meat products in certain areas of Uttar Pradesh

Tanu Singh and Basanti Bist

Present investigation was undertaken to assess the prevalence of verotoxic *Escherichia coli* (VTEC) in meat and meat products in certain areas of UP. Studies were also carried out to detect virulence markers and virulence genes. A total of 300 samples (135 carabeef, 55 chicken, 25fish, 60 chevon, 25 pork) and 150 meat products (20 each of Mutton Patties, Chicken Patties, Mutton Kabab, Carabeef Kabab, Chicken Nugget, Chicken Burger and 10 each of Chicken Sandwitch, Mutton Curry, Fish Pakoda) samples were collected and screened for the presence of virulence genes vt_1 and vt_2 by multiplex PCR. A total of 4 (1.33%) meat samples and 1 (0.6%) meat product sample were classified as PCR positive. Five isolates (3 from carabeef, 1 from a carabeef kabab and 1 from chevon) were positive for VT genes. All 5 VTEC strains harboured vt_2

gene. None of the VTEC isolates contained vt1 gene. All except one VTEC isolate exhibited toxic effects on Vero cells. VTEC strains were examined for additional virulence factors i.e., haemolytic activity and Congo red binding activity. 2 of 5 (40%) VTEC isolates were found positive for haemolysis when tested on sheep blood agar. Among the 5 VTEC isolates screened for congo red dye binding ability 4 (80%) were found positive. Further, the VTEC isolates were tested for 22 antimicrobial agents. VTEC strains exhibited sensitivity to norfloxacin, ciprofloxacin, chloramphenicol, ceftriazone, Amikacin and amoxyclav. The VTEC isolates were 100% resistance to novobiocin, penicillin G, fusidic acid, colistin, tylosin and Methicillin. In conclusion, this study demonstrated that retail meats, mainly carabeef, were contaminated with VTEC strains. The presence of VTEC strains in retail meat is also of concern due to their potential to cause human infections like Haemorrhagic Colitis, Haemolytic Uremic Syndrome and Thrombotic thrombocytopenic purpura.

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17. Epidemiological studies of brucellosis in cattle and buffaloes in Mathura and adjoining areas of Uttar Pradesh and its zoonotic significance

Pragati Swarnkar and Basanti Bist

The study was conducted to know the seroprevalence of brucellosis in cattle and buffaloes of Mathura and adjoining areas with respect of different epidemiological determinants and its public health significance by employing I-ELISA, RBPT and STAT and comparison of I-ELISA with the other conventional test. A total of 568 serum samples of cattle and buffaloes of different age, sex and places from organized and unorganized farms of Mathura and adjoining areas were collected, which were screened for Brucella antibodies using RBPT, STAT and indirect ELISA test, where as 108 serum samples of 14 veterinary students and 94 animal handlers were collected from different places of Mathura district. The Overall prevalence against brucellosis in cattle and buffaloes were found as 9.3%, 6.61% and 5.10% by I-ELISA, RBPT and STAT, respectively. The overall prevalence of brucellosis in human beings was found as 6.48% and 4.62% by RBPT and STAT, respectively. No veterinary student was found reactor for brucellosis where as 7.44% and 5.31% animal handlers were found positive by RBPT and STAT, respectively. Considering I-ELISA as a standard test, the sensitivity of RBPT and STAT was found to be of 50% and 33.9%, and specificity was found to be of 98% and 97% respectively. Concordance between RBPT and STAT with respect to I-ELISA was found to be 94% and 92%, respectively. There was moderate agreement between RBPT and STAT with that of I-ELISA. Thus using combination of test for screening of the cattle and buffaloes against brucellosis were useful rather using a single test.

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18. Isolation and characterization of Verocytotoxic *E. coli* in faecal samples, milk, and milk products and animal handlers from certain areas of Mathura and Vrindavan region

Charul Rajpoot and Udit Jain

A total no. of 500 samples comprising of 250 faecal samples (cattle 102, buffalo 90, calf 38,) 125 raw milk samples, 75 milk products samples (15 each of rasagulla, burfi, peda, paneer and curd) and 50 swabs from hands of animal handlers were collected and assessed for the presence of virulence genes Stx1 and Stx2 by multiplex Polymerase Chain Reaction (PCR). Out of 210 *E. coli* isolates 100 were further processed, Out of these 37 isolates were subjected to verocytotoxic assay and 63 isolates were subjected to PCR. Out of 37 isolates 13 were found to have positive cytopathic effect on vero cell line and 7 *E. coli* out of 63 were found positive for Stx gene. A total

of 109 *E. coli* isolates (63 faecal samples, 30 milk samples and 16 milk products samples) were screened for the presence of Stx genes. Out of which 9 isolates were found to be positive for Stx genes. Among these isolates only one isolate of faecal sample of diarrhoeic cow was found positive for Stx1 and one isolate from faecal sample of healthy cow, two isolates from faecal samples of diarrhoeic cow, 1 isolate from faecal samples of diarrheic buffalo, two isolate from faecal samples of diarrhoeic calf was found to be positive for Stx2 gene. However, no Stx gene was detected from animal handlers. Prevalence of VTEC was highest in faecal samples followed by milk and milk products. In present study the overall haemolytic activity and congo red dye binding ability of VTEC isolates was found to be 55.56% and 88.89% respectively. 5/9 (55.56%) VTEC isolates were found positive for haemolytic activity on sheep blood agar 8/9 (88.89%) VTEC isolates were found positive for congo red dye binding ability. The VTEC isolates were further tested against 13 antimicrobial agents. These VTEC isolates exhibited highest sensitivity to ciprofloxacin (100%), followed by nalidic acid(92.00%), ceftriazone (88.00%), cefotaxime(88.00%) and highest resistance was shown by antibiotics like co-triomoxazole(80.00%), followed by penicillin G(40.00%) and tetracycline (36.00%).

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19. Occurrence of Salmonella organism in foods of animal origin (milk, meat, fish and egg) and water and their public health significance in Mathura district

Rakhi Sharma and Udit Jain

Food borne pathogenic zoonotic or potentially zoonotic bacteria in foods of animal origin (milk, meat, fish and egg) and water are the cause of illness and death for many people. During the study, 370 sample analyzed of different markets of various areas and places of Mathura District as sources of carabeef, chevon chicken, pork and fish meat, samples shown the presence of pathogenic *Salmonella* species. In carabeef 13.33%, chevon 10%, chicken 20%, pork 13.33% and fish 0% in meat samples, egg 26.66%, milk 8% and in animal products handlers 3.33% occurrence was estimated. In similar way highest contamination of *Salmonella* species was recorded in chicken meat and least contamination in fish meat. The study also concluded that, the presence of *Salmonella* in meat samples due to the unhygienic conditions, where and from the meat samples were obtained, either from the pond, lake, river (in case of fish) or from the unhygienic storage and pre- processing and handling conditions (in case of meat and poultry chicken) which makes the food contaminated with pathogenic *Salmonella* species. Raw milk may act as vehicle for Salmonella infection and can cause serious threat. Milk may get cross contaminated with faecal matter during milking. Majority of the isolates of *Salmonella* were found to be sensitive to Ceftriaxone, Ciprofloxacin, Gentamicin and Ofloxacin are sensitive to Salmonella spp. Amikacin, Chloramphenicol, Cefoperazone/Sulbactam Kanamycin are intermediate to Salmonella spp. Ampicillin, Amoxyclav, Cefixime/Clavulanic acid, Tetracycline are resistant to Salmonella spp. Streptomycin are nearly 50% intermediate and 50% resistant to Salmonella spp according to antibiotic sensitivity pattern.

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Ph.D.

1. Detection and identification of *Clostridium perfringens* in food

Ranvijay Singh and Basanti Bist

The presence of *Clostridium perfringens* was studied in various foods (meat and meat products, milk and milk products, juice and water). The results revealed the overall occurrence of *Clostridium perfringens* was 57.9% in different food with the highest prevalence in poultry (94.11%). The meat samples of goat and fried chicken had (58%) and (16.66%) of *Clostridium perfringens*, respectively. Among the milk samples, srikhand (82.1%) had highest presence of *Clostridium perfringens* followed icecream (44.44%), raw milk (33.3%) and pasteurized milk (32.1%). For the isolation and enumeration of *Clostridium perfringens*, four different media were used viz. SPS, TSC, SFP and IMM. IMM showed 100% positivity on the basis of presumptive isolation. Among agar media, SFP and TSC had given more than 90% positivity for *Clostridium perfringens* followed by SPS (83.66%) in poultry samples. The results of selective media in the goat sample showed TSC and SPS to be better than SFP. In the milk sample particularly pasteurized milk and srikhand, SFP was found to be better than SPS in the isolation and identification of *Clostridium perfringens*. The overall occurrence of *Clostridium perfringens* in CMM was 88.31% followed by ATM (32.46%) and IMM (22%) in poultry meat in combination with various selective media. In goat meat, CMM showed prevalence of 52.5%, ATM- 22.5% and IMM-20% *Clostridium perfringens* in combination with selective media. Among virulence markers of *Clostridium perfringens*, lecithinase and hemolytic activity were examined. A total of 149 isolates of *Clostridium perfringens* were screened to observe lecithinase activity, out of which 142 (95.6%) were turned to be positive. In meat samples, goat isolates showed higher lecithinase activity (96.6%) than poultry (92.5%). In milk isolates, lecithinase activity displayed by *Clostridium perfringens* was 100%. For hemolytic activity, 139 strains of *Clostridium perfringens* were observed, 130 (93.52%) had shown hemolysis on blood agar plates. The hemolytic activity in poultry isolates of *Clostridium perfringens* was 94.54% and found greater than goat (86.66%). In raw and pasteurized milk and icecream hemolytic activity was 100% while in srikhand it was 88.88%.One hundred thirty food samples were screened by PCR by single step enrichment to detect the presence of enterotoxin gene in *Clostridium perfringens*. Of the 130 samples, 21(16.15%) samples had enterotoxin gene of *Clostridium perfringens*. Out of 60 samples of poultry, only one sample (1.6%) was positive for enterotoxigenic *Clostridium perfringens*. In 30 and 20samples of goat and srikhand, 8(26.66%) and 12 (60%) samples were positive for enterotoxigenic Clostridium perfringens, respectively. Raw and pasteurized milk and icecream didn't show presence of enterotoxin gene among the samples tested. Among the antibiotic tested, Amikacin cephalosporin group (Cefuroxime, Cephradine, Ceftriaxone, Ceftazidime and Cephoxitim), Piperacillin and chloramphenicol were highly sensitive (80-100%) followed by erythromycin and Gentamycin which were found to be effective between 40 to 60%. The drugs like tetracycline, penicillin and Ampicillin had displayed resistance between 40-70%, while the drugs like co-trimoxazole and Cloaxacillin showed resistance from 80-90%. The *Clostridium perfringens* isolates were nearly 100% resistance to Lincomycin. Phylogenetic analysis of Sequences of PCR Products of *Clostridium perfringens* (16srDNA) by 'DNA Star' computer software programme revealed that *Clostridium perfringens* (G53) showed highest similarity (66.3%) with ATCC, 16sRNA *Clostridium perfringens* followed by genomic DNA (42.9%-Gwalior). The genomic DNA (23.8%-UK) and 16sRNA from canine feces (23.5%-UK) had lower percent of identity with *Clostridium perfringens* (G53). In the phylogenetic study of enterotoxigenic *Clostridium perfringens* (G53 and G60)it was found that similarity with cpe equine- Canada and cpe Europe was 100%. The similarity of cpe (G53 and G60) was found lower with cpe (human-36.9%) and cpe serotype A(31.2%). Isolates from goat (cpe-G53 and G60) had shown 98.7% identity with each other.

Veterinary Surgery and Radiology

SNo.	Titles	Name of Scholars	Name of Major Guide	Years	Page No.
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3.	Studies on ketamine alone and in combination with diazepam, midazolam and xylazine for induction of general anaesthesia in butorphenol-xylazine premedicated horses	Dr. Vivek Malik	Dr. Bharat Singh	2005	212
4.	Studies on bupivacaine, xylazine, ketamine hydrochloride and their combinations for lumbosacral anaesthesia in dogs	Dr. Sheshman	Dr. Bharat Singh	2005	213
5.	Studies on combination of xylazine, bupivacaine and butorphanol for epidural analgesia in dogs	Dr. Deepesh Kumar	Dr. R.P. Pandey	2006	213
6.	Evaluation of xylazine -ketamine and xylazine-propofol for clinical surgery in dogs	Dr. Alok Kumar	Dr. R.P. Pandey	2006	214
7.	Clinical evaluation of different techniques of ovariohysterectomy in bitches	Dr. Anil Kumar Singh	Dr. Bharat Singh	2006	215
8.	Anaesthetic and surgical evaluation of xylazine with diazepam or midazolam and ketamine in cattle	Dr. Ramraj	Dr. Bharat Singh	2006	216
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14.	Studies on diagnosis and treatment of otitis in dogs	Dr. Puja Jain	Dr. R.P. Pandey	2010	220
15.	Studies on halothane anaesthesia in propofol, xylazine, midazolam premedicated and propofol induced dogs	Dr. Tejveer Singh	Dr. Bharat Singh	2010	220
16.	A comparative study of closed interlocking nailing (C.I.L.N.) and open interlocking nailing (O.I.L.N.) in management of canine femur and tibia fractures	Dr. Viram Varshneya	Dr. R.P. Pandey	2011	221
17.	Studies on chemotherapy of mammary tumor in bitches	Dr. Neeraj Yadav	Dr. Bharat Singh	2011	222
18.	Radiographic morphometric studies of distal bones and joints of fore and hind limbs of buffaloes	Dr. Sudhanshu Bansal	Dr. Vivek Malik	2011	222
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20.	Development and clinico-biomechanical evaluation of prosthetic limb in dogs	Dr. Mohd Shiyad K.K.	Dr. Vivek Malik	2012	224
21.	Studies on common dental affection in dogs	Dr. Rahul Kumar	Dr. R.P. Pandey	2012	225

1. Clinico-experimental studies on reconstructive surgery for resected achilles tendon in sheep

Rajneesh Kumar and R.P. Pandey

The present work was planned to study the effect of conservative management of resected Achilles tendon vis-à-vis tendon repair by locking loop method and tendon repair by modified locking loop and suture anchorage at tuber calcis in sheep. The study was conducted on four mixed gender sheep in the age range of 1-3 years, and 18-24 kg body weight. In study group "A" the procedure consisted of transaction of Achilles tendon, suture closure of skin wound, and immobilization of limb in Thomas' splint. In study group "B" the procedure consisted of transaction of Achilles tendon and its reconstruction using modified locking loop method, suture closure of skin wound, and immobilization of limb in Thomas' splint. In study group "C" the procedure consisted of transaction of Achilles tendon and its reconstruction using locking loop method with suture anchorage at tuber calcis, suture closure of skin wound, and immobilization of limb in Thomas' splint. Results consisted of observation for recording alteration in general condition (appetite, posture at recumbency, standing and walking); mechanical parameters (weight bearing on operated limb and weight distribution, angle of hock and mean circumference above the hock) on day 0, 7, 14, 21, 28, 35 and 45 post-operatively and histological study of biopsy samples on day 7, 14, 21 and 28 post-operatively. The salient conclusions of the study were: This was noted that fore limbs bear approximately 60 per cent and hind limbs bear approximately 40 per cent of total body weight. Repair of resected Achilles tendon by using modified locking loop technique with anchorage at tuber calcis had added advantage in maintaining the post operative angle of hock for better weight bearing and early restoration of ambulation. Achilles rupture in light weight animals can be managed by immobilizing the limb promptly in modified Thomas splint supported with a card board but the rate of restora- tion of normalcy is slow. Suture apposition of cut ends of the Achilles tendon resulted in early restoration normal angle of hock, weight bearing on operated limb and histological integrity. Cross linking of the collagen fibers and fiber bundles was relatively more pronounced in locking loop techniques on histological examination. Monofilament nylon proved a satisfactory material for tendon suturing. The results can be recommended for clinical application in selected cases with suitable indications.

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2. Evaluation of various approaches for cystorrhaphy, normograde indwelling catheterization and herbal treatment in buffalo calves

Krishna Kumar Mishra and Bharat Singh

Study was done oneEight clinically healthy male buffalo calves in the age group of 1.5 to 4 years and body weight ranging from 110 to 325 kg to evaluate the paramedian, para anal and infra anal approaches for cystorrhaphy and. normograde catheterization of urethra. These techniques were replicated for five times on these animals in randomized manner. Paramedian laparocystotomy was performed in dorsal recumbency by using "dorsal recumbency frame" whereas para anal and infra anal approaches were performed in sitting posture of the animal. The different clinical, haematological, serum biochemical, and urine analysis parameters were evaluated at zero day and 3rd,5th, 9th and 15th day of post operation from blood, serum and urine, respectively. In paramedian laparotomy sufficient space is available for manipulation and exteriorization of urinary bladder. Whereas in para anal and infra anal approaches it is limited. In paramedian approach, all the areas (vertex, body and neck) of the urinary bladder can be repaired easily whereas in para anal and infra anal approaches only vertex and body of urinary

bladder can be repaired easily and neck area can be repaired with difficulty. Cystotomy incision at the vertex was repaired easily without any hindrance in all the approaches however, it was a little bit easier in para anal and infra anal approaches as the vertex is completely lying out sides, while it is somewhat deep in the paramedian approach from the cutaneous incision. In infra anal approach amount of haemorrhage recorded was more in comparison to paramedian and para anal approaches. Normograde indwelling catheterization was easily performed through small cystotomy incicision in paramedian approach whereas it is difficult in para anal and infra anal appoaches and require a bit larger cystotomy incision. The para anal and infra anal approaches were more comfortable to the animals as compared to paramedian approach as has been observed with over all status reflected by clinical and physiological manifestation of the animals during entire operative period. The adhesion were more in case of infra anal followed by para anal in the retropeitoneal area and were extended to the cystotomy site with surrounding abdominal structures whereas in the paramedian site no adhesion formation was noticed. The cutaneous healing observed at different site was comparable to each other in all aspect without any complication noticed during the post operative period. However, the post operative care required in infra anal group of animals was more in comparison to para anal and paramedian sites. There was no significant change in clinical parameters and haematological serum biochemical and urine analysis parameters studied in the present study.

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3. Studies on ketamine alone and in combination with diazepam, midazolam and xylazine for induction of general anaesthesia in butorphanol-xylazine premedicated horses

Vivek Malik and Bharat Singh

Four clinically, apparently healthy, thorough bred male/female horses of 3-5 years of age and weighing 250-310 kg were randomly numbered and given four treatments with four replicates of each treatment as detailed below. T1: Butorphanol (0.06 mg/kg i.m.) and 10 min later xylazine *(0.5* mg/kg i.v.) and after complete sedation followed by ketamine by slow intravenous injection till effect. T_2: Butorphanol and xylazine as in T, followed by a mixture of ketamine and diazepam (20:1) by slow intravenous injection till effect. T3: Butorphanol and xylazine as in T, followed by mixture of ketamine and midazolam (30:1) by slow intravenous injection till effect. T_4: Butorphanol and xylazine as in T, followed by mixture of ketamine and xylazine (10:1) by slow intravenous injection till effect. First of all effective doses of butorphanol (0.06 mg/kg b.w.) and xylazine (0.5 mg/kg b.w.) were selected by trial and error method. Mean doses of ketamine to produce surgical anaesthesia in T_1, T_2, T3 and T_4 were 1.83±0.18, 1.77±0.09, 1.30±0.12 and 1.76±0.267 mg/kg respectively, while the doses of diazepam, midazolam and xylazine in T_2, T3 and T4 groups were 0.085±0.039, 0.046±0.080 and 0.143±0.015 mg/kg respectively. The clinical study included time of complete sedation, induction period, time of complete anaesthesia, duration of anaesthesia, time of onset of recovery, time of sternal recumbency, time of standing, time of walking with staggering, time of walking without staggering (complete recovery time), degree of analgesia, sedation and anaesthesia with abolition of different reflexes viz. pedal, palpebral, corneal and anal. Muscular and anal sphincter relaxations were also recorded. Physiological study included recording of HR, RR and RT before administration and at complete sedation, maximum depth of anaesthesia, onset of recovery, complete recovery, 6 hours, 12 hours and at 24 hours of drug administration. Haematological study included the estimation of PCV, Hb, TLC, and DLC before administration and at complete sedation, maximum depth of anaesthesia, complete recovery, 12 hours and 24 hours of drug administration. Biochemical study included estimation of serum creatinine, SGOT, SGPT, electrolytes viz. sodium, potassium and chloride before administration and at complete sedation, maximum depth of anaesthesia,

complete recovery, 12 hours and 24 hours of drug administration. Results of the present study revealed that butorphanol- xylazine-diazepam-ketamine and butorphanol- xy lazine-midazolam-ketamine combinations produce excellent analgesia with better muscular relaxation, long duration of anaesthesia and smooth induction and recovery along with better cardio-protective effects in comparison to butorphanol-xylazine-ketamine and butorphanol-xylazine-ketamine -xylazine combinations and can be used safely in orthopaedic operations and those procedures which require better muscle relaxation and comparatively long duration of anaesthesia.

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4. Studies on bupivacaine, xylazine, ketamine hydrochloride and their combinations for lumbosacral anaesthesia in dogs

Sheshman and Bharat Singh

The study was conducted to evaluate the effect of bupivacaine, xylazine, ketamine and their combinations for lumbosacral epidural analgesia in dogs. Six nondescript healthy male *1* female dogs ranging from 2 to 4 years of age and 15 to 20 kg body weiqht were randomly numbered and given six treatments in randomized manner. The treatment details were as follows: (i) Treatment I (T_1): 5 *mg/ml* bupivacaine hydrochloride @ 1 mg/kg body weight given at slow speed epidurallv at lumbosacral space.(ii) Treatment" (T 2): 20 mg/ml xylazine hydrochloride @ 0.5 mg/kg body weight given as in T_1. (iii) Treatment **III** (T3): 50 *m:;1/ml* ketamine hydrochloride @ 3 mg/kg body weight given as in T_1. (iv) Treatment IV (T 4): 5 *mg/ml* bupivacaine hydrochloride @ 1 mg."'g body weight + 50 *mg/ml* ketamine hydrochloride @ 3 mg/kg boay weight were mixed in the same syringe and given as in T_1. (v) Treatment V (Ts): 5 *mg/ml* bupivacaine hydrochloride @ 1 mg/kg body weight + 20 *mg/ml* xylazine hydrochloride @ 0.5 mg/kg body weight were mixed in the same syringe and given as in T_1 (vi) Treatment VI (T 6): 20 mg/ml xylazine hydrochloride @ 0.5 mg/kg body weight + 50 *mg/ml* ketamine hydrochloride @ 3 mg/kg body weight were mixed in the same syringe and given as in T_1. The volume of drugs injected was kept same in all the groups i.e. 0.5 ml/ kg body weight. The effect of these treatments were studied on the clinical, physiological, hematological and biochemical parameters. The initiation of analgesia (min), initiation of sedation, initiation of return of analgesia, initiation of return of sedation, duration of analgesia, duration of sedation, complete return of analgesia, and complete recovery of sedation were recorded. The depth and extent of desensitization at tail, perineal, thigh, flank and inguinal regions were recorded at 0, 5, 10, 15, 20, 30, 45, 60, 80, 90 and 120 minutes after administration of drugs. On the basis of result of various clinical, physiological, haematclogical, biochemical and serum electrolytes parameters it is reveal that there W2.S no deleterious effect on any vital function and organ of the body and these drug regimens can safely be used in routine clinical cases of surgery without any risk except ketamine, which show cataleptic effect in the concentration used for the study. Based on duration of analgesic effect bupivacaine, bupivacaine - xylazine and bupivacaine - ketamine drugs can be used for three to four duration of operations while xylazine and xylazine - ketamine can be used for one-hour duration of surgical operation in the flank and hind limb.

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5. Studies on combination of xylazine, bupivacaine and butorphanol for epidural analgesia in dogs

Deepesh Kumar and R.P. Pandey

The present study was planned to evaluate the effects of lumbosacral epidural anesthesia with bupivacaine, xylazine and butorphanol alone and their combinations in dogs and to find out the suitable drug(s) with their doses for lumbosacral epidural anesthesia in dogs for different

clinical surgical and manipulative procedures. The number of treatments taken were six viz. T_1 (5 mg/ml bupivacaine hydrochloride @ 1 mg/kg body weight), T_2 (20 mg/ml of xylazine hydrochloride @ 0.5 mg/kg body weight, T_3 (5 mg/ml bupivacaine hydrochloride @ 1 mg/kg body weight + 20mg/ml of xylazine hydrochloride @ 0.5 mg/kg body weight), T_4 (5 mg/ml bupivacaine hydrochloride @ 1 mg/kg body weight + 2 mg/ml of butorphanol @ 0.2 mg/kg body weight), T_5 (20mg/ml of xylazine hydrochloride @ 0.5 mg/kg body weight + 2 mg/ml of butorphanol @ 0.2 mg/kg body weight), T_6 (5 mg/ml bupivacaine hydrochloride @ 1 mg/kg body weight + 20 mg/ml of xylazine hydrochloride @ 0.5 mg/kg body weight + 2 mg/ml of butorphanol @ 0.2 mg/kg body weight). The clinical and anesthetic effect and were onset of analgesia which was quickest for T_1 (3.63±0.24 min.) and slowest for T_2 (5.0±0.4 min.). Sedation was not observed in T_1. The onset of sedation was maximally delayed in T_4 (13.5±1.71 min.) where as onset was quickest in T_3 (8.25±0.63 min.). Return of pain response was longest in T_3 (284.75±9.35 min.) followed by T_6 (274.0±9.50 min.), T_1 (247.75±13.80 min.), T_4 (245.50±3.97 min.) and T_5 (65.20±2.62 min.) while was shortest in T_2 (57.0±4.14 min.). Duration of analgesia was longest in T_3 (279.75±9.33 min.) followed by T_6 (269.75±9.77 min.), T_1 (244.0±13.90 min.), T_4 (241.00±3.70 min.) and T_2 (52.0±4.45 min.) while shortest in T_5 (61.25±2.32 min.). Mean duration of sedation was maximum in T_5 (78.50±1.85 min.) followed by T_6 (74.25±2.17 min.), T_3 (68.75±4.23 min.), T_4 (60.75±3.14 min.) and minimum in T_2 (59.75±3.71 min.) Mean time to standing took longest in T_6 (347.25±7.94 min.) followed by T_3 (323.0±18.36 min.), T_1 (304.50±13.05 min.), T_4 (291.0±5.77 min.), T_5 (90.50±1.55 min.) and shortest in T_2 (72.0±5.11 min). Mean complete recovery time was maximum in T_6 followed by T_3, T_1, T_4, T_5 and minimum in T_2. In group T_2, T_3, T_4, T_5 and T_6 it was recorded at 10 minutes and in T_1 at 15 minutes after epidural injection. Bupivacaine did not cause any significant effect on SAP and on electrocardiographic recording, where as T_2 and T_3 produced significant increase in the SAP and T_4, T_5 and T_6 produced significant fall in SAP. Sinus Bradycardia and sinus arrest are the major findings in the entire group except T_1. Haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC), differential leucocyte count (DLC) did not show any marked change. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase, alkaline phosphatase (ALP), serum creatinine increased significantly in all the groups where as total protein decreased in all the groups while no appreciable effect on serum sodium and potassium ion were noticed. Based on duration of analgesic effect bupivacaine-xylazine(T_3), bupivacaine-butorphanol (T_4) and bupivacaine-xylazine-butorphanol(T_6) combinations can be used in lengthy operation whereas, xylazine (T_2) and xylazine-butorphanol (T_5) can be used for short duration of operation in the flank, abdomen, inguinal and hind limb.

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6. Evaluation of xylazine-ketamine and xylazine-propofol for clinical surgery in dogs

Alok Kumar and R.P. Pandey

The study was conducted on 20 selected clinical cases of dogs of either sex, of various breeds and of widely different ages and body weights. The clinical anaesthetic protocols used for surgery at this department were considered as treatments. The dogs were given atropine (0.02mg/kg b.wt i/m) and xylazine @) 0.5mg/kg b.wt intramuscularly (in T_1, T_2 & T_3) and anaesthetized after 10 minutes by: (1) a mixture of xylazine @ 0.25 mg/kg b.wt and ketamine @ 4mg/kg b.wt given intravenously and maintained with 0.125 mg/kg b. wt of xylazine and 2mg/kg of ketamine (single incremental dose) in group T_1 (n=4); (2) a mixture of xylazine @ 0.25 mg/kg b.wt and ketamine @ 4mg/kg b.wt given intravenously and anaesthesia maintained with two incremental doses of a mixture of xylazine 0.125 mg/kg b.wt and ketamine 2 mg/kg b.wt (double incremental dose) in T_2 (n=4); (3) a mixture of xylazine @ 0.25 mg/kg b.wt and ketamine @ 4mg/kg b.wt only, given intravenously in T_3 (n=6) and (4) xylazine @ 1mg/kg b.wt.

intramuscularly followed after 10 minutes by intravenous dose of Propofol @ 4 mg/kg b.wt. in T_4 (n=4) for a variety of clinical surgical and manipulative procedures. Induction to recovery time and duration of surgical anaesthesia were maximum for T_2 followed by T_1, T_4 and T_3 in decreasing order. The recovery time was maximum in T_2 (69.75±1.93) and minimum in T_3 (38.50±1.48), duration of surgical anaesthesia was also maximum in T_2 (43.00±0.91) and minimum in T_3 (19.00±0.37). A significant decrease in HR was recorded in all the groups. Maximum decrease in HR occurred at 20 min post induction. The mean RR vale decreased significantly in all the groups. In group T_1, T_2 and T_3 maximum Decrease occurred at 20 min while In T_4 maximum decrease (one fourth of 0 min. value) in RR occurred at 10 min. RT decreased non-significantly in group T_1 and T_3 while significant decrease occurred in T_2 and T_4. A non-significant decrease occurred in mean haemoglobin value in all the groups. TLC decreased non-significantly in all the groups. Mean values of SGOT and SGPT concentrations increased significantly in all the groups. The SGOT value did not became comparable to mean value of 0 min after 24 hours in all groups except T_4 and the SGPT value became comparable to mean value of 0 min after 24 hours in all the groups except T_2 value. A significant hyperglycemia occurred in all the groups. The mean Glucose Values did not became comparable to mean value of 0 min after 24 hours except in T_3. The mean value of serum sodium concentration increased significantly in all the groups. The mean value of potassium ion concentration decreased significantly in T_1 and T_2 and non-significantly in T_3 and T_4. The significant reduction in chloride ion concentration was observed in all the groups, which ranged within normal physiological limits. Electrocardiographic changes at 60 min after attaining surgical anaesthesia represented the changes at 10 min but with more intensity. At this time T-wave variations were typical of electrolytes disturbance and varying degree of S-A block or A-V block was present. In one animal of T_4, T wave was typically biphasic with ST segment depression indicative of hypokalaemia.

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7. Clinical evaluation of different techniques of ovariohysterectomy in bitches

Anil Kumar Singh and Bharat Singh

The present investigation was conducted on 16 clinical cases, aged 2-6 years and weighing between 14 kg to 25 kg. Ovariohysterectomy (OH) of these animals was done through right flank and midline approach under general anaesthesia. The bitches were premedicated with atropine sulphate (0.044mg/kg b.wt.) and xylazine hydrochloride (0.5mg/kg b.wt.) administered intramuscularly. After 10 minutes a combination of xylazine hydrochloride and ketamine hydrochloride (1:3) was administered intravenously till effect, followed by maintenance of general anaesthesia with the intravenous incremental dosages of same combination as per the need. The animals were restrained either in dorsal recumbency for mid-ventral approach or in left lateral recumbency for right flank approach. Animals of group A and B underwent OH through right flank approach and closure of abdominal wound after operation was carried out in layers. In the first layer the transverse abdominal muscle with its attached peritoneum was closed and in second layer the internal and external oblique muscles of abdomen were closed together in simple continuous pattern. Whereas animals of group C and D underwent OH through mid-ventral approach and closure of abdominal wound after operation was also carried out in layers. Clinical evaluation of different techniques of OH was done on the basis of clinical observations recorded during preoperative, operative and postoperative periods as below: Clinical observations of the temperature, pulse and respiration rates were recorded before operation, at day of operation and after operation on 1st (24hrs), 2nd, 3rd, 5th, 7th and 10th day of operation in all the bitches. Observations during operation included, the time taken for surgery

(duration of operation) and amount of hemorrhage during entire procedure. The different hematological and serum biochemical parameters viz., Hb, PCV, TLC and DLC and serum glucose and serum creatinine were evaluated at preoperative and postoperative i.e. on 1st (24hrs), 2nd, 3rd, 5th, 7th and 10th day after operation from blood and serum, respectively. In the present study, the animals underwent OH through right flank approach (A and B groups), the mean time taken for completion of surgery were 31.25±1.99 and 32.25±1.61 minute, the mean amount of hemorrhage during surgery were 50.00±2.29 and 51.25±1.79 gm. and mean cost of operation were 236.17±7.44 and 250.19±5.41 rupees, respectively. Whereas the animals underwent OH through mid-ventral approach (C and D groups), the mean time taken for completion of surgery were 39.00±2.14 and 44.25±2.17 minute, the mean amount of hemorrhage during surgery were 23.00±2.19 and 25.25±1.65 gm. and mean cost of operation were 280.82±5.15 and301.41±7.60 rupees, respectively. The animals of group D, had maximum postoperative complications with regard to suture biting, suture dehiscence and evisceration as has been observed in No.A16 (Chromic catgut) animal and exudation in No.A15 (Chromic catgut) animal, followed by group C, in which exudation was observed in No.A11 and A12 (Chromic catgut) animals, then group B in which suture biting was observed in No.A5 (Vicryl) animal whereas group A animals were recovered uneventfully without any complication. There was no significant change in hematological and serum biochemical studies in the present study.

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8. Anaesthetic and surgical evaluation of xylazine with diazepam or midazolam and ketamine in cattle

Ramraj and Bharat Singh

The present study was conducted on 24 cattle ranging from 1 to 6 years of age and 80-300 kg b. wt., randomly numbered and given six treatments in randomized manner in two groups consisting of four animals in each treatment groups. The details of different treatments are as follows: Treatment 1 (T1) - Xylazine @ 0.05 mg/kg plus diazepam @ 0.2 mg/kg, b. wt. i.m., Treatment 2 (T2) - Xylazine @ 0.05 mg/kg plus midazolam@ 0.1mg/kg, b. wt. i.m., Treatment 3 (T3) - Xylazine @ 0.05 mg/kg plus diazepam @ 0.2 mg/kg, b. wt. i.v., Treatment 4 (T4) -Xylazine @ 0.05 mg/kg plus midazolam @ 0.1mg/kg, b. wt. i.v, Treatment 5 (T5) - Xylazine plus diazepam as in T3 and after 10 min. followed by ketamine slow i.v. till effect to deepen/extend the duration of anaesthesia., Treatment 6 (T6) - Xylazine plus midazolam as in T4 and after 10 min. followed by Ketamine as in T5. The physiological study included recording of HR, RR, RT, hematological and biochemical study taken before and at 30, 60, 90, 120, 1440 and 2880 min. after the administration of different treatments. The down time, time to onset of analgesia and time to develop peak effect were 13.75±0.62, 17.50±0.64 and 22.50±0.63 min. with i.m. xylazine-diazepam and were significantly more than that of i.m. xylazine-midazolam 12.25±0.85, 14.50±0.60 and 19.00±0.40 min., respectively. The down time, time to onset of analgesia and time to develop peak effect on i.v. administration of xylazine-diazepam and xylazine- midazolam were 2.25±0.14, 7.75±0.47 and 9.50±0.47 min. and 2.00±0.20, 6.75±0.47 and 8.50±0.64 min., respectively, and were significantly less in comparison to i.m. administration of these drugs. The duration of peak effect and duration of analgesia in T1, T2, T3, T4, T5 and T6 groups of animals were 12.50±0.62, 9.75±0.62, 10.50±0.28, 8.50±0.64, 29.50±5.04 and 27.00±5.08 min. and were 21.00±0.40, 19.25±1.37, 19.50±0.64, 15.00±0.91, 37.25±4.26 and 34.50±6.06 min., respectively. The duration of peak effect and duration of analgesia were lower in midazolam treatment groups in comparison to diazepam treatment groups. The dose of ketamine was required to maintain anaesthesia for 37.25±4.26 and 34.50±6.06 min. duration in T5 and T6 groups of animals were 2.32±0.26 and 2.63±0.08 mg/kg, respectively. The recovery time in T1, T2, T3, T4, T5 and T6 groups of animals were 49.00±1.47, 43.00±1.29, 32.50±1.44, 25.50±0.86, 50.75±5.42 and 46.25±2.54 min., respectively. Results of present study on

various clinical, physiological (HR, RR RT), hematological (PCV, Hb, TLC, DLC) biochemical (serum glucose, alkaline phosphatase, urea nitrogen, bilirubin) and serum electrolytes (Na^+, K^+ and Cl^-) parameters did not reveal any deleterious effect on any vital function and organs in the body and these drug combinations can safely be used for different purposes in routine clinical cases as per the need without any risk.

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9. Cardiovascular monitoring in surgical and critically ill canine patients

Amit Kumar and R.P. Pandey

The study was conducted on 30 critically ill dogs. The parameters examined included heart rate, respiratory rate, rectal temperature, haematology (Hb, PCV, PMN%), blood pressure (BP), peripheral venous pressure (PVP), serum electrolytes (Ca, Na, K, P, Cl) and electrocardiography. The subjects underwent surgical treatment and general anaesthesia and some was critically ill. All the dogs were divided into two major groups A and B. All the sub groups contained equal number of dogs. Group A was subdivided into sub group A_1, A_2, A_3, A_4, A_5, A_6 and these sub groups were belonged to various surgical conditions e.g. fracture, ear affections, perineal hernia, urolithiasis, OH or castrations and mammary tumours respectively. Group B was subdivided into B_1, B_2, B_3, B_4, B_5, B_6 and these sub groups belonged to various critical conditions like anaemia, dehydration, nervous signs, infections, paralysis and trauma (with gross infection) respectively. In case of group A parameters were recorded at maximum depth of anaesthesia or during surgery. Significant decrease in SAP was recorded in sub group A_2 in comparison to A_5; while sub groups A_1, A_3, A_4 and A_6 showed significant increase in SAP in comparison to A_5. There were significant increase in SAP in sub groups B_1, B_3 and B_6 in comparison to B_2; while B_4 and B_5 showed significant increase in SAP in comparison to B_2. Mean values of P wave amplitude, duration, P-R interval, QRS complex interval, R wave amplitude and Q-T interval were not differ significantly in between different sub groups of animals of group A. A non-significant difference was observed in P wave duration, P-R interval, QRS complex interval and R wave amplitude. However, a significant increase in P wave amplitude and Q-T interval were recorded in sub group B_2 in comparison to other sub groups of group B. The dogs which belonged to sub group B_2 showed biphasic T wave, deep inverted T wave, sagging of S-T segment, flattening of T wave, variable R-R interval, low amplitude T wave, spiked and large T wave, peaked P wave in ECG tracings. These indicated hypokalemia and correlated well with the serum electrolytes findings in sub group B_2. Sub group B_5 showed respiratory sinus arrhythmia. The dogs which belonged to sub group B_3 showed atrial flutter, variable R-R interval, saw tooth appearance of P wave, negative T wave and notched P wave which may be the indication of left atrial enlargement and chronic vulvular insufficiency, and respiratory sinus arrhythmia. The dogs which belonged to sub group B_6 showed tachycardia with negative deviation of S-T segment which was an indication of hypoxia, and also showed sinoatrial block, ventricular premature complex (VPC) and electrical alteranans which may be the indication of pericardial effusion. The dogs which belonged to sub group B_1 showed variable R-R interval and typical Wenckebach phenomenon-(Mobitz type I) for the non-conducted P wave. The dogs which belonged to sub group B_5 showed respiratory sinus arrythmia. The dogs which belonged to sub group A_1 showed sinoatrial block (Atrial stand still) which was an effect of xylazine in dogs. The dogs which belonged to sub group A_6 showed variable R-R interval, P waves of variable heights. The dogs which belonged to sub group A_4 showed S-A and A-V block that was also interpreted as an effect of xylazine-ketamine anaesthesia. QRS complex of variable amplitude, strikingly tall R waves that were reported to occur in left atrial enlargement and chronic renal hypertension.

10. A comparative study of closed interlocking nailing (C.I.L.N.) and open interlocking nailing (O.I.L.N.) in management of canine femur and tibia fractures

Monika Goyal and Bharat Singh

The present study was planned to find out the incidence of canine fracture cases in the area based on the canine cases registered during the period with effect from January 2003 to December 2006 the incidence of long bone fracture cases in relation to total cases, surgical cases and fracture cases was 3.9, 8.9. 84.74 percent, respectively. During the present study period with effect from October 2006 to April 2007 the incidence of surgical cases in relation to total cases and fracture cases in relation to total cases as well as in relation to surgical cases was 44.74 and 5.77 and 12.90 percent, respectively. The incidence of long bone fracture cases in relation to total cases, surgical cases and fracture cases was 4.53, 10.14 and 78.57 percent, respectively. During the period from Jan. 2003 to Dec. 2006 and Oct. 2006 to April 2007 the age and sex wise, the incidence of long bone fractures, in less than 1 years, between 1 to 6 years and above 6 years of age was 41, 15; 24, 12; and 6, 2 and 27.27, 31.81; 18.18, 9.09; and 4.54, 9.09 percent, respectively. The cases were managed by POP cast with splint, wooden splint in fore limb and Thomas splint along with wooden splint and crape' bandage for tibial fracture intramedullary bone pinning and Crape' bandage and Homoeopathic medication (Arnica -30, Calcare phosphorica 6x, Symphytum-30, and AT-200 a biochemic preparation) were found to be facilitate the fracture healing. For open infected intraarticular fractures involving condyles with small piece, it is observed that they can be best managed with palliative treatment including complete rest, cultural examination, antibiotic administration, antiseptic dressing and calcium, mineral supplements.

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11. Studies on propofol, midazolam and their combinations for clinical anaesthesia in dogs

Jagdish Prasad Kushwaha and Bharat Singh

The present study was conducted on 36 selected clinical cases of dogs of either sex of different breeds and 2-8 years of age and weighing 10-25 kg to study the effects of midazolam and propofol in different combinations on clinicophysiological, haematobiochemical and some haemodynamic parameters and electrocardiography. The animals were divided into six groups and different drug/drug combinations were administered for tranquilization and anaesthesia. Midazolam, 0.5 mg/kg b.wt. i.v. (T_1). Midazolam, 0.5 mg/kg b.wt. i.v. followed by mixture of propofol, Midazolam (1:1 v/v) i.v. till effect used for induction as well as maintenance of anaesthesia (T_2). Midazolam, 0.5 mg/kg b.wt. i.v. followed by mixture of propofol, Midazolam (1:2 v/v) i.v. till effect used for induction as well as maintenance of anaesthesia (T_3). Propofol alone used i.v. till effect for induction as well as maintenance of anaesthesia (T_4). Induction of anaesthesia with propofol i.v. till effect and maintenance with mixture of propofol and midazolam (1:1 v/v) i.v. used in incremental doses for maintenance (T_5). Induction of anaesthesia with propofol i.v. till effect and mixture of propofol and midazolam (1:2 v/v) i.v. used in incremental doses for maintenance (T_6). On the basis of results it can be concluded that (1) Midazolam at the dose rate of 0.5 mg/kg b.wt. i.v. serves as a tranquilizer for short duration of procedure and can be used as an adjunct to local analgesia for minor surgical operations. (2) Propofol alone was found to be safe and suitable anaesthetic agent for clinical use in variety of surgical procedure and (3)Midazolam at the 0.5 mg/kg b.wt. for tranquilization and mixture of propofol and midazolam (1:2 v/v) for maintenance was best drug combination for satisfactory/ balanced anaesthesia in dogs, which can easily be practiced in various clinico-surgical procedure for various duration of surgical interventions as has been also recorded in this study without any deleterious effects on any system and organ of the body.

12. Studies on laparoscopic sterilization techniques in bitches

Kuldeep Singh Gautam and R.P. Pandey

The study was planned to evaluate the three agents viz. (a) carbon dioxide, (b) carbon dioxide followed by filtered room air for maintaining the intra-operative laparoflation and (c) filtered room air alone used for laparoflation; on the basis of physiological, haemodynamic, haematological alterations and effects of laparoscopic ovariectomy and laparoscopic ovario-hysterectomy in bitches; to find out the suitable and less expensive gaseous agent for laparoflation in bitches; to find out a technique for quick laparoscope assisted oophorectomy in pre-pubertal dogs using the agent of laparoflation found suitable on the basis of above findings and to study the type and rate of complications associated with the laparoscopic sterilization techniques in pre-pubertal and adult dogs and to record intra-operative observations. It was found that: Laparoflation with either of the gaseous agents namely, filtered air and carbon di oxide have no significant adverse physiological, behavioural or clinical effect on the animal. Laparoscopic oophorectomy and ovario-hysterectomy can be performed with minimal time, minimal manipulation and quick post operative ambulation and healing albeit after achieving dexterity by practice and be used as an effective measure for animal birth control programme. Extracorporeal oophorectomy has considerable advantage over intra-corporeal technique with less time consumption but with the additional need of two extra ports to deliver the ovaries.Use of filtered air conjunction with CO_2 can cut down cost of procedure in comparison to carbon dioxide in ABC programme in veterinary practice for maintenance of intra operative laparoflation. Similarly filtered air alone is found suitable for laparoflation for diagnostic laparoscopy.

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13. Radiographic and ultrasonographic studies on liver, spleen, kidneys and urinary bladder in common breeds of dogs

Mohd. Nadeem and Bharat Singh

The present study was conducted in dogs, irrespective of their age, body weight and breed and were divided into three groups. Group N (n=7) comprised of apparently healthy dogs, Group A (n=7) included dogs presented to the University clinics for ailments of the GIT and those of Group B (n=7) included dogs with the primary complaint of urinary system. In each animal, age, body weight, body length, height and circumference were recorded. This study revealed that except the age, no other measurements varied significantly. The liver length and width did not differ significantly in groups N and A. The whole spleen could not be visualized in any of the radiographs. However, it could be discerned as a band and its width was measured accordingly. The spleen width did not differ significantly in groups N and A. The T13 vertebra to sternum length (VS length) was measured on radiographs in lateral view and a ratio between VS length and liver length, VS length and liver width and VS length and spleen width were worked out. It was observed that the liver length was 0.91 times the VS length in Group N and 0.88 times in group A, while the liver width was 1.6 times in Group N and 1.49 times in group A. Similarly, the spleen width was 12.33 times less in Group N and 9.95 times less in group A. Kidneys silhouettes could be properly seen in ventro dorsal view using positive contrast radiography. The urinary bladder appeared as pear shaped anechoic area with hyper-echoic far margins due to acoustic enhancement and smoothly marginated echogenic bladder wall in animals of group N. However, the margins appeared irregular in distended bladder in three animals of group B while in one animal of this group a semi-lunar hyper-echoic area with acoustic shadow was observed indicating cystolith. Based on these findings the following conclusions can be made: Radiographic assessment of liver is easier and better in lateral views as compared to

ventrodorsal views, however, identification of focal or diffuse pathological lesions in hepatic parenchyma is difficult in radiography. Radiographic ratio of VS length and liver length can be a valuable diagnostic tool for hepatomegaly. Contrast radiographic procedure aids in better assessment of kidneys as compared to survey radiography. Radiographic ratio of L2 body length and kidney size can aid in diagnosis of renal pathology. Hepatic, splenic and renal parenchyma can be better assessed by ultrasonography. Morphometric assessment of organs is more accurate on ultrasonography. A combination of ultrasound and radiography is superior to any of these modalities used alone.

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14. Studies on diagnosis and treatment of otitis in dogs

Puja Jain and R.P. Pandey

The study was undertaken on 27 dogs presented at the Teaching Veterinary Clinical Complex, College Of Veterinary Science And Animal Husbandry, Mathura for a period of 6 months from 1st November to 31st April 2010 with any kind of complaint related to ears. The influence of age, breed, sex, living environment of pet, skin coat, shape of ears, frequency of ear canal cleaning, anal sac infection, ectoparasiticide and deworming use on the incidence of otitis externa was also studied. The results of the study revealed that the incidence of ear affection was highest in dogs belonging to the age group of 1-5 years and the least in the age group below 1 year. Male dogs showed greater involvement than females. German shepherds, followed by Spitz, and Labrador retrievers were the most frequently affected breeds. Indoor companion pets were mostly affected as compared to outdoor pets. Long haired dogs were more commonly affected than short haired dogs. Dogs having erected ear was more commonly affected followed by floppy ear, droopy ear and semiprick ear. The dogs in which regular cleaning of ear canal was followed had the least incidence. Anal sac infection was present in 40.74% cases. The dogs suffering from otitis showed physical manifestation such as erythema, crust formation, foul smelling otic discharge, head shaking, scratching of ear pinnae with paws, pain on palpation of auricular cartilage, and ulceration of the inner aspect of external ear canal. The dogs harbouring Staphylococccus spp. infection of ears were presented with foul smelling purulent exudates or brownish color ceruminous discharge, and in case of Pseudomonas spp. Infection yellowish to greenish color discharge was presented. Affected dogs were also found with inflammation, head tilted towards the affected site, constant shaking of head, and scratching ear pinnae with paws. In clinical cases of dogs with Mallasezia spp. Ear infections, presence of brownish color foul smelling purulent ceruminous exudates in external ear canal was present. Cells of Mallasezia spp. were seen as oval to pea-nut Gram-positive unicellular yeast cells. Ultrasonography of the Exteranal ear canal with 6.5-8 MHz curvillinear probe after infusion of saline solution as a contrast media into the ear canal may be an accurate, non-invasive, rapid, and widely available method for assessment of ear canal. Out of all maladies of dogs the overall incidence of otits externa stood at 2.86 percent. Direct swab cytology proved an excellent diagnostic aid yielding quick and correct results for deciding the therapy. Ultrasonographic evaluation of tympanic bulla in suspected cases of otitis media was found to have enormous potential as a non-invasive diagnostic aid.

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15. Studies on halothane anaesthesia in propofol, xylazine, midazolam premedicated and propofol induced dogs

Tejveer Singh and Bharat Singh

The present study was undertaken to test the suitability of different preanaesthetics/ combination and to study their effects on propofol-halothane anaesthesia in dogs. The study

was conducted on 24 selected clinical cases of dogs of either sex and of different breeds. Emphasis was given to include animals of similar body wt in same group. The dogs were divided in to four groups viz. T_1, T_2, T_3 and T_4. Weak time, recovery time, sternal recumbency time and standing time were recorded in each group. The quality of sedation, analgesia and muscular relaxation was also recorded during pre-induction, post-induction and maintenance period. Different physiological parameters viz. heart rate, respiratory rate, rectal temperature and body surface temperature and haemodynamic parameters viz. systolic blood pressure, diastolic blood pressure, mean arterial pressure, central venous pressure and haemoglobin oxygen saturation were recorded at time 0 (base line) and at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 120 min after administration of drugs. Response of corneal reflex and pedal reflex were also observed at the same time intervals. Required doses of induction and maintenance agents were also recorded at the end of each anaesthetic trial. Haemoglobin, packed cell volume, total leucocytes count, differential leucocytes count, plasma glucose, creatinine, urea nitrogen, aspartate aminotransferase, sodium and potassium were estimated at time 0 (base line) and at 15, 30, 60, and 120 minutes after administration of drugs. Based on the above study the different combination of anaesthetic protocol was found suitable in the following order: Xylazine (0.25 mg/kg i.m. + midazolam 0.25 mg/kg i.m.) combination was found best in terms of quality of sedation, induction and recovery followed by xylazine (0.5 mg/kg i.m.), midazolam (0.5 mg/kg i.m.) and propofol (2 mg/kg i.v.), respectively, when used as preanaesthetics to propofol-halothane anaesthesia in dogs. Xylazine (0.25 mg/kg i.m.+ midazolam 0.25 mg/kg i.m.) combination and xylazine (0.5 mg/kg i.m.) as preanaesthetics produced more dose sparing effect on induction and maintenance agents with slightly more but transient cardiac depression in comparison to others. No anaesthetic combination produced any serious depressive effect on cardiorespiratory, haemodynamic and haemato-biochemical parameters and they returned to base line at the end of observation period. Atropine sulphate (0.04 mg/kg i.m.)- Xylazine (0.25 mg/kg i.m.)+ Midazolam (0.25 mg/kg i.m.)- Propofol-halothane and Atropine sulphate (0.04 mg/kg i.m.)- Xylazine (0.5 mg/kg i.m.) -Propofol-halothanes were found most suitable anaesthetic combinations for clinical anaesthesia in dogs.

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16. A comparative study of closed interlocking nailing (C.I.L.N.) and open interlocking nailing (O.I.L.N.) in management of canine femur and tibia fractures

Viram Varshneya and R.P. Pandey

The present study was conducted on eight clinical cases (7 animals with one animal having bilateral femur involvement) of femur and tibial fractures in canines, divided into two groups. The animals of Group-A were subjected to Closed Interlocking Nailing, whereas, those of Group-B were subjected to Open Interlocking Nailing as reduction could not be achieved by closed method. The reduction and fixation were achieved under the guidance of C-arm image intensifier. Preoperative radiographs were taken in all clinical cases for assessment of procedure, nail and screw dimensions. Weight bearing in animals of Group-A was Excellent in three dogs and Fair in one, while in Group-B it was excellent in all the animals. Radiography on 30th post-operative day in group-A revealed stable implant and fracture fragments with evidence of mild to exuberant periosteal reaction which was indicative of initiation of fracture healing process. Good bone fragment positioning was observed in three cases. Immediate weight bearing did not disturb the reduction in three cases. However, in one case, the radiograph revealed breakage of proximal screw and a loose fractured segment on the plantar aspect of the distal fragment well incorporated in the callus. In Group-B in almost all the cases, signs of periosteal reaction were evident after 30th day of surgery. Radiography of the animals of this group revealed good

apposition of the fragments and intact implant in all cases. It is concluded that open interlocking was easier to perform than CILN in delayed cases but in fresh cases CILN was easy to perform. Closed interlocking nailing can be the method of choice for diaphysary fractures of femur and tibia in dogs. The distinct advantages of closed nailing are preservation of primary fracture haematoma and early healing. Incidence of screw bending and breakage in large dogs necessitate the use of two screws for locking. Closed interlocking nailing gave acceptable to good results even in multiple fractures of the shaft. Interlocking nailing is distinctly superior method of fracture fixation as it completely negates the possibility of axial rotation and proximal or distal nail migration.

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17. Studies on chemotherapy of mammary tumor in bitches

Neeraj Yadav and Bharat Singh

The present study was conducted on twelve cases of CMNs. The occurrence of CMNs was found to be 35.30% with maximum percentage of cases in the month of September followed by February. Various parameters recorded were age, breed, sex, duration of clinical signs, mode and rate of growth, recurrence, size of neoplasm, number of glands involved, location and consistency of neoplasm, attachment to the skin or body wall, nipple deformities and discharge, mated at first heat, neonatal deaths. All the animals were divided into three groups viz., Group S, Group A and Group C in which Group S animals were subjected to surgery alone (simple mastectomy, enblock dissection and lumpectomy), in Group A animals after surgery undergone to adjuvant chemotherapy with combination of doxorubicin @ 25 mg/m^2 I/V and cyclophosphamide @ 100 mg/ m^2 and 3 doses of this treatment at 7 days interval and in group C animals undergone chemotherapy alone. Radiograph and Ultrasonograph of all the cases was also done. Histopathologial evaluation revealed majority of tumors to be malignant (75%) and benign (25%). Combination of doxorubicin and cyclophosphamide was found to be effective as effective as adjuvant chemotherapy in treatment of CMNs which increases the longevity and survival of animals.

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18. Radiographic morphometric studies of distal bones and joints of fore and hind limbs of buffaloes

Sudhanshu Bansal and Vivek Malik

The present study was conducted on thirty clinical cases of buffaloes of different age groups which were presented at TVCC, DUVASU Mathura for diagnosis and treatment of minor problems for radiographic measurements of different distal bony and joint indices, divided into three groups depending on their age (emphasis was given to select animals of almost similar body weights and height in a particular group). Group A included animals of 1.5 to 3 years of age; Group B: 4 to 6 years and in group C animals of more than 6 years of age were selected. Then digital radiographs were taken of both fore and hind limbs in dorsopalmar/dorsoplantar views and in lateral view using 70 kVp and 30 mAs with FFD of 80 cm as radiographic factors. The studied parameters included bone length, bone diameter, joint space, joint angle and cortex to diameter ratio of metacarpal, metatarsal and phalanges and their joints. Mean±SE values of length of metacarpal of animals of group A, group B and group C was recorded to be 19.36±0.06 cm, 19.90±0.20 cm and 20.50±0.16 cm, respectively and mean±SE values of metatarsal recorded was 22.21±0.17 cm, 22.77±0.15 cm and 24.25±0.10 cm, respectively. Mean±SE values of diameter of metacarpal and metatarsal of group A, B and C was recorded as 3.13±0.03 cm, 3.98±0.07 cm, 4.29±0.02 cm and 3.01±0.02 cm, 3.41±0.06 cm, 3.62±0.08 cm, respectively. The diameter of metacarpal bone was found to be more significant in comparison to diameter of metatarsal of

buffaloes of all three age groups. The length of metacarpal, metatarsal, P1, P2, P3 of hind limbs were found to be higher than that of fore limbs in the animals of all three groups while diameters of metacarpal, metatarsal, P1, P2, P3 of fore limbs were found to be higher than that of hind limbs. Significant increase in the diameter of all the bones was noticed with advancing age with maximum diameter in group C followed by group B and A, respectively. However, C/D ratio were found to be maximum in the animals of group B, followed by that of group C and group A, respectively. There was no significant difference in the values of metacarpo-phalangeal joint angle (JA1) and proximal inter-phalangeal joint angle (JA2) of the fore limbs in any of the group, whereas, it was noticed that the values of JA1 and JA2 of hind limb and distal inter-phalangeal joint angle (JA3) of fore limb in the animals of group A was significantly higher than that of other two groups with the minimum in the animals of group C. Metacarpo-phalangeal joint space (JS1) did not show any significant difference between the medial and lateral sides in forelimb in any of the groups. Whereas, JS1 of both medial and lateral sides in hind limb was wider in the animals of group A followed by group B and group C, respectively. In all three groups mean±SE values of proximal inter-phalangeal joint space (JS2) of both medial and lateral sides did not differ significantly in both fore limbs and hind limbs. In groups B and C, mean±SE values of distal inter-phalangeal joint space (JS3) of both medial and lateral sides were significantly higher in forelimb than that of hindlimb. However, no significant difference was noticed when comparison was made between values of JS3 of both medial and lateral sides of fore limb and hind limb.

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19. B-Mode ultrasonographic evaluation of teat in dry and lactating buffalos

Pramod Kumar and Sanjay Purohit

The aim of the present study was to determine the normal and abnormal ultrasonographic features of teat parameters in dry, lactating and pathogenic (teat affections) buffaloes. For this study 18 buffaloes (six dry and six lactating from dairy farm and six pathogenic from Kothari hospital) were selected. B-Mode ultrasonographic examination of teat by 8-MHz convex transducer was performed in all three groups. Ultrasonographic finding were described and teat canal length, teat end width, teat wall thickness and teat cistern width were measured. The mean teat length was 7.358±0.523 cm and 7.491±0.289 ern, 6.683±0.711 em and 7.775±0.808 em, 7.091±1.055 em and 7.8±1.043 ern in front and rear teats of dry, lactating and pathogenic groups of animals, respectively. Teat diameter was measured by using vernier calliper at 2.5 ern above the tip of the teat in all buffaloes. The mean teat diameter was 2.462±0.167 cm and 2.670±0.144 em, 2.580±0.159 cm and 2.986±0.187 cm, 3.234±0.393 em and 3.147±0.444 ern in front and rear teats of dry, lactating and pathogenic groups of animals, respectively. Teat end shape was round in majority of the animals. For evaluation of internal parameter of teat, ultrasound scans were taken. The teats were scanned in B-mode using a portable ultrasonography machine. Teats were dipped into plastic cup filled with water at room temperature for better visualization of the teat structures. The probe was placed in the wall of the plastic cup using ultrasound gel and held lateral to the teat. The ultrasound images obtained were recorded. The proper images were chosen for measurements and subsequently certain teat parameters were measured. Ultrasonography allows measurement of a wide range of teat tissue parameters, including canal length, teat end width, teat wall thickness and cistern diameter. Teat canal length was measured in millimeters as the distance between the distal and proximal end. Streak canal was observed as a hyperechogenic line at the tip of the teat. Teat canal length was measured 7.543±1.159 mm and 8.170±1.163 mm, 9.768±1.039 mm and 9. I 50±0.866 mm, 9.563±1.557 mm and 11.243±2.423 mm, in front and rear teats of dry, lactating and pathogenic buffaloes respectively. Teat end width was measured in millimeters as a perpendicular to the axis of the teat canal at its proximal end. Teat

end width was measured 16.450±0.938 mm and 17.900±1.021 mm, 18.608±0.801 mm and 19.841±0.666 mm, 21.525±3.437 mm and 21.750±1.944 mm in front and rear teats of dry, lactating and pathogenic buffaloes respectively. Teat wall thickness was measured in millimeters above the proximal end of the teat canal. Teat wall thickness was 7.651±0.480 mm and 7.609±0.413 mm 9.064±0.540 mm and 8.954±0.510 mm, 9.906±1.3868 mm and 9.097±0.955 mm in front and rear teats of dry, lactating and pathogenic buffaloes respectively. Teat cistern width was measured in millimeters one cm above the proximal end of the teat canal perpendicular to the axis of the teat canal. Teat cistern width was 6.530±0.975 mm and 7.619±0.893 mm, 6.750±0.495 mm and 6.630±0563 mm, 10.631±1.738 mm and 10.319±1.682 mm in front and rear teats of dry, lactating and pathogenic buffaloes respectively. The B-Mode ultrasonography technique as described in this study seems to be reliable for determining the normal and abnormal anatomic features of the teat parameters in buffaloes.

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20. Development and clinico-biomechanical evaluation of prosthetic limb in dogs

Mohd Shiyad K.K. and Vivek Malik

The present study was carried out on six dogs of either sex, belonging to various breeds and age group reporting the department of Surgery and Radiology and in the Teaching Veterinary Clinical Complex (TVCC), DUVASU), Mathura. Improved stump socket prosthesis for six dogs of different breeds like Labrador (1), nondescript (2), Pomeranian (1) and crossbreds (2) were developed. The fabrication of prosthesis using aluminium U- channel and hollow pipes of different sizes (U channel 9mm, hollow pipe 9mm, U channel 6mm) and stainless steel springs of three different sizes. Different *in vitro* biomechanical tests of the prosthetic limb were done. These tests proved that the 6 mm aluminium U channel could be used for construction of prosthetic limb for animal weighing 5-35 kg, hollow pipe (9mm) could be used for animal weighing 35-55 kg and 9mm U channel could be used for animals weighing 55-75 kg. Biomechanical tests of springs with G of 80000 mega Pascal showed that spring A can bear up to a weight of 4.1 kg and can deflect about 10 mm. Spring B can deflect up to 15 mm safely and can carry a load of 8.6 kg. Regarding spring C, it can bear a load of 21.1 kg and could deflect up to 50 mm without damage. So these springs can be used in the construction of the prosthetic limb. Clinical evaluation of prosthesis was done on the basis of the weight bearing ability of dogs on the prosthetic limb. A special weighing platform was designed so as to measure the amount of weight animal applying on the prosthetic limb or the support on the prosthetic. Weighing of animal was done at 5 days interval. Based on the weighing done on the different animals after the attachment of the prosthetic limb helped in summarizing that after the attachment of the prosthetic limb the animal gets completely adapted to the prosthetic by 15 days of its attachment. The adaptability and comfort experienced by the animal after prosthetic application and body's natural reaction to the different materials used in the construction of the prosthetic limb were also studied. The owners were provided with two questionnaires to extract feedback from them. Overall performance of the prosthetic limb was found satisfactory and 4 animals out of the 6 got well adapted and were comfortable with the prosthesis and there was no any skin reaction to the materials we used. Video kinematic analysis study was done to assess the degree of movement in the joints of the prosthetic by recording linear parameters and horizontal and vertical motion by angular parameters. The results of the gait analysis of the limb after the attachment of the prosthetic limb helped in finding the functioning of the prosthetic limb. We found that animals were using the prosthesis and were applying load over it and not just dragging it. It was concluded that the stump socket prosthesis developed in this study is a feasible and cost effective modality and it could be used to improve the quality of life of the amputee animals.

21. Studies on common dental affection in dogs

Rahul Kumar and R.P. Pandey

The present study was conducted on a total of 128 dogs of different breeds and different age groups and incidence on various criteria was worked out. Out of these, 45 dogs were found to be suffering from periodontal affections. The Pomeranian breed of dogs showed the highest incidence of periodontal affections (14.06%) followed by German Shepherd (10.16%), Labrador Retriever (4.69%), Dachshund (0.78%), Rottweiler (0.78%), Non descript (3.20%) and Great Dane (\.60%). In the present study the overall incidence of periodontal affections was found to be 35.15% in 128 dogs examined, of which 66 were in 1-4 year age group, 20 in 5-8 year age group, 22 in 9-1 I year age group and 20 in above 12 year age group with 4.54%, 60.00%, 63.63%, and 80.00% incidence of periodontal disease (POD), respectively. Incidence study of dental calculus and crown abrasion also followed the same trend; minimum or nil incidences in young dogs with increasing trend as the age advances and the severity of the problem too showed an increasing trend. The sex-wise incidence of periodontal disease revealed greater involvement of male dogs (62.22%) than female dogs (37.78%). On the basis of body wt. maximum incidence of POD was seen in dogs body wt. between 10-30 kg followed by <I 0 kg and minimum in dogs above 30 kg body wt. General body condition of chosen 45 dogs 22.22% dogs were poor, 31.11% was satisfactory and 46.67% were in good health condition. The study of incidence of dental affections according to Habitat of dogs revealed that 88.89% cases were as companion in indoor animal and I I. I I% dogs were used as security dogs in outdoor. Of total 45 dogs 31.1% only were having exclusive dental and periodontal problem whereas 33.34%, 17.78%, 13.33% and 4.44% were having GIT, urinary, respiratory and cardiovascular health issue, respectively. The POD was present in 82.22% pets purchased from the breeder and 17.78% were bred in the house. Existing home dental care was practiced in 6.67% whereas 93.3% dogs suffering from POD did not have any dental care. In the present work incidence of POD in dogs maintained on pure vegetarian diet was much more in comparison to dogs maintained on non-vegetarian mixed diet. In the same way 68.9% dogs were maintained on homemade food and only 20% of all 45 dogs were exclusively fed with commercial pet foods. Ignorance and lack of awareness of the pet owners for dental care can be seen by the result of incidence according to occurrence of problem and incidence according to duration of problem. In 86.67% problem was longstanding but ignored and 55.56% cases the duration ranged up to 6 months. In 42.22% cases the problem remained ignored and were treated in 26.67% by oral antiseptic and in 31.11% by oral antiseptic and some systemic antibiotic and anti-inflammatory medication. According to owners, instituted treatment was successful in 57.69% cases and 42.31% problem remained the same. The incidence of various dental affections on definitive examination in our study in 45 dogs was found to dental plaque, tartar, gingivitis, teeth mobility, tooth discoloration, caries, dental fistula, and crown abrasion as 55.55%, 80%, 68.89%, 8.89%, 26.67%, 6.67%, 2.22%, I 1.1%, respectively. During treatment ultrasonic scaling was found superior to manual scaling as there were fewer incidences of inadvertent trauma and haemorrhage and time taken was markedly less. Keeping in view the owner's ignorance of dental problems in pets it is observed that regular precise and scientific examination of oral cavity should be a part of routine physical examination after 4 years of age, at least once or twice in a year to prevent major disease of teeth.

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College of Biotechnology

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1. Phylogenetic studies of canine parvovirus in dogs using VP1/VP2 gene

Deepti Singh and Amit Kumar Verma

In this cross-sectional study, out of 100 faecal samples from dogs showing the clinical signs of gastroenteritis (vomition, diarrhoea, and dysentery), amplicons of 160bp could be isolated from 63 samples using CPV-2RT primers. Analysis of prevalence with respect to epidemiological factors viz., breed, sex, age, vaccination status and cohabitation with other dogs was determined and study revealed that breeds, age, vaccination status and co-habitation with other dogs seem to influence the distribution of canine parvovirus infection. It was the highest in Doberman (77.78%) and the lowest in Pomeranian (45.45%). Age wise prevalence of CPV were high in pups (0-<6 months age group) indicated higher susceptibility of pups to CPV. Prevalence of canine parvovirus infection was higher in unvaccinated dogs and dogs sharing their habitat with another dog. Sex had no significant influence on prevalence of canine parvovirus infection. After confirmation of CPV by primers pCPV-2RT, another PCR was carried out with all the 100 faecal samples were used to amplify VP1/VP2 structural gene of CPV genome. Out of 100 faecal samples, 63 were amplified by pCPV-2ab primer set, whereas 54 were positive against pCPV-2b primer set indicating higher prevalence of CPV-2b (54 samples positive) in comparison to CPV-2a (09 samples). A total of eight isolates were sequenced by the private firm for phylogenetic analysis of these with the previous isolates, whose sequences were retrieved from the GenBank. In the Phylogenetic tree, all the field isolates isolated in the present study were grouped in one group along with isolated from China, Brazil, Bareilly, Kerala-2 and vaccine strain indicating the similarity among them. Though the isolates (Pondicherry 1 and 2; Kerala-1) could be distributed in another genetic group. The nucleotide (nt) divergence among the field isolates (Mathura 1-8) sequenced in this study and vaccine strains and other representative isolates (MEV-1, CPV-2a, CPV-2b Pondichery, CPV-2b China, CPV-2b Bareilly, CPV-2b Brazil, CPV-2b Iyrland, CPV-2b Pondichery-2, CPV-2b Kerala) revealed that nucleotide divergence between Mathura isolates and CPV-2b Pondichery-2 isolates was higher (5.8%) compared to that of among recent field isolates (0.00%). To investigate the molecular basis for the observed genetic divergence in the recent isolates, the nucleotide sequence of VP1/VP2 gene was compared. Comparison of majority (consensus) sequences of recent field isolates and the vaccine strains in the VP1/VP2 region revealed common substitution in the recent field isolates in comparison to MEV. Continued epidemiological surveillance and sequence analysis will help in to uncover the presence of mutations and will provide insights into the prevalence of different antigenic variants of CPV.

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2. Polymorphic studies of POU1F1 gene in Barbari goats

Shweta Sharma and Madhu Tiwari

Goat industry is the important part of the socio-economy structure of our country. There are various recognized goat breeds in India of varying potential producing meat, milk and fibre. Among them Barbari breed are highly suited for rearing under restrained and stall feeding conditions by small land holders. This essentiate the need to propagate this breed through marker assisted selection utilizing important candidate genes. POU1F1 gene is one of the gene that can be used as candidate gene for animal selection and breeding. As this gene encodes a pituitary specific transcription factor involved in development and regulating hormone expression in animals and moreover polymorphism of POU1F1 gene had been observed to be associated with important growth and production trait in various Chinese goats. With the objective, the present study was undertaken for studying polymorphism of POU1F1 gene and its

genotypic and allelic frequency in 50 goats of Barbari breed. Approximately, 5 ml of blood from representive goats were collected from jugular vein in 10 ml of vacuitainer tubes containing EDTA as anticoagulant. The genomic DNA was isolated from frozen as well as fresh blood samples by standard protocol of Sambrook *et al.* (1987). The purity was checked spectrophotometrically and the DNA samples ranging from 1.75-1.9 were included for the further study. PCR-RFLP/PstI assay was performed on isolated pure DNA samples by using the primer 'F':5'CCATCATCTCCCTTCTT 3'/ 'R':5'AATGTACAATGTGCCTTCGAG 3' And thereafter, RE digestion was done by *Pst*I R.E. The 450bp PCR product of POU1F1 gene was observed by performing agarose gel electrophoresis. The results of P*st*I/PCR-RFLP assay revealed presence of only TT genotype with a genotype frequency of 1. The allelic frequency of T allele was found to be 1and that of C allele was zero. It can be concluded that PCR-RFLP technique can be readily utilized for initial screening of animal population for identification of monomorphic/polymorphic nature of the gene. Barbari goat population showed monomorphic pattern possessing all homozygous (TT) genotype. The gene frequency of wild type T allele was 1. 3'UTR region of POU1F1 in the Barbari goat breed was found to be a highly conserved region of T/C at 110 position of 3'UTR region. Reproduction in all animals is necessary for continuation of generation and plays an important role in all economic yields in terms of milk and meat. If reproduction rate of animals is high, milk and meat yield will be higher in whole life span. The tendency of twining and triplicate is common in both sheep and goat. Several genes affecting ovulation rate in sheep have been discovered since the first major gene *FecB* (Fecundity Booroola) had been detected in 1980. Marker assisted selection using *FecB* mutation is being used to increase litter size in sheep having considerable economic value to mutton. However such study is not available in Barbari goat. Therefore the present study will be undertaken to study the polymorphism in major fecundity Booroola gene (*FecB*) in Barbari goat with objectives to identify *FecB* gene polymorphism in Barbari goat and to analyze the allelic frequencies of *FecB* gene in Barbari goat. In the present study DNA was isolated from the blood of 53 Barbari goats and the amplified fragments sizes were consistent with the expected size as determined from their gene sequence information. T-ARMS-PCR revealed the presence of only mutant carrier (AG) genotype among the screened 53 Barbari goats. In heterozygous goat, all three different sized products, namely, 1178 bp (common outer), 1097 bp ('A' allele specific) and 136 bp ('G' allele specific) were amplified. Animals with wild type homozygous (AA) and mutant (GG) genotype could not be identified. The genotypic and allelic frequencies at *FecB* locus in Barbari goat were calculated by standard procedure (Falconer and Mackay, 1996). The frequency of heterozygote genotype (mutant carrier) was 1.0 while there was absence of mutant genotype (GG) and wild genotype (AA) in studied population of Barbari goats. The frequency of A (wild) and G (mutant) alleles were 0.5. The conclusions were that Tetra- Primer amplification refractory mutation system- Polymerase chain reaction (T-ARMS- PCR) method can be used to detect *FecB* mutation in Barbari goat breed of India. Barbari population showed monomorphic pattern possesses all heterozygote genotype. The allelic frequency of mutant (G) and Wild (A) type nucleotide were 0.5 for each allele. Introgression of the *FecB* allele from Barbari goat to non-prolific Indian goat breeds can improve fecundity in other goat breeds. This study showed that *FecB* mutant allele is present in Barbari goats, on other hand due to screening of small sample size there would be possibility that *FecB* mutant allele might be present in homozygous state in Indian Barbari goat breed.

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3. Studies on Polymorphism of Major Fecundity Booroola Gene (FecB) in the Indian prolific Barbari Goat

Harsh Kumar and Deepak Sharma

India has the rich repository of goat genetic resources having 20 well characterized breeds of goat with distinct features. Barbari is a major dual purpose breed of India and utilized for milk and its meat. Reproduction in all animals is necessary for continuation of generation and plays an important role in all economic yields in terms of milk and meat. The tendency of twining and triplicate is common in both sheep and goat. Intensive research has come out on different prolific sheep breed to identify the genes involved in controlling ovulation rate and prolificacy. Several genes affecting ovulation rate in sheep have been discovered since the first major gene *FecB* (Fecundity Booroola) had been detected in 1980. In the present study,the polymorphism in major fecundity Booroola gene (*FecB*) in Barbari goat with objectives to identify *FecB* gene polymorphism in Barbari goat and to analyze the allelic frequencies of *FecB* gene in Barbari goat. DNA was isolated from the WBC pellet using RBC lysis buffer. The concentration of the DNA was estimated spectrophotometrically and reading at OD_{260} and OD_{280} represented the purity of the DNA and ranged between 1.8 and 1.9 indicating purity. The DNA revealed single band near the wells on 0.7% agarose gel electrophoresis visualized under gel documentation system. The amplified fragments of the candidate gene were run on 2.5% agarose gel for 4 hours and documented using gel documentation system. T-ARMS-PCR Revealed the presence of only mutant carrier (AG) genotype among the screened 53 Barbari goats. In heterozygous goat, all three different sized products, namely, 1178 bp (common outer), 1097 bp ('A' allele specific) and 136 bp ('G' allele specific) were amplified. Animals with wild type homozygous (AA) and mutant (GG) genotype could not be identified. The genotypic and allelic frequencies at *FecB* locus in Barbari goat were calculated by standard procedure (Falconer and Mackay, 1996). The frequency of heterozygote genotype (mutant carrier) was 1.0 while there was absence of mutant genotype (GG) and wild genotype (AA) in studied population of Barbari goats. The frequency of A (wild) and G (mutant) alleles were 0.5.

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4. Comparative profiling of seminal plasma and sperm membrane proteins in cattle and buffalo

Shilpi Dixhit and Vijay Pandey

The aim of the present study was to elucidate the composition of seminal plasma and sperm membrane proteins as well as to study the effect of cryo-preservation of semen on apoptotic like changes in spermatozoa of Haryana cattle and Bhadawari buffalo bulls. Six each sexually mature Haryana cattle and Bhadawari buffalo bulls having age of 2-4 years were used as semen donor. Six ejaculates from each bull were collected in morning hours using artificial vagina. Volume of each ejaculate of semen was directly measured in milliliters (ml) and concentration of spermatozoa (10^6/ml) in the neat semen was determined by the haemocytometer method. Immediately after collection, semen samples were centrifuged at 5000 rpm for 10 minutes at 4°C and the supernatant (seminal plasma) was separated. The sperm pallets were lysed by boiling with SDS-tris solution and centrifuged at 5000 rpm for 10 minutes for extraction of membrane proteins of spermatozoa. The proteins of seminal plasma as well as sperm membrane extract was estimated by Lowry method. SDS-PAGE was carried out in vertical slab gel electrophoresis system for protein profiling of both seminal plasma and sperm membrane extract of cattle and buffalo semen. The apoptotic index of sperms in neat and cryo-preserved semen were determined by staining the sperm pellet with Hochst stain and propidium iodide and degree of fluorescence is determined under fluorescent microscope. The mean ejaculate volume was

estimated 4.03±0.22 ml in cattle and 2.95±0.18 ml in buffalo bulls. The mean sperm concentration was observed to be 1736.94±60.46 $X10^6$/ml in cattle and 1678.05±86.68 $X10^6$/ml in buffalo bulls. The statistical analysis of the result did not show significant difference in ejaculate volume and sperm concentration in cattle and buffalo bull semen. The mean±SE concentrations of seminal plasma protein were observed 7.86±0.34 mg/dl in cattle and 4.63±0.16 mg/dl in buffalo bull semen. The seminal plasma protein concentration showed a significant difference ($p<0.01$) in cattle and buffalo bull semen. The mean±SE concentrations of sperm membrane extract protein were observed 2.81±0.25 mg/10^9 sperms in cattle and 4.42±0.63 mg/10^9 sperms in buffalo bull semen. The concentration of sperm membrane extract protein showed significant difference ($p<0.05$) in cattle and buffalo bull spermatozoa. Electrophoretograms obtained by polyacrylamide gel electrophoresis of seminal plasma proteins revealed 13 protein bands ranging from 6.5 kDa to 204 kDa while sperm membrane proteins revealed 17 protein bands ranging between 6.5 to 174 kDa in Haryana cattle semen. Seven protein bands of molecular weight 6.5, 8.5, 26.5, 43, 66, 70, and 84 kDa were observed common in seminal plasma and sperm membrane of cattle. The SDS-PAGE of buffalo sperm membrane proteins revealed 14 protein bands ranging from 16.0 kDa to 205 kDa and seminal plasma showed 24 protein bands ranging between molecular weight 6.0 to 200 kDa. Nine protein bands of molecular weight 20, 26.5, 36.5, 38, 44, 66, 70, 72 and 84 kDa were observed common in seminal plasma and sperm membrane of buffalo. In present study, the mean±SE apoptotic index of Haryana cattle and Bhadawri buffalo was observed 5.27±0.15 and 17.33±0.33 in neat semen and 10.83±0.22 and 25.00±0.21in post-thaw semen, respectively. Though no significant effect was observed in apoptotic index in neat semen of both the species however higher numbers of apoptotic sperms were observed in buffalo as compared to cattle bull semen. Significantly higher apoptotic index was observed in post thaw buffalo semen as compared to neat buffalo semen as well as cattle cryopreserved semen.

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5. Assessment of different activation protocols for production of parthenogenetic goat embryos

Juhi Pathak and S.D. Kharche

In the present study the effect of different chemical activation protocols on cleavage rate of *in vitro* matured goat oocytes and the development of parthenogenetic embryos produced from different chemical activation protocols were observed. 934 goat ovaries were collected in normal saline and were transported to laboratory within 4 hrs. 2094 good quality oocytes were collected by puncturing of follicles and were matured for 27hrs in maturation media (TCM-199). Matured oocytes were subjected to chemical activation treatment. Chemicals for oocytes activation comprised a) 7% ethanol for 5min+2.0mM 6-dimethyl amino purine (6-DMAP) for 4 hr, b) 7% ethanol for 5min+10µg/ml cycloheximide (CHX) for 4 hr, c) 7% ethanol for 5min+2.0mM DMAP+10µg/ml cycloheximide (CHX) for 4 hr in two different madia (KSOM and mCR2aa). To study embryo development, chemically activated oocytes were randomly divided and cultured in two different media viz. KSOM and mCR2aa media for upto 12 days. In this study maturation rate of 95.03% was observed. In KSOM, the cleavage rate of chemically activated *in vitro* matured goat oocytes in Gr.1, Gr. 2, Gr. 3 and Gr. 4 were 0.00%, 42.83%, 58.62% and 74%, respectively. Whereas, in mCR2aa media, the cleavage rate of chemically activated *in vitro* matured goat oocytes in Gr.1, Gr. 2, Gr. 3 and Gr. 4 were 0.00%, 54.42%, 44.55% and 51.69% respectively. Furthermore, when we observed embryo development following different activation treatments, the blastocyst production was only observed in mCR2aa medium

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6. Differential expression of TLR3 and TLR4 in caprine Brucellosis

Pallavi Chaturvedi and V.K. Gupta

Brucella melitensis causes chronic infections in goats that can result in abortion, still-births which leads to infertility. Initial host defense to bacterial infection is executed by innate immunity, and therefore the main goal of this study was to examine the contribution of Toll-like receptors (TLRs) during *Brucella melitensis* infection. Research into intracellular sensing of microbial products is an up and coming field in innate immunity. Toll-like receptors (TLRs) recognize *Brucella* spp. and bacterial components and initiate mononuclear phagocyte responses that influence both innate and adaptive immunity. Recent studies have revealed the intracellular signalling cascades involved in the TLR-initiated immune response to *Brucella* infection. TLR2, TLR4 and TLR9 have been implicated in host interactions with *Brucella*; however, TLR9 has the most prominent role. Further, the relationship between specific *Brucella* molecules and various signal transduction pathways needs to be better understood. In present study, the role of TLR3 and TLR4 was determined in *B.melitensis* infection in goats. The expression of TLR3 and TLR4 was analyzed on different tissues of goats viz; brain, spleen, liver and peripheral blood mononuclear cell. It was found that the different tissues expressed a high level of TLR4 while the expression of TLR3 was low on all the tissues used in the present study. It was concluded that the TLR4 expression is critical in *B.melitensis* infection in goats.

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7. Characterization of MC1R gene of goats by PCR-RFLP and HRM analysis

Swati Dubey and P.K. Rout

MC1R (Melanocortin 1 receptor) gene is a highly polymorphic and responsible for coat color, fibre quality and wool quality. MC1R also regulates melanogenesis (skin pigmentation), which protect the body from harmful effect of UV radiation and thermal stress. Melanocortin also regulates several physiological functions such as inflammation, adrenal steroidogenesis, energy homeostasis, feeding behavior and exocrine function and red hair color. As there is no information available on the polymorphic pattern of MC1R gene in Indian goats, therefore the present study was undertaken to characterize MC1R gene in Barbari and Jamunapari goat breeds. The polymorphic pattern of the MC1R gene was analyzed by PCR-RFLP and HRM analysis. The genomic DNA was extracted from 30 blood samples of each Barbari and Jamunapari goats. The quality of DNA was checked biophotometrically and by agarose gel electrophoresis respectively. The amplified PCR product was 592bp (E6-E7) and the restriction analysis was carried out by the *MspI*, at 37 ℃. PCR-RFLP pattern showed same genotype (345+247 bp) in all the analyzed samples indicating the presence of only homozygous dominant allele E^D in both the breed. The HRM analysis was carried out in the E5-E6 region and showed two different genotype in all the analyzed samples indicating the presence of heterozygous SNP. HRM analysis showed two different genotypes in both breeds in the analyzed samples.

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8. Comparative evaluation of different serological tests for diagnosis of caprine brucellosis

Ajay Singh and V.K. Gupta

In the present study *Brucella melitensis* was isolated from the (6) aborted goats. The isolates of *Brucella species* were maintained in serum dextrose agar and Brucella selective medium. Initially, *B. melitensis* were identified and confirmed on the basis cultural, morphological, biochemical characterization and dye tests. Dye test revealed isolates as *Brucella melitensis* biovar 3. For the confirmation Brucella genus was identified on the basis of 1412 bp 16SrRNA PCR product which were found in all the isolates. Brucella species was confirmed on the basis of 723bp Omp31PCR product and that was present in all the Brucella isolates. Different serological examination were performed for circulating antibodies in sera samples and also assessed by the serum PCR. In total 300 sera samples of goats were used for serological examination. The overall seroprevalence was found 11.33% (34) positive, 17.66% (53) doubtful in Serum Tube Agglutination Test (SAT), 42.66% (128) in Rose Bangal Plate Agglutination Test (RBPT), 24.33% (73) in rOmp31 based Enzyme Linked Immunosorbent Assay (rOmp31 ELISA) and 17.33% (52) in dot-Enzyme Linked Immunosorbent Assay (dot-ELISA). rOmp31 ELISA positive 73 sera samples were used in serum PCR and 40 samples were found positive with the positivity of 54.80%. The serological tests used were studied in different combinations to evaluate their diagnostic potential. Results showed that out of 300 samples tested 180 samples were found negative in all the tests used in the study. Only 40 samples were tested positive in all the tests. Forty two samples were showed positive reaction in rOmp31 based ELISA and SAT both. Combination of rOmp31ELISA and RBPT showed 6 samples positive together. No sample was found positive in the combination of rOmp31 ELISA, SAT and RBPT. Combination of rOmp31 ELISA, SAT and d-ELISA showed 20 samples positive. In another combination of rOmp31 ELISA, RBPT and d-ELISA, 38 samples were positive. Only 50 samples were positive in combination of rOmp31 and d-ELISA. These serological tests were further studied for comparative sensitivity and specificity. Results showed that the sensitivity percentage of different tests varied in between 56.25-100%. Whereas, specificity ranged in between 75-100%. Omp31 PCR produced 100% specificity and sensitivity.

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9. Detection and prevalence of rotavirus infection in diarrhoeic bovine calves

Tripti Singh and Rashmi Singh

The rotaviruses, member of genus Rotavirus within the family Reoviridae, are the leading cause of diarrhoea in cow and buffalo calves worldwide. To understand the epidemiology and types of BRVs circulating in this region present study was carried for the detection of rotavirus infection from faeces of diarrhoeic calves by RNA-PAGE and ELISA. One hundred faecal samples were collected from diarrhoeic cattle and buffalo calves from organized dairy farms in Mathura region. Ten percent faecal suspension was made in PBS, pH 7.2. After this, the extraction of viral RNA was done by phenol: chloroform or TRI reagent. The extracted RNA was subjected to RNA-PAGE and silver staining of the gel was carried out for visualization of the bands. Detection of rotavirus by antigenic ELISA Kit was also performed for bovine group A rotavirus antigen. RT-PCR and multiplex nested PCR was done for G and P genotyping. Twelve samples were positive for the Group A rotavirus in PAGE. These samples showed the 11 segments of the RNA genome of the virus in the pattern of 4:2:3:2 characteristic for the Group A rotavirus. Based on the migration of segment 10 and 11, all the positive samples were characterized as long electropherotypes. Differences in the migration pattern of class I segments was observed. In type 1 pattern all the four segments (1, 2, 3 and 4) migrated separately. In type 2 pattern segment 2 and 3 co-migrated. In all the samples class III segments (7, 8 and 9) moved as a single segment. One sample showed the presence of an additional band between gene segment 5 and 6. Multiple bands were present in two samples. All RNA-PAGE samples were found to be positive for Group A rotavirus antigen. Twelve percent prevalence of Group A bovine rotavirus was found in the present study in Mathura region. The maximum prevalence 14.08% was observed in ILFC, DUVASU, Mathura followed by 12.5% in Hasanand Gaushala, Vrindavan, Mathura and lowest of 4.7% in Mahavan Gaushala, Mathura. On detailed analysis of samples it was found that only samples were positive from cow calves and no buffalo calf sample was positive for rotavirus infection. Out of 12 positive samples nine were from female calves and three were from male calves. The samples were positive in the months of August- November and below 1 month of age. Eleven out of twelve PAGE/ELISA positive samples produced the expected amplicon for Group A specific VP6 gene. One sample could not be amplified for Group A specific VP6 gene. On genotyping with G3, G6, G8 and G10 specific primers for VP7 gene and P[1] and P[11] specific primers for VP4 gene, G6 genotype was found in 3 samples followed by G10 in one sample. Mixed genotype of G6G10 was observed in three samples. Four samples remained untypeable for G-genotyptes. In P typing only one isolate was found to be positive for P[1] and rest all of the samples remained untypeable. Overall, the results of present study indicate the G6 as a major G-genotype in this region and confirm bovine RVA circulating here.

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