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University of Peradeniya

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2023 Undergraduate Research Symposium Volume III

# PROCEEDINGS

## Undergraduate Research Symposium

2023

Volume III

Faculty of Veterinary Medicine and Animal Science
University of Peradeniya

# Proceedings

## Undergraduate Research Symposium
**(Batch 2016/17)**

## Volume III

**Faculty of Veterinary Medicine and Animal Science**
**University of Peradeniya**
**Sri Lanka**

25th September and 5th October 2023

# Proceedings of the Undergraduate Research Symposium of the Faculty of Veterinary Medicine and Animal Science–Volume III



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Sri Lanka

## Message from the Vice Chancellor, University of Peradeniya

I am delighted to address you during the third undergraduate research symposium organized by our esteemed faculty here at the University of Peradeniya. The Faculty of Veterinary Medicine and Animal Science holds a special place within the academic landscape of Sri Lanka as the sole institution offering the Bachelor of Veterinary Science (BVSc) degree. Your journey began in 1948 in Colombo, and in 1952, you shifted the operations to Peradeniya. Over the years, we have proudly graduated 2103 veterinarians. These individuals have significantly contributed to our country's livestock and animal health sectors, spanning companion animals, livestock, poultry, aquatic life, zoo inhabitants, and wild animals. These veterinarians shoulder the crucial responsibilities of animal healthcare, welfare, and ensuring economic viability, all while actively safeguarding public health through their roles in controlling zoonotic diseases, ensuring food safety, and addressing the challenge of antimicrobial resistance.

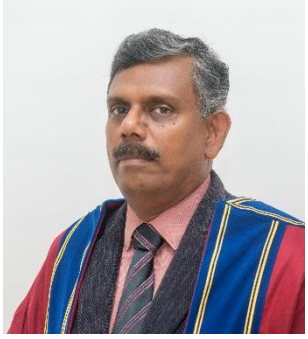
The scope of a veterinarian's work is multifaceted and demanding, requiring active engagement in various research activities. Therefore, our budding veterinarians must be introduced to the research world early in their careers. This symposium is the perfect platform for our students to present their findings before a distinguished scientific community. This experience will lay a strong foundation for them to embark on productive research and academic endeavors throughout their careers. Moreover, this event offers a valuable opportunity for our students to interact with esteemed personalities from the veterinary profession and allied sectors, fostering connections and learning experiences that will shape their future.

I congratulate the Dean, the Faculty of Veterinary Medicine and Animal Science staff, and the organizing committee for their hard work and dedication in bringing this symposium to life. Your efforts are instrumental in nurturing the next generation of veterinary professionals and researchers.

As we commence this third undergraduate research symposium, I wish you all a fruitful conference filled with insightful discussions, innovative ideas, and valuable connections. May this event contribute significantly to the growth and advancement of veterinary medicine and animal science in our country and beyond.

Prof. M.D. Lamawansa
Vice-Chancellor
University of Peradeniya

## Message from the Dean, Faculty of Veterinary Medicine and Animal Science



I am pleased to extend my greetings to the Faculty Undergraduate Research Symposium (UGRS) of 2023. The inclusion of a research project as a mandatory component of the BVSc degree program, introduced during the curriculum revision of 2000, represents a significant milestone. In the past, student research projects and their associated presentations did not confer the recognition they deserve within our faculty. It is heartening to acknowledge that the inaugural Undergraduate Research Symposium in 2020 marked the beginning of a well-deserved appreciation for the hard work of our students. As the sole higher education institution in the country offering a professional veterinary degree, our faculty firmly believes that research is indispensable for the advancement of animal health, as well as for the growth of the livestock and poultry sectors. Moreover, this knowledge plays a vital role in safeguarding the welfare of wildlife and companion animals, while fostering new insights in the diverse fields linked to these sectors. Situated within a Sri Lankan premier university, the Faculty of Veterinary Medicine and Animal Science (FVMAS) is deeply committed to instilling a culture of research among its undergraduate students. Consequently, the Undergraduate Research Symposium has provided an exceptional platform for students to showcase their scholarly work and presentation skills to the broader academic and veterinary communities. In the current year, student research projects were completed under tight budget constraints, and I extend my gratitude to the dedicated academic staff members who provided guidance and supervision to the students despite the prevailing financial constraints. This emphasises the faculty's commitment to fostering a research culture among its students. In conclusion, I would like to extend my best wishes to all the final-year students for their upcoming presentations. I sincerely hope that the invaluable experience gained during your project endeavours will serve as a crucial moment in your lives, potentially altering the course of your academic and professional journeys.

Prof. Anil Pushpakumara
Dean
Faculty of Veterinary Medicine and Animal Science

## Message from the Keynote Speaker

It is my great pleasure to be present as the keynote speaker of the undergraduate research symposium, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya on the 5th of October 2023. It is indeed an honour and a privilege to stand in front of emerging veterinarians and eminent professionals in the University. There is no doubt that we need more and more veterinarians to uplift our country, especially with sound research literacy and high-grade presentation skills.

Antimicrobial resistance is a burning issue in humans and veterinary medicine. Both emerging and remerging diseases pose a great challenge to scientists, which have generated a pressing need for new antimicrobials, new strategies, or alternative methods to overcome the overwhelming issue in the field of medicine. According to FAO, antimicrobial resistance (AMR) is the ability of microorganisms to persist or grow in the presence of drugs designed to inhibit or kill them. Antimicrobial resistance threatens human health, animal health, animal welfare, the environment, food safety, nutrition economic development, and equity within societies. Therefore, antimicrobial resistance is a complex issue contributing to multiple sectors such as human health, animal production, aquaculture, the environment, agriculture, and pharmaceutical industries. Furthermore, one health approach to the mitigation of AMR has been identified as the only route with a successful end.

As veterinarians, we have a significant role in the mitigation of antimicrobial resistance in livestock, companion animals, and aquaculture. Food safety is an important responsibility of veterinarians; we are determined and hold huge accountability to make healthy food for the country and for the export market. In addition, the health of companion animals with the minimum risk of zoonotic infection in small animal practices has been identified as a priority in current veterinary practices. Therefore, combating against antimicrobial resistance is a part of the professional responsibility of veterinarians. As one health partners, our veterinarians equally contributed to the last AMR strategic action plan for 2017-22, A new AMR strategic action plan has been proposed for 2023-28 by the Ministry of Health with the collaboration of the Department of Animal Production and Health, the Ministry of Fisheries, the Department of Agriculture, and the Ministry of Environment under seven identified priorities. As the veterinary community in the country, we have an immense responsibility to support and carry out activities to minimize the risk of AMR in animals and aquaculture.

I would also like to take this opportunity to offer my best wishes to the organising committee of the undergraduate research symposium. I hope it will be an unforgettable day for budding veterinarians.

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# Farm Animal Production and Health

## Session-I

# A Preliminary Study on the Prevalence of *Theileria orientalis* in Three Veterinary Ranges in Kandy District

**Wesley J.J.[1], Jayasekera N.K.[2*], Iddamaldeniya S.S.[3]**

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*Theileria orientalis* is a tick-borne protozoan parasite that affects cattle all over the world. The goal of this research was to investigate the prevalence of *T. orientalis* in cattle populations in the Gangawatakorale, Galaha, and Harispattuwa veterinary ranges of the Kandy district. Over the course of two weeks, sixty blood samples were collected from cattle. The study employed both blood smear preparation and PCR assay for identification of *Theileria* species. Blood smears were made using blood collected from the tail vein and stained with Leishman stain for microscopic examination. *Theileria* piroplasms were identified based on their pleomorphic shapes, including rod, comma, and signet ring shapes. DNA extraction was performed using the QIAGEN DNeasy Blood and Tissue kit, and PCR analysis was conducted to identify *Theileria orientalis* using species-specific primers targeting the MPSP gene. The PCR reactions were run on a thermal cycler and the amplified DNA fragments were analysed using agarose gel electrophoresis. The microscopic examination of blood smears revealed that 3.33% (2/60) of the samples tested positive for *Theileria* piroplasms, with the rod shape being the most prevalent. The PCR analysis showed that 6.66% (4/60) of the samples were positive for *Theileria orientalis* infection, as indicated by the presence of a 776 bp band similar to the positive control. Two samples that were positive in PCR testing were negative in the blood smear identification. This work sheds light on the prevalence of *T. orientalis* in the Gangawatakorale (one smear positive and two PCR positives), Galaha (one smear positive and two PCR positives) and Harispattuwa (both smear and PCR negative) veterinary ranges of the Kandy district. The findings contribute to our understanding of the parasite's epidemiology and molecular features, allowing for better disease management and control techniques. Further studies are warranted to explore the impact of genetic diversity on disease dynamics and to develop targeted interventions for the effective control of *T. orientalis* infections in cattle populations.

**Keywords:** Cattle, PCR Assay Prevalence, *Theileria orientalis*

# Evaluation of Adult Bovine Serum as a Locally Available, Less Expensive, Alternative for Fetal Bovine Serum in Cell Culture Studies

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Foetal Bovine Serum (FBS) is a widely used growth supplement in the *in-vitro* culturing of animal and human cells tissues, and organs. It is the very first growth supplement that has been used since the commencement of cell culture studies in the world due to the presence of micro and macronutrients, along with unknown growth factors. The development of science, animal welfare, and ethical concerns led to look for an alternative to FBS as it is a highly expensive substance in the international market. The objective of the study was to assess the effect of Adult Bovine Serum (ABS) as a locally available, less expensive, alternative for FBS in cell culture studies. Tissue samples were obtained from cattle and goats using a minimally invasive (ear notching) procedure. ABS was prepared using freshly collected blood from clinically healthy, adult, unvaccinated cattle. Serum was separated from blood, filter-sterilized, and stored at -20 $^0$C until media preparation. Two cell culture experiments were done to compare the effects of FBS and ABS on cell growth at the initial cell culture stage and subsequent passaging. All culture flasks were incubated at 37$^0$C in a 5% $CO_2$ incubator. In the first experiment, a tissue sample (bovine) was mechanically and chemically digested and cultured in commercially available FBS-containing DMEM2 medium and ABS-containing DMEM2 medium (n=3). An equal amount of digested tissue sample was used in each culture flask. In the second experiment, confluent stage goat cell culture was harvested by trypsinization and washed twice using DMEM1 to remove the remaining FBS. The prepared sample was divided into equal parts and subcultured in DMEM1 (which does not contain any serum), FBS containing DMEM2, and ABS containing DMEM2 (n=4). In the initial experiment, both FBS and ABS added culture flasks showed similar cell growth rates and came to the confluence within the same period. In the second experiment, the serum-free culture flask showed slower cell growth and a slower cell attachment to the bottom of the culture flask. FBS or ABS containing culture flasks showed comparatively higher rates of cell growth and came to the confluence at the same period. Therefore, this study has shown that ABS can be used as a functionally similar, less expensive alternative for FBS in cell culture studies.

**Keywords:** Adult Bovine Serum, DMEM1, DMEM2, Foetal Bovine Serum, In-vitro

*Acknowledgements: University Research Grant no; 390-2023*

# A Preliminary Study of Molecular Prevalence of *Theileria annulata* in Three Veterinary Ranges of Kandy District.

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*Theileria annulata* is a tick-borne protozoan parasite that causes severe tropical theileriosis in cattle, leading to significant economic losses in the livestock industry. As *Theilaria annulata* is highly pathogenic in cattle, and the molecular prevalence has not been studied in the Kandy district of Sri Lanka, this study aimed to investigate the molecular prevalence of *Theileria annulata* in healthy cattle in the Kandy District, Sri Lanka. Three veterinary ranges; Gangawatakorale, Harispathuwa, and Galaha were chosen. Convenient sampling was performed on twenty healthy cattle from each veterinary range, representing intensive, semi-intensive, and small-scale farms in the study area. Two blood samples were obtained from each selected cattle: a blood drop from the tip of the tail and three ml of blood from the jugular vein into EDTA tubes to make a blood smear and to undertake molecular analysis, respectively. Leishman stained blood smears were observed under a light microscope to identify *Theileria spp* morphologically. Intra-erythrocytic piroplasm phases of *Theilaria* were easily discernible in one of the sixty blood smears and the sample represented Gangawatakorale. All 60 blood samples were subjected to DNA extraction using DNeasy$^{R}$ Blood and Tissue Kit and PCR was carried out using *Theileria annulata* specific primers: Tams1 F 5'ATGCTGCAAATGAGGAT 3', Tspms 1 R-5' GGACTGATGAGAAGACGATGAG 3'. Then the agarose gel electrophoresis was performed to detect the presence of *Theileria annulata* specific 785 bp DNA fragment. The same sample that was positive for *Theileria* species in the blood smear yielded a *Theilaria annulata* specific DNA fragment. According to this study the molecular prevalence of *Theilaria annulata* in healthy cattle in the study area of the above mentioned three veterinary ranges is 1.67%. Findings of the current study are consistent with the previous literature in other areas of Sri Lanka. Sequencing and phylogenetic analysis of the detected *Theilaria annulata* amplicon will be conducted and the study will be continued with a larger sample size to reveal true molecular prevalence of *Theilaria annulata* in healthy cattle in the Kandy district of Sri Lanka.

**Keywords:** Cattle, Kandy PCR, Prevalence, *Theileria annulata*

# The Evaluation of Uterine Position of Non-Pregnant Cows with Their Parity Number in Two Large-Scale Dairy Farms

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The reproductive tract position (RTP) in dairy cows and heifers can have significant implications on their fertility. The position and alignment of reproductive organs play a crucial role in successful conception, embryo development, and pregnancy maintenance. The purpose of this project was twofold; first, to determine the prevalence of reproductive tract positions, and second, to conduct a retrospective investigation of the number of pregnancies with respect to RTP. Rectal palpation and ultrasound examinations were used to determine the uterine position of 123 non-pregnant cows (53 heifers and 70 lactating cows) from two commercial up-country dairy farms. The study was conducted during a predetermined time duration. Each cow's parity which might be either primiparous (first calving, n = 25) or multiparous (more than one calving, n = 45) was noted. During the evaluation, the uterus's location was allocated to RPT 1, 2, or 3. Pearson Chi-Square Test was used to evaluate the relationship between uterine position and parity number. The results of the study did not reveal a significant relationship between the parity number and RTP of non-pregnant cows (P=0.127) in dairy farms. Even though there was a lot of heterogeneity in uterine position, our small sample size prevented us from drawing any firm conclusions. Future research that includes a larger sample size may yield more solid and confirmatory findings. However, this study didn't clearly demonstrate an association between the reproductive tract position in the pelvic cavity and the parity number of heifers and lactating cows. This study emphasizes the significance of using a sufficient sample size in scientific studies, especially when examining intricate biological interactions. Although our results did not provide the anticipated insights into uterine position and its association with parity, they emphasized the need for a future study utilizing a large and more respective sample.

**Keywords:** Dairy Cow, Fertility, Parity, Reproductive Tract Position, Non-Pregnant

# Effect of Sugarcane-Derived Polyphenols on *In-vitro* Ruminal Digestion of Guinea Grass (*Megathyrsus maximus*)

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Plant-derived natural polyphenols have a great potential to enhance forage digestibility and improve dairy cattle productivity. This study aimed to evaluate the effects of sugarcane-derived polyphenols (Polygain™) on the in-vitro ruminal digestibility of Guinea grass, a commonly used forage in Sri Lanka. The study employed an *in-vitro* gas production technique which involved processing 40 mL of ruminal fluid from sheep on a Guinea grass-based diet with a fermentation buffer, $CO_2$ bubbling, maintaining a constant temperature of 39°C, and measuring gas volumes at specific time intervals (2, 4, 6, 8, 10, 12, and 24 hours) over a 24-hour incubation period. Four different inclusion levels of Polygain™ (0%, 0.1%, 0.2%, and 0.3% of feed dry matter) were tested in triplicate for Guinea grass samples. Organic matter digestibility (OMD) and Metabolizable energy (ME) content were estimated from the net gas production (GP). The effects of the different Polygain™ levels on in-vitro feed digestibility were compared using one-way ANOVA. The results demonstrated a significant increase in *in-vitro* GP, OMD, and ME with increasing concentrations of Polygain™ ($P < 0.001$). The mean gas production values for 0%, 0.1%, 0.2%, and 0.3% Polygain™ were 25.7 mL, 46.7 mL, 75.2 mL, and 104.9 mL, respectively. Similarly, OMD values increased from 43.5 g/100g dry matter to 113.9 g/100g dry matter, and ME values increased from 6.3 MJ/kg dry matter to 17.1 MJ/kg dry matter as Polygain™ levels increased. In conclusion, the incorporation of sugarcane-derived polyphenols in the diet of Guinea grass significantly improved its in-vitro ruminal digestibility. The observed increase in GP, OMD, and ME highlights the potential of sugarcane-derived polyphenols as a natural and effective feed supplement for ruminants, offering a sustainable alternative to labour intensive and time-consuming mechanical treatments for enhancing forage digestibility.

**Keywords:** Dairy cattle, Digestibility, Guinea Grass, Polyphenol, Sugar Cane

# Observations on Management Practices of Jaffna Local Sheep

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Sheep farming plays a pivotal role in the rural livelihoods and food supply chain of Jaffna, Sri Lanka. However, the management practices pertaining to Jaffna Local Sheep (JLS) remain relatively underexplored, thereby constraining the full optimization of sheep farming in the region. This study sought to investigate the prevailing JLS management practices within the Jaffna peninsula. Face-to-face interviews were conducted with 10 farmers, addressing various aspects of JLS management, including herd profile, feeding strategies, housing conditions, farming objectives, cost considerations, breeding methodologies, lambing procedures, disease management protocols, and sheep identification practices. Sheep populations across the surveyed farms exhibited considerable variability, ranging from modest herds of 50 to more substantial herds of 1500 sheep. Notably, the predominant feeding strategy involved free grazing without supplementary nutrition, with limited water availability during the night. A significant shift was observed in the primary purpose of JLS farming when compared to past available information, transitioning from manure production to a greater emphasis on meat production. This shift underscored the pressing need for enhanced production performance to meet evolving agricultural demands. However, challenges persisted, with lower production efficiency and reduced lamb survival rates, possibly as a result of traditional breeding and lambing practices. Health issues, particularly pneumonia outbreaks and inadequate tick control, presented additional hurdles to successful JLS management. Furthermore, farmers displayed a notable reluctance to adopt appropriate sheep identification methods. Although overall management costs remained relatively low, the study conclusively emphasizes the necessity for improvements in feeding practices, breeding methodologies, disease management, and sheep identification techniques to optimize production, ensure sheep health, and bolster profitability in JLS management. In conclusion, this study emphasizes the critical importance of educating farmers about best practices, highlighting the value of veterinary care, and promoting improved housing and nutritional strategies to enhance the overall productivity of JLS. This, in turn, will contribute to the long-term success and sustainability of the industry.

**Keywords**: Disease, Feeding, Jaffna Local Sheep, Management, Production

# A Preliminary Study on the Prevalence of *Babesia bigemina* in Three Veterinary Ranges in Kandy District

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Tick-borne protozoan parasite *Babesia bigemina*, which infects cattle globally, is economically significant. Babesiosis is detectable in cattle even in the absence of symptoms of infection, but it persists in a subclinical state in carrier cattle who can act as tick reservoirs and facilitate the natural transmission of the disease. This is a major problem causing constant disease transmission and implications in disease control. The aim of this study was to determine the subclinical molecular prevalence of *B. bigemina* in the cattle population in three selected veterinary ranges. Over a duration of two weeks, 60 blood samples were collected and 60 blood smears were prepared from cattle in Gangawatakorale, Galaha, and Harispaththuwa veterinary ranges. The microscopic examination of blood smears can confirm the diagnosis in clinically ill cattle. Blood smears of the investigated cattle population revealed a prevalence of 0% for *B. bigemina*. The best test for diagnosing acute babesiosis is a Giemsa stained blood smear, but due to their low sensitivity, they cannot be used to determine which animals are the carriers. Over the current diagnostic procedures, molecular testing, including PCR assays, offer a very sensitive and specific way for the diagnosis of subclinical Babesiosis. Polymerase chain reaction (PCR), rapidly amplifies a specific segment of DNA and identifies gene sequences. Apical membrane antigen (ama-1) gene based PCR assay was used to measure the molecular prevalence of *B. bigemina* infection. The PCR results showed that 1.66% of the cattle in the investigated population tested positive for *B. bigemina*, indicating a molecular prevalence of the *B.bigemina* infection. The Subclinical disease prevalence and of *B. bigemina* in three veterinary ranges are significant findings of this study.The research expands our knowledge of the epidemiology of *B. bigemina*, allowing for more effective disease management and control strategies.

**Keywords**: *B. bigemina*, Molecular Testing, Prevalence

# Utilization of Defatted Cocoa Mass as a Nutrient Rich Ingredient to be Used in Feed Formulation for Cattle

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The utilization of defatted cocoa mass as a low-cost protein and fat source in livestock feed has gained significant attention. This research aimed to evaluate the nutritional properties of defatted cocoa mass and assess its suitability as a raw material in feed formulation for livestock. The defatted cocoa mass was obtained from a local cocoa industry and was used in formulating cattle feed, partially replacing soya bean meal, coconut poonac, and rice polish. Nutrient compositions of both cocoa and the resulting cattle feed were evaluated using established proximate analysis protocols. Then, the predicted values and values obtained from laboratory analysis were compared to verify the actual nutrient composition. The energy contained in the final mixture would be enough for the energy requirement in any stage of cattle. The proximate analysis revealed that defatted cocoa mass contained a substantial amount of fat (~20%). The proximate analysis of feed revealed the following results, moisture:1.66%, protein:18.06%, fat:21.19%, fibre:11.20%, ash 2.6%, and nitrogen-free extractives 45.29%. The cattle feed was formulated using a trial error method and the composition of the feed is: Soya bean meal 10% (w/w), Coconut meal 20% (w/w), Cocoa powder 25% (w/w), Rice polish 45% (w/w). After the formulation of the novel feed, the feed was mixed and the subsequent proximate analysis of mixed feed revealed that predicted values (Calculations) of the formulated feed were well correlated with a slight deviation from the values obtained by proximate analysis. The predicted values of formulated cattle feed are as follows: Dry matter 90.076%, Protein 19.346%, Fat 12.566%, and Fibre 11.675%. The values obtained from proximate analysis were: Dry matter 90.47%, Protein 18.97%, Fat 16.79%, and Fibre 14.21%. Defatted cocoa mass can be used as a low-cost protein and fat source. Therefore, it can be beneficial for farmers economically and reduce dependence on expensive conventional protein, and fat ingredients. However, further research is necessary to evaluate the palatability, digestibility, and long-term effects of feed in different livestock species.

**Keywords:** Cattle Feed, Defatted Cocoa Mass, Feed Formulation, Low Cost, Proximate Analysis

# Farm Animal Production and Health

# Session-II

# Investigation of an Outbreak of Recurrent Subclinical Mastitis in a Goat Farm in the Central Province, Sri Lanka

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Mastitis is a costly disease in dairy goat herds worldwide. An outbreak of recurrent subclinical mastitis in a large-scale goat farm in the Central Province was investigated in this study. The milking animals in the farm were affected with subclinical mastitis for several months and were refractory to antibiotic treatment. However, the antibiotic treatment was not based on culture results or antibiotic sensitivity. *Mycoplasma* species are recognized as significant pathogens responsible for mastitis in goats. Several studies have been conducted to evaluate the causes of ruminant mastitis in Sri Lanka. However, mycoplasma as the causative agent for subclinical mastitis in goats has not been reported before. Therefore, the objectives of the current study were to demonstrate that *Mycoplasma* species can also act as causative agent for subclinical mastitis in dairy goats. A total of 26 milk samples were collected from a dairy goat farm in Gampola veterinary range, Central Province, Sri Lanka during two visits (10 samples from first visit and 16 samples from second visit respectively). The samples were examined using microbiological culture, biochemical tests and PCR to investigate *Mycoplasma* species. The first set of samples (10 samples in total) were negative for bacterial and fungal growth. Out of the 16 samples from the second visit, nine yielded bacterial growths, dominated by *Klebsiella* spp, followed by *Staphylococcus aureus* and *Bacillus* species. All the samples were negative for fungal growth. Out of the nine samples that were positive for bacterial growth, two were positive for *Mycoplasma* genus by PCR specific for 16SrRNA gene. This is the first study to confirm the presence of *Mycoplasma* genus in the milk from Sri Lankan dairy goats, however, due to budgetary limitations, it was not confirmed whether it's a pathogenic species. Further studies on the phylogenetic analysis of *Mycoplasma* species in Sri Lankan goat farms are required to confirm the occurrence, epidemiology, and specific species of *Mycoplasma* involved in Sri Lanka.

**Keywords:** Caprine Mastitis, Class Mollicutes, Epidemiology of Caprine Mastitis, Molecular technique, Subclinical Mastitis

# Evaluating the Efficacy of Single Dose eCG for Inducing Superovulation in a Buffalo at the Veterinary Teaching Farm, Udaperadeniya

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Limited research on superovulation in crossbred buffaloes in Sri Lanka has shown varying degrees of success. This study aimed to evaluate the efficacy of a single-dose equine Chorionic Gonadotropin (eCG) protocol for inducing superovulation in buffaloes, a process known to be more complex in buffaloes compared to dairy cattle. Due to the lack of the cyclic buffaloes in the Udaperadeniya Veterinary Teaching Farm, the study utilized only two female buffaloes, with one as the control. The primary objective was to assess the success of the protocol by monitoring ovarian structures by scanning, including follicles and corpus lutea. The protocol commenced with an initial scan on day 0, followed by the administration of intra-vaginal progesterone devices (CIDR) and oestradiol benzoate. On day 4, one buffalo received 1000 IU of eCG while the control received none. On day 8, the progesterone device (CIDR) was removed, and both buffaloes were given PGF2α (0.5mg). Further scanning was performed on days 6, 8, 18 (according to the protocol) and 27 (extra day due to no corpus luteums in ovaries in previous scanning days). Scanning results revealed that the single dose of eCG failed to induce multiple ovulations in the treated animal, possibly due to the suboptimal dosage of the equine Chorionic Gonadotropin hormone. The recommended 2000 IU of eCG could not be administered due to the unavailability of the hormone in the market. Consequently, it is concluded that better results may have been observed if the full dosage was used, and further investigations into the response of buffaloes to multiple injections of low-dose eCG are warranted.

**Keywords:** Buffaloes, CIDR, eCG, Estradiol Benzoate, PGF2α, Superovulation

# Reproductive Performance of Jaffna Local Sheep in Jaffna Peninsula

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Jaffna Local is an indigenous sheep breed that possesses unique genetic traits adapted to the local environment. Ensuring their optimum reproductive performance is vital for preserving genetic diversity and improving the performance of this breed. Sequence mismatch analysis of a previous study revealed that the uni-model distribution in Jaffna Local sheep indicates haplotype expansion. But there is little or no research on the reproductive performance of Jaffna Local sheep. Therefore, the current study aimed to evaluate the reproductive parameters such as conception rate, lambing rate, litter size, and inter-lambing interval in Jaffna Local sheep raised in the Jaffna district of Sri Lanka and to find out the relationship between the reproductive parameters and factors affecting reproductive performance. The data on the reproductive parameters were collected from the veterinary offices and direct interviews with farmers in the Jaffna district. 50 farmers were randomly selected to do a questionnaire survey. The results of the present study revealed that the mean age of puberty of ewes was 8.8 (± 0.1) months with a range between 7-12 months. The mean of first mating age was 8.5 (± 1.2) months while the mean first lambing age was 14.4 (±1.1) months with a range of 12-16 months. The mean gestation length was 151.9 (± 5.5) days with a range of 140-162 days, the mean inter-lambing interval was 6.5 (± 0.5) months with a range of 6-7 months, and the mean litter size was 1.0 with a range between 1-2. The mean weight at puberty of an ewe was 15.8 (± 4.9) Kg with a range between 10-25 kg. The annual conception rate was 90% and the lambing rate was one lamb/ year. The mean age at puberty of a ram was 5.9 (±0.6) months with a range of 5-7 months, the mean weight at puberty of ram was 17.4 (±3.2) Kg with a range between 12- 22 Kg, and the mean market weight was 24.0 (±3.4) Kg with a range between 20-30 Kg. The study revealed that there are relationships among these reproductive parameters and other factors such as management practices, type of nutrition and body condition score ($p < 0.05$). Overall reproductive performance of Jaffna Local sheep was lower compared to other exotic sheep breeds. The reproductive performance of the local sheep breed can be improved by proper management practices, good nutrition and improving the body condition score.

**Keywords:** Jaffna Local, Management, Reproductive Performance, Sheep

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# Evaluation of Non-Genetic Factors Affecting the Expression of Oestrus and Rate of Pregnancy in Cross-Bread Jersey Cows in Point Pedro Veterinary Range in Jaffna District

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Reproductive traits are critical for production, herd replacement, and the overall profitability of dairy farming. The present study determined how age, parity, milk yield, dry period and nutritional status (BCS) of cows, and ambient temperature influence the oestrus and pregnancy rate of cross-bread Jersey cows in Point Pedro veterinary range in Jaffna district. The association between these parameters and the expression of oestrus and rate of pregnancy in these animals were examined on 15 dairy farms using 194 Jersey crossbred dairy cattle over an eight-month period. Data were collected on three continuous variables (dry period of a cow, age of a cow, and mean daily milk yield in the early lactation period of a cow) and three-factor variables (parity, body condition score (BCS), and environmental temperature on artificial insemination (AI) performing date). Furthermore, data regarding animals showing oestrus, date of AI (without repeat AI), date of 1st pregnancy diagnosis (PD) for those inseminated cattle, and results of PD examination were also collected. All the data were analysed by binary logistic regression models using the StataSe version 14 in two analyses. Analysis-1 revealed that the expression of the oestrus had a significant relationship ($P \leq 0.05$) with milk yield and BCS of the Jersey cross cattle ($R^2 = 0.92$). Cows with BCS levels above 3.25 were eight times more likely to enter oestrus than cows with BCS values between 2.75 and 3.25. The likelihood of cattle expressing oestrus is reduced by 0.32 times for every additional litre of milk produced. Age, parity, the length of the dry period, and the environmental temperature had no effect on the oestrus expression of cows. However, there was a tendency to increase oestrus with increasing parity ($0.05 \leq P \leq 0.10$). Analysis-2 revealed that the rate of conception had a significant relationship ($P \leq 0.05$) with parity, BCS, and milk yield. The probabilities of cattle being pregnant were 36 times higher in the second and third parties than in the first parity, and they were 90 times higher in the parities above the third parity. In addition, every additional litre of milk produced by the cow considerably lowered the likelihood of pregnancy in Jersey crossbred cattle by 0.36 times. Compared to cows with a BCS between 2.75 and 3.25, cows with a BCS above 3.25 had a 27-fold higher chance of becoming pregnant. The results of this study showed that, in the Jaffna region of Point Pedro Veterinary Surgeon, oestrus in cattle is influenced by BCS and milk production, while pregnancy is influenced by BCS, milk yield, and parity. Furthermore, high milk production has a negative impact on reproductive performance.

**Keywords:** Cross-bread Jersey, Jaffna, Reproductive performance

# Locally Made Double Opening Selfed Headlock System for Cattle Farms

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Systems for locking the head of cattle are essential instruments in livestock management that are used for a variety of tasks, including handling, feeding, and veterinary procedures. The design and function of a typical cow headlock system are briefly described in this abstract, with special emphasis placed on how important it is for improving farmer safety, animal welfare, and operational effectiveness. The cow headlock system is a mechanical device put in place within a feeding or livestock enclosure. The system's design incorporates robust materials and ergonomic elements, ensuring longevity and ease of use for livestock handlers. The self-locking feature automatically secures the animal's head upon entry, reducing the need for manual intervention and minimizing the risk of accidental release. It consists of separate headlocks that limit mobility while allowing cattle to insert their heads to access the feed. Sturdy metal frames with adjustable neck gaps are often part of the design, allowing the accommodation of cattle of various kinds and sizes. Furthermore, the headlock system enhances feeding management practices. It also allows farmers to control the feed intake of individual animals, preventing overeating or feed wastage. The adjustable neck spaces accommodate the animal's growth, ensuring a comfortable fit while avoiding injuries or discomfort caused by ill-fitting restraints.

**Keywords:** Cattle, Control Abscess, Feeding, Headlock, Locally

# A Cost-Effective Automated Manure Scraper Machine for the Cattle Shed at the Veterinary Teaching Farm

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Automated manure scrapers are widely used currently in large-scale dairy farms. Automated manure scraping mechanisms have many advantages over manual methods of manure scraping in dairy farms such as reducing odors, reducing ammonia emissions, reducing pathogenic concentration in the floor, increasing efficiency and reducing labor cost. In this study, a cost-effective and efficient automated manure scraper was designed for the veterinary teaching farm. A prototype of a more advanced manure scraper is also made with the limited resources and through the assessment of this automated manure scraper, a more effective and permanent automated manure scraper will be designed. In this project, parts of the permanent automated manure scraper machine were made. The motor and associated gear box setting, the cable system and power supply were made permanently. Only the scraper machine will be remodelled using more durable materials. To achieve higher efficiency on permanent manure scraper machines, data will be collected, and performance evaluation will be done. The new scraper machine will reduce the cost and labor intensity that is used for manure scraping in the veterinary teaching farm.

**Keywords:** Automated Scraper, Cattle Shed, Cost-effective, Cow Manure, Large Scale

# Effects of Probiotics and Prebiotics in Cow Milk Yield and Compositions

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Prebiotics and probiotics are extensively used in livestock production to improve overall animal performance by enhancing immunological function and gut health. Most dairy farmers in the nation, including those at the veterinary teaching farm, struggle with poor milk yield per animal. The new symbiotic product which contained the *Lactobacillus*, *Bacillus subtilis*, *Bifodobacterium* and *Enterococcus* as probiotics and fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) as prebiotics may be used to increase milk production. Therefore, the aim of this study was to determine the effects of the symbiotic mixture containing probiotics and prebiotics (at the ratio of 9:1) on the milk production and milk composition of cows reared in the veterinary teaching farm. The experiment employed 14 early to mid-lactating Jersey-Friesian crossbred cows at the age of 6 years at their third parity. Seven animals were chosen as the treatment group and seven animals as the control group. The current research on probiotics and prebiotics in dairy cows showed increased daily milk yield and significant changes in milk composition. The mean milk output did not change in the first week, but after nine days, the treated cows showed a significant increase. Treated cows produced an additional 0.5, 1.2, and 2 litres of milk on days 10, 13, and 22 respectively, because of the regular use of symbiosis. At the beginning of the experiment, the milk fat percentages of treated cows were noticeably low. In contrast, starting on day four after the symbiotic treatment, the fat levels in the milk collected from the treated cows gradually increased, whereas the overall fat content of the control group varied and noticeably decreased. On day 22, there was no discernible difference between the treated and untreated cows' milk fat percentages. Symbiotic-treated cows had similar milk composition to control groups, with no significant differences in solid non-fat, protein, lactose, salt, pH, conductivity, or density. High milk yields and good fat levels may be obtained if we consistently feed this symbiotic mixture to dairy cows. Further research on this symbiotic combination at different doses over an extended period could provide intriguing and productive results.

**Keywords**: Dairy Cows, Milk Composition, Milk Yield, Prebiotics, Probiotics

# A Preliminary Study on the Synchronization of Dairy Heifers in a Large-Scale Upcountry Dairy Farm

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Getting a heifer to calf at 27 months of age begins right from the day she is born. Every stage in the heifer-rearing process leading up to calving must be closely monitored to achieve the desired age at first calving. The farm aims to breed them between 15 and 18 months, making sure calving takes place between 25 and 27 months. The study was conducted on a farm in need of pregnant heifers to expand their milking herd for a newly built dairy project. There are many synchronization protocols available to synchronize heifers, each with varying success rates. Artificial insemination (AI) on synchronized heifers can be performed either based on observed estrous signs or at a fixed time. Timely ovulation in fixed-time AI (FTAI) can be achieved by using either Estradiol benzoate (OEB) or Gonadotropin-releasing hormone (GnRH). All heifers were rectally palpated and checked by ultrasound (US) scanning when necessary. Heifers that have attained an acceptable body weight and possess a reproductive tract of suitable size for their age are selected to be included in one of the synchronization protocols. The study was conducted in March 2023. Reproductive tract position (RTP) in the pelvic cavity was determined when heifers were rectally palpated. Body Condition Score (BCS) of all the treated heifers was also recorded. Heifer that had a Corpus Luteum (CL) was assigned to prostaglandin-based estrus synchronization and those having active ovaries were assigned to progesterone-based synchronization protocol (Progesterone + Estradiol benzoate). Animals injected with prostaglandin ($PGF_{2\alpha}$) were observed for heat signs and inseminated if they were in heat. Those who were not shown heat were injected with a second $PGF_{2\alpha}$ dose 14 days later and inseminated when they were in heat(n=47). Heifers that received intra-vaginal progesterone device (CuMate) D 0 were also injected with 2 mg estradiol benzoate (n=24). The device is removed on D8 and injected 500 mcg $PGF_{2\alpha}$. Two milligrams (2 mg) of estradiol benzoate were given on D9, and fixed-time artificial insemination was done on day 10. The pregnancy was checked at 35 days post-insemination using ultrasound scanning. The results revealed that pregnancy rates in Farm 1 and Farm 2 for $PGF_{2\alpha}$ and progesterone treatments were 38.7%, 28.6%, 21.1%, and 66.7%, respectively. Based on these findings, it is evident that heifers can be successfully synchronized, leading to an acceptable conception rate after the synchronization process. The progesterone-based treatment protocol in Farm 2 gave encouraging results and warranted further investigation. No significant associations were found between pregnancy status and synchronization protocols ($P = 0.100$) or between pregnancy status and RTP ($P = 0.171$).

**Keywords:** Artificial Insemination, Fixed-Time AI, Heifer, Synchronization

# Coprological Study on Gastrointestinal Parasites of Milking Cows in Selected Areas of Polonnaruwa District

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Gastrointestinal parasites cause severe production losses in dairy cattle worldwide. Similarly, in Sri Lanka, milk production losses were reported mainly due to gastrointestinal parasitism. The parasitic burden may vary depending on the deworming history, age, sex, breed and nutritional status of the milking cows. A study done in the Gampaha district revealed that, out of the 163 cattle and buffalo fecal samples examined, 13.39% of animals were positive for one or more types of parasitic eggs. Dry zone farmers in the country, despite the presence of low milk-producing cattle and multiple parasitic diseases, contribute immensely to the total milk production in the country. The Polonnaruwa District contains many dairy farming villages with great access to veterinary services, however, no studies were done in order to evaluate the gastrointestinal parasite burden of the dairy cows in the region. This study was designed to evaluate the gastrointestinal parasitism of dairy cows in selected dairy farms within the Polonnaruwa District. A total of 30 fecal samples were collected from dairy cattle from selected dairy farms in the Polonnaruwa District. The collected samples were examined using the salt floatation technique and McMaster counting chamber technique to identify the presence of parasitic eggs and protozoan oocysts. The results indicated the presence of ≥100 EPG only in 7 samples tested and all had strongyl-type eggs. The Pearson's correlation between the EPG and the milk yield indicated no significant relationship between the daily milk production and EPG levels ($p=0.2299$). The information gathered during sample collection indicated that the animals were regularly dewormed and received proper care from a range veterinarian. Based on the findings, this study concludes that there is minimal risk of gastrointestinal parasitism among the dairy cattle populations evaluated for EPG in the Polonnaruwa district.

**Keywords:** Coprological study, Gastrointestinal, Milking cows, Parasites

# Companion Animal Health

# Session-I

# Determination of Associations between the 5-Point Body Condition Score of Dogs and the Feet Lesions Suggestive of Pododermatitis and the Bacterial/Yeast Density of Interdigital Skin

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Pododermatitis is a common inflammatory condition affecting the interdigital skin, foot pads, nail beds, and nails of dogs. Pododermatitis can seriously affect the quality of life of dogs as it limits mobility. The gross lesions of canine pododermatitis include redness, swelling, and thickening of paw pads. Obese or overweight dogs carry a high risk of developing chronic diseases. Body condition score (BCS) is a simple and easy-to-perform method to determine the body fat and lean muscle mass in dogs. Excess body weight produces excessive pressure on the feet. Therefore, obese or overweight dogs are at a higher risk of developing pododermatitis than dogs with ideal BCS. Further, the excessive pressure on the feet of overweight or obese dogs may mechanically alter the interdigital microenvironment facilitating the overgrowth of yeast and bacteria leading to pododermatitis. Therefore, the current project was designed to determine whether there is an association between the BCS of dogs determined by the 5-point BCS system and the presence of pedal and interdigital gross lesions suggestive of pododermatitis. It was also assessed whether an association was present between BCS, and the density of bacteria/yeast compared to hind feet. For this purpose, BCS of 50 medium/large breed dogs were determined and the association between BCS and gross lesion on feet suggestive of pododermatitis was assessed using Sommer's d test. The same test was used to determine the association between BCS and yeast/bacterial density of interdigital skin. The findings of the present study revealed that there is a positive non-significant association between BCS of dogs and the presence of lesions suggestive of pododermatitis in fore and hind feet (Forefeet, SD value = 0.40, $p$=0.09, hind feet, SD value=0.47, $p$=0.09). Further, it was identified that there is a significant association between higher BCS and higher yeast density on interdigital skin (Fore feet, SD value = 0.61, $p$=0.04, hind feet, SD value=0.59, $p$=0.04). The strength of association regarding bacterial density was moderate and insignificant (Fore feet, SD value = 0.54, $p$=0.06, hind feet, SD value=0.56, $p$=0.08). Overall, the current findings suggest that higher BCS increased the yeast density in the interdigital area, and that there are insignificant positive associations with bacterial density and gross lesion scores. Thus, further studies are necessary for confirmation.

**Keywords:** BCS, Feet lesions, Interdigital skin, Pododermatitis

# Effects of Conjugated Fatty Acids on Fasting Glucose and Osmotic Fragility of Red Blood Cells (RBC) in Experimental Mice

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Conjugated fatty acids, particularly Conjugated Linoleic Acid (CLA; 18:2, 9c,11t & 10t, 12c) and Conjugated Linolenic Acid (CLNA; 18:3, 9c, 11t, 13t), have numerous health benefits. However, potential hypoglycaemic effects remain unexplored. Long-term anti-diabetic medications have side effects. Hence, alternatives and dietary management is crucial for diabetic patients. The impact of conjugated fatty acids on such diets lacks clarity. Thus, this study was performed to evaluate the effect of conjugated fatty acids on fasting blood glucose levels in hyperglycaemic mice which were fed a sucrose rich diet. Further it examined their effect on red blood cell (RBC) fragility. 15 ICR weaned female mice were acclimatised to a high sucrose diet (10% w/w) for 7 days. These mice were divided into three groups: Test A (coconut fat), Test B (CLA) and Test C (CLNA). Based on their dietary intake, daily gavage of 3% experimental fat (CLA, CLNA rich bitter gourd fat or coconut fat) was started immediately following acclimatisation. Animals were allowed to access water and feed *ad libitum* during the experimental period. On the 7$^{th}$ and 14$^{th}$ days after starting the experimental diets, a calibrated glucometer was used to monitor the fasting blood glucose levels. On the 14th day, osmotic fragility of CLNA-fed and mice fed with coconut fat were measured according to standard protocols. By the application of the Graphpad Prism program, one-way and two-way ANOVA tests were used for data analysis. Findings revealed that CLA had a potent acute hypoglycaemic action in hyperglycaemic mice after 7 -days of gavage-based supplementation. The effect of CLNA was not that strong. CLA group was discontinued due to increased mortality of mice after the 7$^{th}$ day. Continuation of the CLNA group revealed that the hypoglycaemic action of CLNA was inconsistent. Initial results indicated that CLNA might negatively affect fasting blood glucose levels after 7 days. Interestingly, the osmotic fragility test showed that CLNA can increase osmotic fragility of RBC, suggesting possible implications for its other biological activities, such as reported cytotoxic effects on cancer cells. The results of this study reveal that conjugated fatty acids exert some therapeutic actions and those need to be further evaluated.

**Keywords:** CLA, CLNA, diabetes mellitus, osmotic fragility

# Isolation and Culturing of Canine Bone Marrow Stem Cells: A Step-by-Step Guidance and Report of Initial Findings

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Canine bone marrow stem cells (BMSCs) have emerged as promising therapeutic candidates within the realm of regenerative medicine. This comprehensive research endeavours to facilitate the cultivation and expansion of canine BMSCs, while simultaneously conducting an in-depth assessment of their inherent properties and prospective applications. The central focus of this study revolves around the meticulous collection and processing of bone marrow from the humerus of three dogs. Following the collection phase, the extracted bone marrow was mixed with DMEM. This bone marrow-DMEM composite was subjected to a rigorous centrifugation process utilizing solution containing sodium diatrizoate (9.1% W/V) and Polysaccharide (5.7% W/V), (lymphoprep, Germany) yielding a density gradient medium. Through this centrifugation mechanism, the diverse cell populations were effectively stratified based on their respective densities. This segregation resulted in the formation of distinct layers characterized by specific cell types, thereby enabling the strategic isolation of targeted cell populations, subsequently earmarked for further comprehensive scrutiny and analysis. These isolated cell populations were subjected to a meticulous five-day culture within DMEM supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin 0.1mg/ml streptomycin. Furthermore, the culture medium was enriched with essential osteogenic compounds, namely ascorbic acid(20µl/1ml), β-glycerophosphate(43µl/ml), and dexamethasone(2µl/ml). In parallel, this research delved into the perceptions and cognitions of veterinary students, involving a cohort of 78 participants. This evaluation, was conducted through a structured questionnaire. It was revealed that a notable 60% of participants exhibited an awareness of stem cell research and its multifarious applications. Evidently, a significant majority 86% expressed an affirmative disposition towards recommending stem cell treatments in the event of their availability. Remarkably, a substantial 80% expressed a penchant for specializing in treatments involving stem cells. However, comprehensive investigations and extensive characterization of the isolated cell populations are warranted. This approach is essential to fully harness the potential of this innovative methodology for therapeutic applications within the broader domain of veterinary medicine.

**Keywords:** Bone marrow, Canine, Regenerative medicine, Stem cells, Veterinary

# Efficacy of Autologous Platelet-Rich Plasma (PRP) for the Treatment of Chronic Infected Wounds in Dogs and Cats: A Comprehensive Evaluation and Clinical Application Study

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Autologous platelet-rich plasma (PRP) has demonstrated its potential in enhancing wound healing and tissue regeneration. It is commonly utilized as a source of growth factors for general wound healing and bone fracture treatment. PRP contains a high concentration of platelets and growth factors, which are condensed in small volumes of plasma. This leads to faster healing times in various preclinical and clinical conditions. This study aims to investigate the cellular concentrations of PRP and its clinical application. Blood samples were collected from four randomly selected dogs with no pre-existing medical conditions. The in-house double centrifugation method was employed to obtain PRP. After processing, the cellular composition of PRP was measured, including platelets, white blood cells, neutrophils, lymphocytes, and monocytes. The results revealed markedly elevated platelet (36.6% elevation) concentrations and decreased WBC concentrations (51.61% reduction) in all PRP samples compared to whole blood. Differential counts showed considerably lower concentrations of neutrophils (36.87% reduction), lymphocytes (42.22% reduction) and monocytes (82.14% reduction) in PRP compared to whole blood, while Eosinophils and Basophils did not demonstrate any remarkable difference. Furthermore, this study evaluates the effects of consecutive applications of autologous PRP in dogs (n=2) and cats (n=1) with large chronic skin wounds presented at the Veterinary Teaching Hospital, Peradeniya. The percentage of contraction, re-epithelialization, and healing in all treated patients indicated no complications or side effects associated with consecutive PRP treatments, and all wounds exhibited improvement in closure and re-epithelialization. Clinical cases of various chronic wounds in dogs and cats were observed, including a cobra bite lesion, a burn lesion, and an idiopathic lesion. Autologous PRP was applied in these cases, resulting in a significant improvement in healing within a relatively short treatment period. The findings of this study suggest that repeated autologous topical PRP treatments have a positive effect on large cutaneous chronic wounds of different aetiologies. However, an extended time frame is necessary with control samples to confirm the findings. Accordingly, PRP treatment represents a simple, cost-effective, and viable alternative to promote healing processes in chronic large wounds in dogs and cats.

**Keywords**: Autologous platelet rich plasma, Cats, Chronic wounds, Dogs, Wound healing

# Prevalence of Ear Mite Infestation of Dogs and Cats from Selected Small Animal Practices in Badulla District

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Otitis Externa is a significant condition commonly encountered in veterinary practice, requiring accurate diagnosis for successful treatment. One predominant cause is parasitic otitis, which can be easily treated. Ear mites (*Otodectes cynotis*) are parasites that infest the ear canals of dogs and cats. These highly contagious mites spread through direct contact or shared living spaces. Symptoms include itching, scratching, head shaking, and a waxy discharge may be leading to secondary bacterial infections and severe symptoms as loss of hearing. A previous study done in the Gampaha District demonstrated the prevalence of ear mite infestations in dogs as 13.20% and in cats as 22.72%. However, this is the first study regarding the prevalence of ear mite infestations and main predisposing factors for the disease in Badulla District. Thus, the study was designed to determine the prevalence of ear mite infestation in dogs and cats from selected small animal practices in the Badulla District. In total, 31 canine samples and 23 feline samples were collected from all cats and dogs who were presented to selected set of small animal clinics in the district within the month of May 2023. The samples were collected and examined for ear mites, data on breed, hair coat length, living premises, age, bathing frequency, cleaning frequency of premises, living environment, contact with other animals, and previous tick treatments through owner interviews. The prevalence of ear mite infestations showed 29.03% in dogs and 26.08% in cats, with a higher prevalence in dogs than cats. The data obtained from the study performed in the Gampaha District had lower prevalence of ear mite infestation while a higher prevalence was recorded in Badulla District. However, no statistically significant associations were found between the predisposing factors considered and ear mite infestation. Further studies with larger sample sizes are recommended to gain more comprehensive understanding of ear mite infestation in dogs and cats in this area.

**Keywords:** Badulla, Cats, Dogs, Ear mite infestation

# Prevalence of Equine Piroplasmosis in Donkeys in Mannar Town, Sri Lanka

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The donkey, which belongs to the family Equidae is an important animal used for transport and draught purposes. Equine piroplasmosis is caused by *Theileria equi* and/or *Babesia caballi*. It is a tick-borne disease. This study was conducted to investigate the presence of equine piroplasmosis caused by *Theileria equi* and *Babesia caballi* in free-roaming donkeys in Mannar, Sri Lanka. A total of 32 apparently healthy donkeys from Mannar Donkey Clinic and Education Centre (n=25) and Mannar town area (n = 7) were included in the study. Blood samples were collected from the jugular vein of these donkeys, and thin blood smears were prepared from the blood drops collected from the ear tip for microscopic examination. Based on microscopic examination of stained smears, the results of the study revealed that 7 (21.8%) of the 32 donkeys were positive for *T. equi* and two of the 32 donkeys were positive for *B. caballi*. This study will support the welfare and health of donkeys in Mannar and help to control this disease spreading to other equids like horses. Polymerase chain reaction and clinical pathological studies will be carried out in the near future. Further this study will contribute to the development of specialized approaches for the diagnosis, management, and treatment of this disease in donkeys and other equids.

**Keywords:** *Babesia,* Donkey, Equine Piroplasmosis, *Theileria*, Tick-Borne disease

# A Longitudinal Study of Periodontal Disease in Dogs Using Different Indices

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Dogs are commonly impacted by periodontal disease, which has serious detrimental implications on their overall oral health. This study aimed to investigate the prevalence of periodontal disease in domestic dogs in the Central Province of Sri Lanka and to explore possible associations with age and gender. Each tooth of dogs selected using convenience sampling between the ages of three months and twelve years was assessed using a periodontal probe. Based on a careful inspection and the presence or absence of gingival bleeding, the gingival index was assessed. Probing depth was measured by gently inserting a periodontal probe between the free gingiva and tooth surface until resistance was felt. The furcation index was determined by sliding the periodontal probe down the free marginal groove until it touched the long axis of the teeth. The mobility index was assessed based on tooth movement under gentle pressure. Finally, the periodontal grade was assigned based on the results of all the indices. The scatter plot analysis provided preliminary evidence of a potential association between advancing age and the severity of periodontal disease. Among the 50 conveniently selected dogs of various ages, all exhibited at least one tooth with early periodontal disease (100% prevalence). Gingivitis, the early stage of periodontal disease, was found to be associated with all cases of caries. Only 12% of the dogs, including all patients over 10 years old, were found to be in stage 2. In terms of probing depth values, stage 2 had the highest frequency (23 occurrences) and was observed in both young and old patients. The furcation area showed no pathology in 78% of dogs under 10 years old, indicating healthy conditions related to the furcation index. However, data related to the mobility index were limited (16% of the total examined), making it a challenge to establish age-related characteristics. Based on the periodontal grade, 37 dogs (74%) were still in the early stage of periodontal disease, while only one dog (4%) reached the last stage. The findings highlighted the need for age-specific interventions and emphasise the importance of regular dental care to minimise the impact of periodontal disease in the canine population.

**Keywords:** Age, Furcation index, Gingivitis, Periodontal Disease

# Detection of Cryptosporidium in Owned Dogs in and Around Kandy

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Cryptosporidiosis, caused by the protozoan parasite Cryptosporidium, is a gastrointestinal disease affecting both humans and animals. The clinical significance of this organism remains uncertain due to its detection in healthy dogs. This study aimed to detect Cryptosporidium oocysts in dogs presented to the Veterinary Teaching Hospital and the Government Veterinary Hospital in Peradeniya, with or without clinical signs of cryptosporidiosis, to understand the occurrence and clinical relevance of this parasite. Fecal samples were directly collected from the rectum of 103 dogs and subjected to Sheather's sucrose flotation test. Following direct microscopic examination of the supernatant, smears were prepared and stained with modified Ziehl-Neelsen stain. Out of the 103 faecal samples, 14 tested positive for Cryptosporidium oocysts, indicating an occurrence rate of 13.6%, which is higher than reported rates in other countries. Interestingly, only four of the 14 Cryptosporidium-positive dogs had diarrhoea, suggesting clinical cryptosporidiosis. However, concurrent Trichuris infection and strongyloidosis were detected in two of these dogs, implying that most infected dogs may remain asymptomatic carriers. Further studies focusing on dogs with diarrhoea are warranted to better understand the clinical significance of Cryptosporidium. It is essential to mention the potential for false-negative results due to intermittent oocyst shedding or low oocyst load. It is important to use molecular-based testing to enhance sensitivity and accuracy of detection of cryptosporidium. Adult dogs, particularly those over five years were more likely to shed Cryptosporidium oocysts, while puppies younger than one year had a lower incidence of infection. Male dogs had a higher infection rate, likely due to increased exposure while freely roaming. In conclusion, this study reveals the presence of Cryptosporidium in owned dogs in the Kandy District of Sri Lanka. Given that the majority of dogs were asymptomatic or had concurrent infections with other diarrhoeal parasites, the clinical significance of Cryptosporidium remains uncertain. However, due to its zoonotic potential, regular dog screening and appropriate hygienic practices should be adopted to minimize public health risks.

**Key words:** Cryptosporidiosis, Owned Dogs, Kandy District

# Companion Animal Health

# Session-II

# Assessment of the Geographical Distribution, Disease Burden and Characteristics of Highly Fatal Acute Respiratory Distress among Young Dogs in Sri Lanka

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Acute respiratory distress syndrome (ARDS) is a life-threatening condition that results in hypoxaemia. ARDS could be caused by various aetiologies and involves damage to the alveolar epithelium and capillary endothelium, leading to increased permeability and impaired gas exchange. ARDS is not commonly reported in dogs. However, over the last decade, a highly fatal disease characterized by acute respiratory distress in young dogs has been detected in Sri Lanka. Affected dogs show acute dyspnoea, pallor, or cyanosis of mucous membranes, and die within 24-48 hours. Aetiology of the disease is still not known. The geographical distribution of the disease and the occurrence of the disease are yet to be elucidated. This study determined the geographical distribution and seasonality of ARDS affecting young dogs in Sri Lanka. We collected data through an online questionnaire and telephone interviews with veterinarians across different provinces. Additionally, we extracted data from the Veterinary Teaching Hospital records for time series forecasting. The results indicated high disease incidence rates in Kandy and Badulla districts, with seasonal peaks in June-August and December-January. The disease has mostly affected dogs in Gampola, Yatinuwara, Gangawata Korale, Pasbage Korale (Nawalapitiya), and Kundasale in the Kandy district, as well as Badulla, Bandarawela, Haputhalae, Kandaketiya, and Passara in the Badulla district. All veterinarians who have encountered cases of the disease reported that it primarily affects young dogs below 6 months of age. This research is significant as it sheds light on a previously unknown disease affecting young dogs in specific areas of Sri Lanka. Understanding its geographical distribution, seasonality, and potential risk factors can lead to better disease management and preventive measures. Further research and identification of the aetiological agent are crucial to effectively combat this highly fatal disease in young dogs.

**Keywords:** Acute Respiratory Distress Syndrome (ARDS), Cyanosis, Geographical distribution, Hypoxaemia, Young dogs

# Histopathological Changes Seen in Canine Liver During Possible Hypoxic Damage

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Hypoxic hepatitis occurs when there is an imbalance between the oxygen supply and oxygen demand in the liver, leading to inadequate oxygen and nutrition supply which results in cell injury. The liver is highly sensitive to the oxygen supply due to its high metabolic activity and the significant cardiac output it receives. Hypoxic hepatitis can be caused by pulmonary failure, dehydration, heart failure, abnormal heart rhythm, infection and severe bleeding. Pneumonia is a common condition that can lead to hypoxia and reduced tissue perfusion in both dogs and humans by limiting the space for gas exchange due to the production of exudates. Hepatic hypoxia results in cytoplasmic and nuclear changes in hepatocytes and bile ducts. The study involved observing stained sections of lung and liver samples from 20 pneumonic dogs and comparing them with 20 normal lung and liver samples from non-pneumonic dogs The ages of the control groups and test groups were chosen from a similar age category as much as possible. In the study, histopathological changes in the liver of dogs with hypoxic hepatitis were examined and no gross lesions were seen except for pneumonia. The major cytoplasmic changes observed in hepatocytes included cytoplasmic vacuolation, bile pigments, glycogen granules and loss of cell margins. Nuclear changes were pyknosis, karyorrhexis, karyolysis and eccentric nuclei. Additionally, the study observed histopathological changes in bile ducts, including bile duct hyperplasia, epithelial cells detachment from the basement membrane, necrotic debris inside the lumen and fibrosis. The severity of pneumonia correlated with the percentage of cytoplasmic and nuclear changes observed in the liver. Severe pneumonia cases showed a higher extent of changes compared to moderate and mild cases. The study also noted zonal lesions in the liver, which provide insights into the relationship between hypoxic changes and liver damage. The centrilobular area was significantly affected by severe pneumonia cases, followed by moderate and mild cases. The study highlights the occurrence of hypoxic hepatitis or ischemic hepatitis in dogs with pneumonia.

**Keywords:** Hepatitis, Histopathology, Hypoxia, Ischemia, Pneumonia,

# Histopathological Observation in Canine Myocardium During Possible Hypoxic Damage

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Myocardial hypoxia occurs when oxygen supplementation becomes lower than the oxygen demand of myocardial tissue. Myocardial hypoxia causes damage in myocardial tissue which can be identified in histopathological sections. Myocardial ischemia is a cause for myocardial hypoxia which occurs due to functional or structural alteration in myocardial perfusion. Pneumonia can lead to myocardial hypoxia through various mechanisms. This study aimed to investigate the occurrence of myocardial damage in dogs with pneumonia-induced hypoxia. In this study twenty (n=20) pneumonic lung samples (test group) and twenty (n=20) normal lung samples (control group) were taken from dead dogs. Pneumonic and normal lung samples were taken from age matched dogs and no gross lesions were observed except pneumonia in pneumonic dogs. Myocardial tissues were carefully harvested from animals in both groups, and all samples were subjected to histopathological examination. The observed changes in the myocardial tissues of pneumonic dogs were compared to those of the control animals. Lesions observed in human ischemic heart disease patients such as fragmentation of cardiac myocytes, wavy cardiac myocytes, increased eosinophilia, absence of nuclei, pyknotic and caterpillar nuclei were also observed in myocardial of test group. The finding suggests a similar condition to human ischemic heart disease in dogs. This study highlights the importance of recognizing myocardial hypoxia as a potential complication of pneumonia in dogs. Further research is required to elucidate the underlying pathogenesis of myocardial damage during hypoxia in canines, which could lead to the development of effective treatment strategies and preventive measures.

**Keywords:** Ischemic Heart Disease, Myocardial Ischemia, Myocardial Hypoxia, Pneumonia, Pyknotic Nuclei.

# Morphological Characterization of Fleas Collected from Dogs and Cats in Kandy District of Sri Lanka

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Fleas are obligate haematophagous, wingless, laterally compressed insects that feed on a wide variety of hosts. Fleas are important because they cause discomfort and blood loss in domestic animals while acting as vectors or intermediate hosts for a variety of pathogens. Fleas are medically significant because they transmit zoonoses and many people develop allergic dermatitis following flea bites. *Ctenocephalides canis* and *Ctenocephalides felis* are thought to be present as ectoparasites in domestic dogs and cats in Sri Lanka. Because there is no literature to support this hypothesis and many other flea genera can infect domestic animals, this study aimed to morphologically characterise fleas collected from dogs and cats. Following an extensive literature search a morphological identification key was developed for microscopic identification of fleas. Altogether 32 samples were collected from 28 dogs and 4 cats in the Kandy district, with each sample containing a maximum of 10 adult fleas. Each flea was morphologically identified using a stereomicroscope and the morphological identification key. Only two species were morphologically identified in the samples: *Ctenocephalides orientitis* and *Ctenocephalides felis*. When individual fleas were examined, 137 (72.11%) were identified as *C. orientitis* and 41 (21.58%) as *C. felis*. The morphological key developed herein was insufficient to identify another 12 (6.3%) fleas because they showed hybrid characteristics. When the hosts are considered, 16 dogs and 4 cats were infested with a single flea species. However, 11 dogs were infested with mixed flea species. In contrast to the hypothesis, *C. orientis* appears to be the most common flea species infesting dogs in the Kandy district of Sri Lanka, followed by *C. felis*. The small sample size limits the ability to draw conclusions about the predominant flea species infesting cats in the study area. Hybrid flea species have been reported worldwide; however, molecular characterization is required to confirm each species and its hybrids. A comprehensive investigation recruiting a large sample size spread across different geographical locations, including both owned and stray dogs and cats is recommended to capture all flea species infesting dogs and cats in Kandy district, Sri Lanka.

**Keywords:** Cats, *Ctenocephalides orientis, Ctenocephalides felis*, Dogs, Fleas

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# Assessment of Diagnostic Accuracy and Inter-Pathologist Agreement in Diagnosing Canine Round Cell Tumours via Tele Cytology Performed Using Images Captured by a Smart Phone Camera

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Neoplasia is a common disease encountered by veterinarians. Rapid and accurate diagnosis of neoplasia is essential for a better disease outcome. Cytology is an inexpensive and minimally invasive diagnostic method that can be effectively used to diagnose neoplasia. Many veterinary practices in Sri Lanka are equipped with basic instruments to perform in-house cytology but often lack expertise to interpret results. Physical distance between veterinary practices and pathologists greatly affects rapid results interpretation. Tele cytology facilitates rapid interpretation of cytology results. It includes acquisition of digital images, transferring images to a pathologist in a distant location and obtaining results through networking. Smart phone cameras can be used as an alternative to conventional image capturing devices used for tele cytology. This project was designed to evaluate whether tele cytology with smart phone cameras can be effectively used to diagnose round cell neoplasia in dogs. For this purpose, five round cell tumors (lymphoma, mast cell tumors, TVT, plasmacytoma and histiocytoma) were imaged using a smart phone camera and sent to two pathologists in distant locations via emails. The diagnoses provided by the pathologists were used to determine inter-pathologist agreement using kappa tests. In addition, the diagnoses provided by the pathologists were compared with the histopathological diagnosis of the same tumor and the level of agreement was determined using the same statistical method. The overall inter-pathologist agreement in diagnosing round cell neoplasia was good and statistically significant ($\kappa$=0.88, $p$=0.03). The agreement between diagnoses by pathologists and histopathology was fair to moderate ($\kappa$=0.58, $p$=0.05). When the agreements were assessed separately for different tumor types, both inter-pathologist as well as between the pathologist's diagnosis and histopathology were excellent for "lymphoma, mast cell tumors and TVT" ($\kappa$=1, $p$=<0.001). The agreements were poor at highest diagnostic level for plasmacytoma and histiocytoma. Further, use of cytology images obtained at low power and high power together to attempt at a diagnosis was more effective than using low power images alone. Overall, these findings suggest that tele cytology performed with smart phone cameras is an effective way for rapid diagnosis of canine round cell neoplasia.

**Keywords:** Diagnostic accuracy, Round cell tumour, Smart phone , Tele Cytology

# Determination of Gastrointestinal Parasitic Infections in Free Roaming Puppies under Six Months of Age in Selected Locations in the Central Province

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Free roaming dogs and puppies play a major role in the transmission of diseases among domesticated animals and pose potential threats to public health. One of the common routes of disease transmission is through the oral-fecal route. This study aimed to comprehensively evaluate the types of gastrointestinal parasites and their eggs or cysts present in free roaming puppies who are less than six months of age. The study further explored the zoonotic potential of these parasites and their eggs, emphasizing the importance of community awareness for public health. A study was conducted in Central province, which involved the random collection of fecal samples from thirty (n=30) free-roaming puppies under six months of age. The collected fecal samples were processed through centrifugation, and parasite identification was performed using fecal floatation analysis. The parasites were observed directly under a light microscope. Out of the 30 samples 28 were found to be positive for the presence of gastrointestinal parasitic eggs. The most prevalent species identified were *Toxocara, Ancylostoma* and *Dipylidium* spp Two samples tested negative for parasitic eggs. The parasite load varied, ranging from 2 to 150 eggs per gram of feces. The prevalence of three zoonotic parasites (*Toxocara, Ancylostoma* and *Dipylidium* spp) emphasizes the potential risk these free-roaming puppies pose to both human and animal health. The findings of this study underscore the urgent need to address the issue of free-roaming puppies and the potential for disease transmission they carry. Therefore, this study warrants the necessity in continuation of future research to identify the preventive mechanisms of disease distribution among dogs and human population through the feces of puppies.

**Keywords:** Gastrointestinal Tract (GIT), Puppies, Stool samples

# A Hospital Based Study on Detection of Canine Filariasis and Potentially Ivermectin Resistant Filarial Strains

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Canine filariasis is a mosquito-borne infection. The common genera of filaria responsible for canine filariasis are *Dirofilaria*, *Brugia*, and *Dipetalonema*. These genera are zoonotic. Ivermectin is one of the drugs that has been widely used in veterinary practice to treat canine filariasis. As ivermectin is prescribed commonly for dogs and oral dosage forms are available over the counter, development of resistance in parasites against ivermectin has been a concern. The objectives of this study were to determine the occurrence of canine filariasis, identify responsible species, and assess the presence of ivermectin-resistant filaria in dogs visiting Sapugaskanda Veterinary Hospital (SVH). All dogs presented to the SVH between May 22$^{nd}$ and July 6$^{th}$, 2023, with clinical signs such as cutaneous ulcerations and the formation of subcutaneous nodules, soft tissue swelling and before ivermectin treatment for skin conditions were included in the study. The dogs included were subjected to a blood drop examination to determine the microfilaremic status. A thick blood film was prepared, and the dogs were treated with 0.06mg/kg of oral ivermectin for 7 days. Owners were interviewed verbally to gather background information, history of ivermectin use, and were advised to bring the animals in for a follow-up visit 7 days after completion of oral treatment. A second blood sample was taken to perform Knott's technique. In total, 2018 canine cases were presented to the hospital within the allotted time, and 267 of these cases satisfied the inclusion criteria, however, only 30 dogs were tested positive for microfilaremia. This correlates to an 11.24% occurrence of microfilaremia in dogs reported with the listed clinical signs to the SVP. *D. repens* was identified morphologically in every positive sample. Among the positive dogs, males outnumbered females (53%) and 63% of these dogs have lived outdoor. Only three of the treated dogs paid follow-up visits, and Knott's technique revealed two of them infected with filaria, and these were considered as potentially ivermectin-resistant filaria. Molecular characterization revealed that these two potentially ivermectin-resistant filaria as *D. repense*, and molecular screening for ivermectin resistance is underway. The current study found a very low occurrence of canine filariasis and the identification of potentially ivermectin-resistant filaria was hindered as the owners did not return for follow-up. A large-scale study, involving stray dogs and prolong ivermectin-treated dogs will reveal the true prevalence and increase the likelihood of detecting ivermectin-resistant phenotypes.

**Keywords:** Dogs, Filaria, Ivermectin-resistance, Sapugaskanda
*Acknowledgements: University Research Grant (URG/2022/67/V)*

# Poultry Production and Health

# Session-I

# Establishment of Polymerase Chain Reaction (PCR) Protocol to Detect *Mycoplasma gallisepticum* in Poultry

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Chronic Respiratory Disease (CRD) poses a significant global threat to the poultry industry, prominently linked to the pathogen *Mycoplasma gallisepticum* (MG). CRD in poultry manifests through distressing symptoms like coughing, sneezing, and nasal discharge, with MG's role as a respiratory tract colonizer intensifying the disease's impact. Due to MG's challenging cultivation, molecular diagnostic techniques are pivotal. To address this need, this study endeavours to establish and optimize a conventional Polymerase Chain Reaction (PCR) protocol for efficient MG detection within the Sri Lankan poultry sector. This technique facilitates the amplification of MG-specific pvp-A gene sequences, enabling precise detection. The optimization process involves aligning primers with conserved regions of the MG genome, refining annealing temperatures, and optimizing cycling parameters based on a comprehensive literature review and the target PCR product size. Positive controls comprising previously validated MG DNA samples assure the protocol's accuracy. Eight samples were collected which were exhibiting respiratory symptoms from a chosen poultry farm in the Central Province. Nucleic acid extraction followed by conventional PCR using a hot start kit and distinct solutions was performed. The PCR was conducted with an initial denaturation step at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was carried out at 72°C for 10 minutes. Gel electrophoresis aimed to yield the 374–695bp size PCR product. The obtained PCR product corresponded to expectations, although the presence of smaller, nonspecific products in the gel image signifies further optimization requirements. The occurrence of such nonspecific products can be attributed to factors like annealing temperature and primer specificity. In conclusion, this study marks the establishment and preliminary optimization of a PCR protocol for MG detection in the poultry sector. Yet, additional refinement is essential to augment the protocol's robustness. This research addresses a critical gap in disease management, presenting a reliable tool for early MG detection, contributing to the industry's resilience.

**Keywords:** Chronic Respiratory Disease (CRD), Conventional PCR, *Mycoplasma gallisepticum* (MG), Optimization, Poultry

# Associated Management Risk Factors of Chronic Respiratory Disease in Commercial Layers Presented to Veterinary Investigation Center, Wariyapola

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Chronic Respiratory Disease (CRD) is a significant and economically important ailment that affects the poultry industry, and it is caused by *Mycoplasma gallisepticum*. To investigate the associated management risk factors of CRD in commercial layers, a study was conducted at the Veterinary Investigation Center in Wariyapola. The study extended over five weeks and examined 14 cases, where CRD was confirmed based on gross pathology and clinical signs. Farmers were provided with a questionnaire to assess various farm management practices that could contribute to the prevalence of CRD, including housing, flooring, rearing systems, temperature fluctuations, stocking density, feeding methods, feed type, litter moisture, litter raking frequency, disinfection between flocks, history of previous disease outbreaks, deworming practices, and vaccination against CRD. The study revealed that all farms used open-sided houses (100%) and deep litter management (100%) with an all-in-all-out rearing system (100%) while 58% of farms had a controlled feeding method and 85.7% used self-mixed feed. Though none were vaccinated against CRD, 71.4% regularly dewormed their flocks. About 50% of farms experienced temperature fluctuations and lacked frequent litter raking. Additionally, 71.4% had high stocking density, and 78.6% had high litter moisture. Half of the CRD-diagnosed farms had recent disease outbreaks, and 78.6% resorted to antibiotic treatment. The study results provide valuable insights into the current management practices adopted by poultry farmers and how these practices may influence the prevalence of CRD. Understanding these risk factors can help farmers implement effective preventive measures to control and reduce the incidence of CRD in commercial layer flocks.

**Keywords:** Chronic Respiratory Disease, Commercial Layers, Layer Management, *Mycoplasma gallisepticum*, Sri Lanka

# Detection of Infectious Laryngotracheitis Virus in Commercial Layer and Broiler Farms in Kurunegala and Kandy Districts

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Infectious Laryngotracheitis (ILT) is a highly contagious respiratory disease that affects poultry, causing significant economic losses in the poultry industry worldwide. It is caused by Gallid Herpes-1, a member of the family *Herpesviridae.* This disease is characterized by respiratory distress, gasping, coughing, nasal discharges, and expectoration of bloody mucus. This study aimed to determine the prevalence of ILT in commercial layer and broiler farms in the Kurunegala and Kandy districts of Sri Lanka using the conventional polymerase chain reaction (PCR) technique. A total of 18 pooled samples of trachea and lungs were collected from dead birds showing respiratory signs in different poultry farms in both districts and subjected to conventional PCR targeting the p32 gene of the ILT virus. Out of the 18 samples, three were found to be positive for ILT, indicating an overall prevalence rate of 16.67% in the studied population. When analyzing the prevalence in individual districts, a higher prevalence rate of 33.33% was observed in the Kurunegala district, while no positive cases were detected in the Kandy district (0% prevalence). These findings highlight the presence of ILT in commercial layer and broiler farms in the study area, with a moderate overall prevalence. The higher prevalence observed in Kurunegala district suggests it is a potential hotspot for ILT transmission, warranting further investigation and targeted control measures. The absence of ILT cases in the Kandy district indicates a difference in disease prevalence between the two regions, likely influenced by various factors such as biosecurity practices and management strategies. This study emphasizes the importance of implementing effective prevention and control measures, including improved biosecurity protocols, vaccination strategies, and early detection methods to mitigate the spread and impact of ILT in poultry populations. Further research is required to understand the underlying factors contributing to the varying prevalence rates observed in different districts and develop region-specific strategies for ILT management.

**Keywords:** Infectious Laryngotracheitis, Kandy, Kurunegala, Polymerase Chain Reaction, Poultry,

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# Molecular Detection of Chicken Anemia Virus (CAV) in Inclusion Body Hepatitis (IBH) Infected Broilers in the Kurunegala District, Sri Lanka

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Infectious diseases pose a major threat to the poultry industry, and among them, immunosuppressive diseases are important as they suppress the immunity of birds in a flock. Chicken Anaemia Virus (CAV) infection is one of the most common immunosuppressive diseases among chickens which can be present as an underlying disease and lead to many other diseases. Inclusion body hepatitis (IBH) is one of the viral diseases present in chickens which is caused by Fowl Adenovirus (FAdV) and results in increased mortality in the birds of the flock and thereby reduces productivity. This study was conducted to explore the presence of CAV in broiler farms with confirmed IBH cases. Twelve thymus samples from four medium to large-scale broiler farms in the Kurunegala district were selected following IBH infection confirmation. IBH infection was confirmed by gross lesions, histopathology and conventional Polymerase chain reaction (PCR) in a parallel ongoing study. Total DNA was extracted from thymus samples using the QIAGEN DNAeasy® mini kit. PCR was performed using the CAV1: 5'-GCA GTA GGT ATA CGC AAG GC-3' and CAV2: 5'-CTG AAC ACC GTT GAT GGT C-3' primers targeting a 186 bp region on the highly conserved VP2 coding gene of the CAV. DNA extracted from a live CAV vaccine was used as the positive control. The PCR products were then analyzed by electrophoresis in 1.5% agarose gels and images were captured. Nine out of the twelve samples were positive for CAV. Our study showed that CAV was present in broiler birds either as a primary causative agent aiding the establishment of secondary diseases like IBH or that both CAV and IBH-causing Fowl adenoviruses were co-circulating in broiler farms in Kurunegala district and that was subjected to our study. This emphasizes the importance of following a proper vaccination protocol for broiler breeders as well as for broiler birds to prevent CAV disease. A further elaborative study is needed to investigate the co-existence of both these diseases in broiler farms in the country.

**Keywords:** Broiler, CAV, IBH, Immunosuppression, Viral diseases

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# Assessment of Foodborne Pathogens, Antimicrobial Resistance, and Antimicrobial Residues Associated with Branded Frozen Chicken at Retail Level

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Foodborne bacterial zoonoses pose a global health challenge. These bacteria frequently contaminate animal-origin products, with a significant and direct risk of transmission through poultry and related products, particularly chicken meat. Frozen branded chicken meat is one of the most commonly available and consumed meat varieties in Sri Lanka. In the present study, to assess the occurrence of *E. coli*, *Salmonella*, and *Campylobacter*, to detect resistance profiles of isolated *E. coli* and *Salmonella*, and to test antimicrobial residues, 21 branded frozen whole/half chicken with the package were purchased from supermarkets in the Kurunegala municipal council area. The 21 samples comprised of three samples from each brand (different batches), representing seven brands. Meat packages were observed for quality certifications. Microbiological testing was performed according to ISO standard methods with slight modifications. Antimicrobial susceptibility testing and antimicrobial residue screening were performed using CLSI, 2019 guidelines, and the Modified EU Six Plate Test respectively. The results indicated that 85.71% (n = 18/21) of the tested samples were contaminated with *E. coli*, 14.28% (n = 3/21) with *Salmonella*, and 19% (n = 4/21) with *Campylobacter*. While the prevalence of *Salmonella* contamination resembled previous research in Sri Lanka, the rates for *Campylobacter* and *E. coli* were higher, but still lower than in several other countries. Notably, *E. coli* isolates exhibited high resistance rates: 77.77% (n = 14/18), 55.55% (n = 10/18), 50% (n = 9/18), 50% (n = 9/18), 22% (n = 4/18), 5.55% (n = 1/18) for ampicillin, tetracycline, sulphamethoxazole and trimethoprim, ciprofloxacin, chloramphenicol, and amoxicillin-clavulanic acid, respectively. All *Salmonella* isolates exhibited resistance against tetracycline and chloramphenicol. None of the samples tested were positive for antimicrobial residues. Except for one brand, other brands had quality certifications such as HACCP, ISO22000, and GMP. According to this preliminary study, frozen branded chicken produced by large-scale processors with quality certification systems in place are not devoid of microbiological health hazards. This study sheds light on the need to implement good manufacturing practices "from farm to fork", continuous monitoring of the quality control systems along with public awareness campaigns and proper handling of animal products to ensure food safety.

**Keywords:** AMR, Chicken, Foodborne pathogens, Residues,

# Molecular Detection and Phylogenetic Analysis of Fowl Adenovirus (FAdV) Serotypes Present in Northwestern, Western and Central Provinces of Sri Lanka

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Fowl Adenovirus (FAdV) infections have become an emerging disease in poultry industry during the past few years in both global and local contexts. Molecular detection of FAdV and its serotypes is important in the prevention and control of diseases caused by FAdV in poultry farms. Limited literature on FAdV makes this prevention and control challenging. Hexon gene is widely used in the detection of DNA originating from FAdV with molecular techniques such as PCR. This method precisely counts positive cases with or without clinical signs. This study aimed to detect the DNA originated from FAdV from May to July in 2023 from selected poultry farms in the Northwestern, the Western and the Central provinces of Sri Lanka. Further, this study aimed to obtain the DNA sequences of detected DNA targeting hexon gene of FAdV from 2021 to 2022 from those farms and construct a phylogenetic tree to determine evolutionary relationship of DNA sequences obtained for hexon gene of FAdV with the same DNA sequences available in NCBI GenBank. In this study, kidney, liver, spleen, gizzard and heart samples were taken from dead birds suspected to be affected with FAdVs. Primers specific to FAdV and its serotypes were used to perform the PCR to detect DNA originating from FAdV and its serotypes. Samples were analysed for FAdV DNA using a previously established PCR protocol. All samples tested were negative for DNA originating from FAdV from May to July 2023. DNA sequences of the partial hexon gene were obtained from the positive DNA isolated from 2021 to 2022 from a previous study. The sequences obtained were aligned and BLAST using MEGA XI and NCBI BLASTn tool respectively. Sequencing results reconfirmed the previous results obtained through PCR. Phylogenetic analysis revealed the relationship between the sequence of local isolates and sequences obtained from different geographical locations of the world. Further studies on molecular and pathological characterization are needed to understand the FAdV infections in Sri Lanka, develop methods such as effective vaccination protocols and control economic losses to the poultry industry.

**Keywords:** Fowl Adenovirus, Hexon Gene, Phylogenetic Analysis, Sanger Sequences, Serotypes

# Molecular Detection of Avian Metapneumovirus (aMPV) in Birds Showing Respiratory Signs in Selected Commercial Poultry Farms Located in the Northwestern and the Central Provinces of Sri Lanka from May to June 2023

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Poultry respiratory diseases caused by bacterial and viral pathogens pose a significant threat to commercial poultry. Avian metapneumovirus (aMPV) is a prominent viral pathogen responsible for chronic respiratory infections in turkeys and chickens. This study aimed at the molecular detection of aMPV in birds exhibiting respiratory signs in selected commercial poultry farms located in the Northwestern and the Central provinces of Sri Lanka. A comprehensive review of the literature revealed the genetic diversity of aMPV, with four serotypes (A, B, C, and D) identified based on the G protein sequence of ART genome. The virus primarily spreads through direct and indirect contact and aerosol transmission via respiratory droplets. Clinical symptoms of aMPV infection include respiratory issues such as coughing, sneezing, nasal discharge, and difficulty in breathing. The damage to respiratory epithelium caused by the virus can lead to secondary bacterial infections, exacerbating the respiratory condition. The severity and distribution of pathological changes vary depending on the stage of infection and individual factors. To detect aMPV, 18 samples were collected from oropharynx of birds showing respiratory signs in commercial poultry farms. RNA extraction was performed, followed by multiplex quantitative polymerase chain reaction (qPCR) using a Biocheck aMPV commercial kit (USA). This qPCR assay enables the simultaneous detection of aMPV serotypes A, B, C, and D. The results of the study revealed absence of detectable viral RNA of aMPV in the samples obtained from selected poultry farms from May to June 2023 and confirmed that aMPV infections are not a possible cause for observed respiratory symptoms. It is important to consider factors such as the time frame of sample collection, and viral shedding patterns that may influence the results. This study highlights the importance of molecular surveillance and detection techniques for the accurate diagnosis of respiratory diseases in poultry. Further investigations and molecular diagnosis are warranted to explore possible causes for the observed respiratory symptoms in the affected birds. Identifying the precise etiology of respiratory diseases is essential for implementing appropriate control and prevention strategies. While this study answered the main research questions, it is essential to acknowledge its limitations such as small sample size and limited time frame.

**Keywords:** AR, Avian Metapneumovirus (aMPV), Multiplex qPCR, Poultry

# A Pathological Study of Dead on Arrival Birds in a Broiler Processing Plant

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Broiler birds that die between loading at the farm and slaughtering are described as 'dead on arrival' (DOA) birds. Higher DOA rates indicate potential health and welfare issues in the poultry business. Therefore, to reduce the percentages of these (DOA) birds, it is important to understand the risk factors associated with their deaths. In this study, a detailed pathological investigation was carried out in a well-established poultry processing plant in the Central Province to identify the most common factors associated with DOAs. Over a period of seven months, 196 DOA birds were subjected to postmortem, and detailed macroscopic and microscopic pathological examinations were done. All macroscopic lesions were recorded, and tissues were immediately collected to 10% neutral buffered formalin for histopathology. Twenty-six macroscopic lesions were identified and further categorized as external and internal lesions for clarity. According to this study, the most common external lesions were bruises (41.33%) and fractures (7.14%) followed by pecking wounds (3.06%). The most common internal lesions found were lung congestion (46.43%), pericarditis (40.82%), air sacculitis (29.08%), perihepatitis (23.47%), polyserositis (21.43%) and liver necrosis (19.39%). All histopathological findings matched with the macroscopic lesions. Bruises and lung congestion were the most common findings that are mostly associated with transport stress. However, lesions like pericarditis, air sacculitis, and perihepatitis were also found in significant numbers indicating that there may be underlying infectious diseases in birds that died during transport. These findings indicate the importance of good health status as well as paying more attention to the catching and crating process which is crucial in decreasing the percentage of DOA broilers in the future.

**Keywords:** Broiler, Central province, Dead on Arrival Birds

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# Poultry Production and Health Session-II

# Associated Management Risk Factors of Infectious Bronchitis in Commercial Layers Presented to the Veterinary Investigation Centre, Wariyapola

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Infectious bronchitis (IB) is a highly contagious respiratory disease that poses a significant threat to commercial layer flocks, resulting in substantial economic losses within the poultry industry. This study aimed to identify the management risk factors associated with IB in commercial layers presented to the Veterinary Investigation Centre, Wariyapola. The study period encompassed five weeks, and 15 cases were included in the analysis. Various farm management factors were examined, including the housing system, flooring system, rearing system, adequate ventilation, stocking density, litter moisture, temperature fluctuations, feeding method, feed type, regular vaccination for IB, routine deworming, recent disease outbreaks, and frequent litter raking. The results revealed that the majority of farms had open-sided houses (93.3%), following a deep litter management system (100%), and adhered to all-in-all-out rearing (86.7%). Furthermore, a substantial proportion of farms (66.7%) adopted a controlled feeding method, while 86.7% used a self-mixed feed type. It was found that 53.3% of the farms did not vaccinate against IB in the last three months, but 80% of the flocks were regularly dewormed. Additionally, 53.3% of the farms experienced temperature fluctuations and followed frequent litter raking, whereas 26.6% had high stocking density. Furthermore, many IB-diagnosed farms (53.3%) had disease outbreaks within the last three months and had not received an antibiotic treatment during the previous three months. The findings of this study will provide insights into the management practices that may contribute to the prevalence of IB in commercial layer flocks, allowing poultry farmers to implement adequate disease preventive measures.

**Keywords:** Commercial layer Infectious bronchitis, Management, Preventive, Wariyapoala

# Optimizing Hatchery Performances of a Cobb 500 Broiler Grandparent Female Line Flock: A Retrospective Study

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The global demand for white meat, particularly poultry, is on the rise. Sri Lanka is also experiencing a surge in broiler production to meet this demand. The genetic quality of grandparent stock plays a key role in broiler growth and productivity. Successful broiler grandparent production depends on the performance of the parent stock on the grandparents' farm. Fertility, hatchability, and egg hatching quality are critical factors influencing broiler chick quantity and quality. Monitoring and optimizing grandparent stock's reproductive performance ensures a steady supply of healthy broiler chicks, supporting the commercial broiler industry's success and sustainability. This study is conducted to evaluate the apparent fertility, temperature fluctuations in egg storage, and hatchability records of the Cobb 500 broiler grandparent flock female line that showed low hatchability from March to July 2023. Comprehensive records of fertility rates, hatchability rates, and successful hatching of fertile eggs were collected. Laying performances, and body composition were thoroughly assessed by a veterinarian and found to meet established standards. Therefore, the focus of the investigation shifted towards assessing the hatching egg storage, incubation process, and transport. All the data were obtained from farm and hatchery records, while temperature records were obtained from the areas where the hatching eggs were stored and transported. At the same time, the eggs that were taken from the incubation setter were placed on a light source to determine the inside solid mass and transferred to the hatcher machine, while the eggs that claimed to be infertile were broken. The broken eggs were evaluated according to the standard chick embryo development stages. The findings demonstrated that before the implementation of necessary corrections, the egg transport vehicle experienced temperature variations beyond the acceptable standard, leading to a deviation from the expected V-shaped trend in the hatchability graph. Based on the findings from egg breakout analysis, there was an observable escalation in the count of rotten and infertile eggs, while a notable increase was recorded in early deaths compared to both mid-term and late-term mortalities. The issue regarding hatchability and fertility dropped due to not adhering to standard temperature guidelines, and fertility and hatchability have increased by nearly 10% upon temperature correction. These results emphasize the critical importance of consistent and appropriate temperature management in optimizing hatchability outcomes in egg storage environments, particularly during transportation.

**Keywords:** Broiler Production, Egg Storage, Fertile Eggs, Fertility, Hatchability,

# Factors that Affect Lice Infestation in Commercial Layer Chicken and Village Chicken in a Selected Area of Dummalasooriya Veterinary Range

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Poultry are a main component of the animal production industry in Sri Lanka. Layer chickens are reared commercially for eggs in high-density, large-scale operations where birds are held in cages. Village chickens are free-ranging, and this low-input management system is present among rural and semi-urban communities in Sri Lanka. Backyard poultry are frequently exposed to and harbour many parasites, including lice. We studied the effect of management system (commercial vs. backyard) and host factors (colour, age, body condition score) on the diversity and intensity of lice infestation. Six birds from each of the five commercial and backyard poultry farms in a selected area of the Dummalasooriya Veterinary Range were sampled (n=30 for each management type). We applied 1% propoxur powder on the body of the chickens and lice obtained were enumerated by species. Seven species of lice, namely, *Menopon gallinae*, *Goniodes dissimilis*, *Goniocotes gallinae*, *Lipeurus tropicalis*, *Lipeurus caponis*, *Oxylipeurus dentatus* and *Menacanthus* spp. were present. Only *O. dentatus* was limited to village chicken. Species diversity indices (Margalef and Shannon diversity) were slightly higher for lice in village chicken when compared with commercial chicken. The Shannon evenness index was similar for lice in chicken kept under both management types. A generalised linear model showed that the management system ($p = 0.0007$), colour of the plumage ($P < 0.0001$) and the interaction between these two factors ($p = 0.02$) significantly affected the intensity of lice infestation. Further, older birds ($p = 0.007$) and those with poor body condition ($p < 0.0001$) had significantly higher numbers of lice. This study provides evidence that both the diversity and intensity of lice infestation in chicken is affected by the management system as well by host-related factors.

**Keywords:** Backyard Poultry, Commercial Layers, Diversity Indices, Ectoparasites, Lice, Sri Lanka.

# Survey on Haemoparasites in Commercial Layer and Backyard Chicken in Dummalasooriya Veterinary Division

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Poultry sector is considered a fast-growing, well-organized animal production subsector in Sri Lanka. Kurunegala district is a leading district where most of the poultry farms are established and which provides a considerable contribution to the national requirement of meat and eggs. Poultry are usually reared in Sri Lanka under two management systems; intensive (commercial layers and broilers) and extensive (backyard poultry). Parasitic diseases may have negative impacts on the local poultry industry as they might cause production losses. The objective of this study is to examine haemoparasitic conditions among the commercial layers and backyard chicken and to assess the awareness of the farmers about leucocytozoonosis, one of the haemoparasitic conditions that most frequently recurs in Dummalasooriya veterinary division in the Kurunegala District. The study was carried out by examining Leishman's stained thin blood smears obtained from combs of 10 birds from five commercial and five backyard poultry farms. A questionnaire survey was also conducted among both backyard and commercial layer farmers to collect data on leucocytozoonosis. Results revealed that 40% (40/100) of the birds were positive for microfilaria and 60% (60/100) of the birds were absent for microfilaria and other haemoparasitic diseases. There were two types of microfilaria, of which one type was sheathed and the other was unsheathed. Of the 40 microfilaria positive birds, 27 had unsheathed type, 2 had sheathed type and 11 had both types of microfilariae. The prevalence of microfilaria varied according to different risk factors such as the presence or absence of marshy lands and waterlogged areas in the vicinity of the farm, body colour, body condition, age and type or strain of the bird. In Dummalasooriya area, the awareness of leucocytozoonosis was 100% among commercial layer farmers while it was 10% among the backyard poultry farmers. The awareness of clinical signs, control methods and other factors related to leucocytozoonosis varied among farmers. The results implied that there is a knowledge gap between commercial layer and backyard poultry farmers about the haemoparasitic disease conditions. Further investigation with more samples representing all seasons of Sri Lanka coupled with more sensitive diagnostic techniques would provide better understanding about haemoparasitic infections among poultry in Sri Lanka.

**Keywords:** Backyard Poultry, Commercial layers, Dummalasooriya, Hemoparasites, Sri Lanka

# Prevalence of Haemoparasitism in Backyard Chickens in Selected Veterinary Ranges in the Kurunegala District

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Backyard poultry plays an important role in the economies and malnutrition management of low-income families. Backyard poultry producers face numerous challenges and among them, the significant losses they face due to infectious diseases is prominent. In backyard chickens, parasitism is unavoidable, and it can have a mild but long-term impact on production because haemoparasitic diseases in backyard poultry have received little attention in Sri Lanka. Therefore, the aim of this study was to determine the prevalence of haemoparasites in healthy backyard chickens in selected veterinary ranges in the Kurunegala district and to morphologically characterize the identified hemoparasites. A total of 50 blood samples were collected from 25 backyard poultry flocks located in the veterinary ranges of Wariyapola, Kurunegala, and Ridigama. To visualize heamoparasites, thick and thin blood films were prepared from these samples and stained with Leishmann. The morphological key developed from previous literature was used to identify hemoparasites in the samples. The study found an overall heamoparasite prevalence of 18% (9/50) in the study area. Only 2/9 of the birds had mixed haemoparasite infections. Three genera of haemoparasites were identified, with Plasmodium (10%) species being the most common. The prevalence of each Leucocytozoon and Trypanosoma species was estimated to be 6%. Thus, using the stained smear method, haemoparasitism can be detected in healthy backyard chickens in the Kurunegala district, and plasmodium species appear to be the most common haemoparasite. Low sample sizes, convenient sampling, and traditional staining-based pathogen identification may have an impact on determining the true prevalence. To obtain more accurate data, a comprehensive study with increased sample size, randomized sampling, and molecular detection methods are recommended.

**Keywords:** Backyard, Chickens, Haemoparasites, , Kurunegala, Plasmodium

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# Pathological Study of Liver lesions in Dead on Arrival Broiler Chickens

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The death of birds between the catching stage at the farm and unloading from their crates at the processing plant is described as Dead on Arrival (DOA). The death of broiler chickens may happen after loading at the farm, during transportation to the processing plant or during lairage. DOA is an important indicator of animal health, welfare and economic loss; with high DOA, there can be high economic loss to the farmer, so all DOAs are condemned. Therefore, it is important to investigate the causes of death to minimize DOA numbers. Over the period from December 2022 to June 2023, a total of 180 dead on arrival of broilers were subjected to postmortem and sampled in a well-established poultry processing plant in the central province. The birds were sampled on 6 random days. Internal organs were examined, and special attention was paid to any abnormalities in the liver. For histological investigation, samples from abnormal livers were collected in to 10% neutral buffered formalin bottles and histological tissue cassettes. Out of 180 carcasses, 104 showed abnormalities in the liver. Out of these104 cases, 58% had liver congestion, 53% had perihepatitis, 39% had multifocal to coalescing necrotic patches on liver, 23% had liver rupture, 3% had hepatomegaly, and 2% had fatty liver condition. Most birds had more than one abnormality in the liver. Liver congestion and perihepatitis were the most common findings that are mostly associated with transport stress and infectious causes. The current study provided evidence that the main liver lesions found are associated with both infectious and non-infectious causes. However, most lesions represented typical lesions of infectious causes emphasizing the importance of controlling diseases at farm level. A more elaborative research is required to determine the effects of farm health condition on welfare prior to slaughter.

**Keywords:** Dead on Arrival Broilers, Liver Lesions

*Acknowledgements: University Research Grant (URG/2021/43/V)*

# Comparison of Occurrence of Mites, Ticks and Fleas in Commercial and Backyard Layers in Dummalasuriya Veterinary Division

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Poultry sector in Sri Lanka is an essential part of the country's agricultural sector and plays a significant role in boosting the overall economy. Kurunegala district is a leading district where most poultry farms are located. There are two main poultry farming systems in Sri Lanka: commercial and backyard. Ectoparasites can result in production losses in poultry. This study was conducted using five commercial and five backyard poultry farms at Dummalasuriya Veterinary Division in the Kurunegala district in order to compare the occurrence of poultry fleas, ticks and mites in backyard and commercial layer farms, identify the effectiveness of using different types of traps to find red mite infestation and identify the level of awareness among the farmers on red mite infestation. Birds were observed visually for fleas and ticks while four different types of traps (square shaped, rectangular shaped and long & short tube-shaped traps) were placed for three consecutive days to detect red mite infestations and feathers were taken from vent area of birds to detect feather mites. Furthermore, the study was supported by a questionnaire to determine the level of awareness of farmers on red mite infestation. The study revealed that the poultry was infested with ticks, fleas and mites. The majority of the farms (60%) were infested with mites, and only 10% of the farms were infested with ticks and fleas. Feather mites were common in commercial farms (44% prevalence) than in backyard farms (24% prevalence) and the feather mites belonging to the superfamily *Analgoidea* were identified. Even though no red mites were found, some other unidentified organisms were found inside the traps and depending on the number of organisms found, long tube traps were found to be the best type of trap out of the four types of traps used. The questionnaire used for the study revealed that though 60% of the commercial farmers were aware of the red mite infestation, none of the backyard farmers (0%) were aware of it. The results depicted that there is a knowledge gap between commercial and backyard farmers on red mite infestation. In order to confirm the prevalence of red mites, a yearlong study must be carried out as red mite infestation is influenced by climatic changes, and the number of days the traps are kept can be increased up to seven in order to increase the chance of capturing red mites.

**Keywords:** Dummalasuriya Veterinary Division, Fleas, Mites, Mite-traps, Ticks

# Public Health, Food and Animal Feed Security Session-I

# Characterization of Commensal Bacteria Isolated from the Preputial Cavity of Boars and Determination of Their Antimicrobial Susceptibility

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Characterization of normal microflora in preputial cavity of boars may lead to better comprehensive understanding of swine physiology and reproductive health. The colonization of pathogenic bacterial organisms causes ascending urinary infections in boars. *Actinobaculum suis, Escherichia coli, Staphylococcus* spp. are the most common pathogenic organisms present in preputial microflora. *Actinobaculum suis* is the primary pathogen for porcine cystitis-pyelonephritis in pigs. Farmers involved in the swine industry tend to use antibiotics without prescriptions and such malpractices lead to the development of antimicrobial resistance. Moreover, developing South Asian countries like Sri Lanka lacks regulations on antimicrobial drug usage in animal farms. Therefore, swine industry in Sri Lanka can be considered as a potential source for AMR (Anti-Microbial Resistance) occurrence and spread. The main purpose of this study is to identify preputial microflora and determine its antimicrobial susceptibility. Accordingly, samples were collected from 22 boars regardless of their age, breed, and geographical location. The preputial washes were cultured on blood agar and MacConkey agar. Colony morphology and biochemical tests were used to tentatively identify bacterial organisms. Several bacterial organisms such as *E. coli*, *Bacillus spp, Staphylococcus spp, Pseudomonas spp, Citrobactor spp, Corynebacterium spp, Proteus spp,* and *Klebsiella spp* were isolated. A number of ten isolates from five different organisms were subjected to the Kirby Bauer disc diffusion test according to CLSI guidelines. Finally, our results revealed that bacterial isolates of Enterobacteriaceae group are the most common bacterial organism in preputial cavity of boars. According to the antimicrobial susceptibility test, there is a significant trend in the development of resistance to antibiotics such as Ampicillin, Co-amoxiclav and Oxytetracycline. Further, one isolate of *Proteus* spp. showed multidrug resistance while *Pseudomonas* and *Klebsiella* showed resistance against two antimicrobial drug classes indicating its severity. Therefore, this study justifies the need to continue future research to reveal prevailing bacterial species and their AMR mechanisms.

**Keywords:** Antibiotics, Control, Microflora, Resistance, Swine

# Characterization and Determination of Antimicrobial Susceptibility Profile of Gram-Positive Cocci Isolated from Wounds and Ear Infections of Cats and Dogs in Kandy

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Wounds and ear infections are common clinical presentations in small animal practice. Currently, antimicrobial drugs are frequently used for the management of wounds and ear infections in dogs and cats. With the rising usage of antimicrobial drugs, antimicrobial resistance (AMR) is emerging and has become one of the major public health concerns. Previous studies have shown Gram positive cocci as the most abundant bacteria in wounds and ear infections in cats and dogs. Therefore, it is essential to examine the current pattern of AMR towards Gram positive cocci, especially for *Staphylococcus* species isolated from wounds and ear infections. Also, people in contact with dogs and cats have a higher risk of developing AMR. So, the aim of our study is characterization and determination of AMR profiles of Gram-positive cocci in wounds and ear infections of cats and dogs. A total of thirty clinical samples were obtained from wounds and infected ears from twenty-three dogs and seven cats presented to the Veterinary Hospital, Gatambe, Peradeniya for the isolation of Gram-positive cocci bacteria. Samples were cultured on sheep blood agar and Gram stain was done for all the isolated colonies for the identification and the differentiation of the Gram-positive cocci. Twenty-six bacterial colonies were identified as Gram positive cocci. Among them, one *Streptococcus* isolate, and 25 *Staphylococci* isolates were detected by biochemical tests. Identified *Staphylococcus* species were subjected to Kirby Bauer disc diffusion test according CLSI guidelines to see their AMR profiles for ciprofloxacin, cefuroxime, amoxicillin, doxycycline and neomycin. Accordingly, all the isolated samples from dogs showed 72.22% resistance for amoxicillin, 22.22% resistance to cefuroxime, 11.11% resistance to ciprofloxacin, 11.11% resistance to doxycycline and 5.55% for neomycin. In cats, highest resistance was shown to amoxicillin (42.85%). In contrast, resistance to cefuroxime, doxycycline and neomycin were 28.5%, 14.28% and 14.28% respectively. No resistance was observed towards ciprofloxacin (0%) in cats. Our findings show a significant trend in the development of resistance to commonly used antibiotics such as amoxicillin and cefuroxime and a significantly high intermediate resistance to neomycin among the isolates from cats. Therefore, the study indicates the importance of implementing strict regulations on antimicrobial usage and prescription in the veterinary field and continuation of further studies on mechanisms of antibiotic resistance.

**Key words:** Antimicrobial resistance, Cats, Dogs, Ear infections, Gram positive cocci, Wounds

# Antibacterial Efficacy of Selected Hand Sanitizer Products Available in the Local Market in Kandy, Sri Lanka

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Commercial hand sanitizers are a convenient and affordable option for hand hygiene. This study aimed to evaluate the antibacterial efficacy of selected locally available hand sanitizers. Seventeen products were characterized by sterility testing and *in vitro* kill curve assay using *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27953. Approximately $1.5x10^8$ CFU/ml bacterial suspensions were mixed with equal volumes of sanitizers and viability was assessed for 30 minutes. The reduction of culturable bacterial load on hands of 10 volunteers who were trained on hand hygiene using sanitizers were assessed using thumbprint method. According to label information, 11 products contained isopropyl alcohol (IPA) 75%-80% v/v, one product contained 1% IPA with 20% "herbal extracts" and another product contained undisclosed quantity of IPA. Two products contained ethyl alcohol (ETA) 75% v/v, one product was labeled as "contains IPA and ETA 75% v/v". The remaining product was labeled as "contains alcohol". Three products did not have registration from the National Medicines Regulatory Authority (NMRA). All but the product containing 1% IPA with 20% "herbal extracts" was sterile. This product was contaminated with *Pseudomonas* spp. ($7.1x10^4$ CFU/ml), and therefore was excluded from the kill curve and thumbprint experiments. It was not registered with NMRA but labeled as produced by "ISO 9001-2015 certified company". In the kill curve assays, 10 out of 16 products completely inactivated *E. coli* immediately, while 4 products needed 1 minute, and one product needed 5 minutes exposure to achieve complete inactivation. Thirteen products completely inactivated *S. aureus* immediately, while one product needed 1 minute, and one product needed 5 minutes exposure to achieve complete inactivation. Fifteen products completely inactivated *P. aeruginosa* immediately. The remaining product labeled "containing 75% v/v ETA and kills 99.9% germs" failed to inactivate all three bacteria tested, even after 30 minutes exposure. This product was not registered with NMRA. In the thumbprint method, only 13 out of 16 products reduced at least 50% of culturable bacteria on the hands (range 60% - 90%) even though the label claims of nine products indicating "kills 99.9% germs". The lowest performing product (~10% efficacy) was the product that failed the kill curve assay. The other two low performing products included the product containing undisclosed quantity of IPA and the product labeled as "contains IPA and ETA 75% v/v". The findings highlight the need for stringent regulation of the quality of the hand sanitizers in the local market.

**Keywords:** Antibacterial Efficacy, Hand Hygiene, Hand Sanitizers, Kill Curve Assay, Thumbprint Method

# Phenotypic and Genotypic Characterization of Carbapenems Resistant *E. coli* isolated from Clinical Samples of Human and Companion Animals in Sri Lanka

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*Escherichia coli* (*E. coli*) is a common bacterium causing urinary tract infections in humans and animals. The high prevalence of extended-spectrum beta-lactamase (ESBL) among urinary pathogenic *E. coli* (UPEC) is well-documented and such infections are often treated with carbapenems. The emergence of carbapenem resistance in *E. coli* poses a significant public health concern as most of them are multidrug resistant. However, epidemiological and molecular genetic data on carbapenem-resistant *E. coli* is limited in Sri Lanka. This study aimed to determine the production of ESBLs, carbapenemases, and metallo-beta-lactamases (MBLs) in a collection of carbapenem-resistant *E. coli* isolated from both humans (n=21) and dogs (n=4). The presence of plasmid-mediated carbapenem and quinolone resistance genes, namely *blaKPC, qnrA, qnrB,* and *qnrS*, in the isolates were assessed by PCR. Out of the 25 isolates tested, 23 were resistant to ertapenem, and 12 were resistant to both imipenem and meropenem. Nine isolates exhibited resistance to all three carbapenems, while co-resistance to two carbapenems was detected in four isolates. The isolates from dogs (n=4) were only resistant to ertapenem. Only 4/25 carbapenem-resistant *E. coli* isolates tested positive for ESBL production using the double disk synergy test. The Modified Hodge test identified 12 (48%) *E. coli* isolates as carbapenemase producers, while the combined disk test detected MBL production in 12 isolates. This highlights the presence of carbapenem resistance mediated by MBLs in a substantial portion of the isolates. Carbapenem resistance in *E. coli* is largely attributed to MBLs, and the high occurrence of this resistance is a major concern in the South Asian region. The *qnrB* gene was detected in a significant proportion (76%) of the carbapenem-resistant isolates, while *qnrS* was found in only one isolate, and *qnrA* was absent in all isolates. Correlation analysis revealed a strong positive correlation between the presence of *qnrB* genes and ertapenem resistance. Presence of MBL producing carbapenems resistant *E. coli* with MDR poses significant public health concerns. The findings highlight the need for surveillance, molecular characterization and the development of targeted control strategies to mitigate carbapenem resistance.

**Keywords:** Carbapenems Resistant *Escherichia coli,* Combined Disc Test, Extended Spectrum Beta Lactamases, Metallo-Beta-Lactamases, Modified Hodge Test

# Evaluation of Antibiotic Resistance Among *Escherichia coli* Isolated from Different Levels at Mahaweli River in Central Province: Sri Lanka

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Antimicrobial resistance is a growing public health threat. The goal of the current study was to assess the antimicrobial resistance in the bacterial population in selected locations of the *Mahaweli*, the largest river in Sri Lanka using *E. coli* as an indicator organism as a part of an ongoing project conducted at the China-Sri Lanka Joint Research and Development Centre, University of Peradeniya. Water samples were collected from the inlets of 13 drinking water treatment plants in the Central province. *E. coli* was isolated using filtration method using xx filters according to manufacturer's instructions. Altogether, 28 isolates were analyzed for antibiotic resistance using disk diffusion method according to CLSI 2020 standards. Nine antibiotics representing seven different classes including amoxicillin, amoxicillin-clavulanate, ceftazidime, ceftriaxone, ciprofloxacin, chloramphenicol, sulfonamide, streptomycin and tetracycline were used. The highest resistance was observed for penicillins. Amoxicillin resistance was 68% (n=19) followed by amoxicillin-clavulanate 60% (n=17). Penicillin resistance was followed by tetracycline (n=7), quinolones, cotrimazole and chloramphenicol equally (n=5), 3rd generation cephalosporins (ceftazidime and ceftriaxone) and streptomycin equally (n=4). Altogether, 43% of the isolates were resistant to at least one class of antibiotics. Though preliminary, these resistance profiles match the overall antibiotic usage pattern of the country. Penicillins, quinolones and tetracyclines are the most used antibiotics in both human medicine and animal husbandry in Sri Lanka and AMR to these drugs is expected to be high in Sri Lanka. Isolates from six sampling sites were only resistant to penicillin while all three isolates from one sampling site and one isolate each from two different inlets did not show resistance to any of the antibiotics tested. Eight isolates out of 28 (29%) showed multi drug resistance (MDR). Most MDR isolates [88% (7/8)] were resistant to penicillin and tetracycline. Additionally, three isolates showed resistance for two drug classes.

**Keywords:** Antimicrobial Resistance, Multidrug Resistance, Mahaweli River, Sri Lanka

*Acknowledgements: University Research Council, University of Peradeniya (Multidisciplinary Research Grant No280:2022)*

# Assessing the Inhibitory Potential of N-Acetylcysteine on Biofilm Formation by *Escherichia coli* Isolated from Urinary Tract Infections of Humans and Dogs

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*Escherichia coli* (*E. coli*) is the most common organism responsible for urinary tract infections (UTIs) in both humans and animals. Certain strains of uropathogenic *E. coli* (UPEC) have the capability to form biofilms, leading to persistent infections and increased resistance to antimicrobial agents. Therefore, the formation of biofilms by UPEC poses a significant challenge to healthcare professionals. Recent studies have shown the potential of N-acetylcysteine (NAC) as an antibiofilm agent against certain bacterial pathogens. This study evaluated the inhibitory potential of NAC on biofilm formation by UPEC. A total of 47 *E. coli* isolates were selected, including 33 from humans and 14 from dogs, from a previous study. Additionally, *E. coli* ATCC25922 was included as a positive control. Two-fold serial dilutions of NAC were prepared in trypticase soy broth (TSB), ranging from 2 mg/ml to 0.015625 mg/ml. Bacterial suspensions (10 µl) were then inoculated into 90 µl of TSB broth, either without NAC or with varying concentrations of NAC, in microtiter plates. The biofilm-forming ability of the organisms and the inhibitory effect of different NAC concentrations on biofilm formation were assessed using the crystal violet assay. When evaluating the biofilm-forming ability of the isolates, four were categorized as weak biofilm producers, 34 as moderate biofilm producers, and nine as strong biofilm producers. The results indicated that NAC reduced UPEC biofilm formation in a dose-dependent manner. Notably, when treated with 0.5 mg/ml of NAC, over 50% of the isolates showed a 30-60% reduction in biofilm formation, consistent with findings in other studies. Moreover, higher concentrations of NAC exhibited the potential for even greater reductions in biofilm formation. However, most isolates still displayed a 30-40% decrease in biofilm formation across different NAC concentrations. In conclusion, this study highlights the inhibitory effect of higher concentrations of NAC on the formation of biofilms by UPEC. Nevertheless, further research is needed to elucidate the underlying mechanisms of NAC's action against bacterial biofilms.

**Keywords:** Biofilms, N-Acetylcysteine, UPEC

# A Preliminary Study on Levels of Cadmium (Cd) and Lead (Pb) in Locally Grown Cocoa Beans from Upcountry Areas of Sri Lanka

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Cocoa *Theobroma cacao,* cultivated in Sri Lanka, was introduced by European colonial rulers in the 1800s. Cocoa nibs have a great potential for carrying a possible risk in heavy metal accumulation, especially Cadmium (Cd) and Lead (Pb). The higher bioavailability of Cd in soils has caused a higher accumulation of Cd in cocoa beans. Thus, the current study was conducted to analyze the levels of Cd and Pb in locally grown cocoa beans collected from three upcountry locations in Sri Lanka where cocoa trees are found in home gardens. The samples were collected as pooled samples of dry or fresh beans from those areas namely Kothmale, Gelioya and Kundasale. The processing of samples was performed according to standard procedures. The concentration of Cd and Pb were measured using atomic absorption spectrophotometry. The results revealed that the levels of the Cd and Pb in locally grown cocoa beans range from 0.048-0.81 ppm and 0.097-0.175 ppm respectively. Thus, those are within or very close to safe limits for human consumption according to the regulations imposed by international regulatory bodies. The spectrophotometric analysis showed that the concentrations of Cd in the cocoa mass obtained from cocoa beans were lower than the maximum limit of 0.8 ppm. In terms of Lead, the reported values were closer to the upper limits of 0.1 ppm. These ranges indicate that the cocoa grown in upcountry, Sri Lanka has a great potential to be exported into the international markets as an ingredient in the chocolate industry or to be used in the animal feed industry. However, further studies are highly recommended to be performed by covering more upcountry locations and increasing the sample size.

**Keywords:** Cadmium, Cocoa beans, Heavy Metal, Lead, Spectrophotometer

# Public Health, Food and Animal Feed Security Session-II

# Antioxidant Capacity of Different Extracts of *Osmium tenuiflorum*

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*Ocimum tenuiflorum*, commonly known as Holy Basil, Krishna tulsi or Maduru tala is a well-known medicinal plant widely used in traditional Ayurvedic medicine. It has gained significant attention due to its potential health benefits, including antioxidant activity. Antioxidants play a significant role in neutralizing the effects of free radicals and are known to be effective in preventing free radical formation. This study was carried out to investigate the antioxidant capacity of different extracts of leaves *Ocimum tenuiflorum* using DPPH assay with vitamin C as a standard. Plants were collected from the Mallavi area of Mullaitivu district in Northern Province and authenticated at the National Herbarium, Department of National Botanical Garden, Peradeniya. Leaves were washed, air dried, and extracted to five different solvents Ethanol, 50% Ethanol, and water at 27ºC, 60ºC, and 100ºC. The antioxidant activity of each sample was expressed in terms of $IC_{50}$. The highest antioxidant activity was obtained from the boiled water extract with the $IC_{50}$ of 0.58 mg/ml. The $IC_{50}$ of other extracts was 0.84 mg/ml in 50% ethanol extract, 1.41 mg/ml in water at 60ºC, 1.89 mg/ml in water extract at 27ºC, and 1.98 mg/ml from 100 % ethanol extract. The study revealed that the boiled water extract of *Ocimum tenuiflorum* leaves exhibited the strongest antioxidant activity, while the ethanol extract demonstrated the lowest antioxidant activity among the five extracts investigated. Hot water allowed the beneficial compounds from leaves to be extracted resulting in a flavoured beverage with potential health benefits. This study has sparked an interest in utilizing leaves for making tea.

**Keywords:** Antioxidant Capacity, DPPH Assay, *Ocimum tenuiflorum*

# Optimization of Gamma-Aminobutyric Acid Production by *Lactobacillus fermentum* Strain 133

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Gamma-aminobutyric acid (GABA) is a four-carbon non-protein amino acid that has several physiological roles due to its function as an inhibitory neurotransmitter in the central nervous system. GABA plays a crucial role in relieving depression and restlessness, reducing blood pressure and suppressing cancer cell proliferation, etc. The production of GABA naturally by lactic acid bacteria during fermentation can have certain potential benefits compared to using commercial GABA supplements. The objective of the current study was to investigate the optimum bacterial culture conditions for GABA production in the strain *Lactobacillus fermentum* 133. The potential for GABA synthesis in MRS broth culture medium was evaluated using a spectrophotometric method. To increase the rate of GABA production by the *Lactobacillus fermentum*, three key variables, pH (4.5 to 6.5), temperature (32 to 42°C), and monosodium glutamate concentration (43 to 63 mmol/$dm^3$) were optimised by Response Surface Method (RSM) following Central Composite Design (CCD). The highest amount of GABA obtained was 197.7 µg/ml. The optimal conditions for GABA production by *Lactobacillus fermentum* were a temperature of 34.76 °C, a pH of 5.14, and 47.69 mmol of monosodium glutamate, at a 48 hour incubation period. This strain with the optimal culture conditions determined in this study could be used for the biotechnological production of GABA or applied in food fermentation for the development of naturally GABA-enriched foods.

**Keywords:** Gamma-aminobutyric acid, *Lactobacillus fermentum*, Monosodium glutamate ,Optimization, Response Surface Method

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# A Preliminary Study on the Effect of Fermentation and Parboiling on the Nutritional Value of *Setaria italica* (Foxtail millet)

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Foxtail millet (*Setaria italica* (L.) Beauv.) (Thana haal) is one of the earliest cultivated millets in Sri Lanka. The people of the northern part of ancient Sri Lanka consumed large amounts of this millet, but the demand for this millet began to decline as they shifted to rice and wheat. The goal of the current study was to investigate the nutritional value of raw, parboiled, and fermented foxtail millet. The powdered form of raw and parboiled flour of foxtail millet was fermented with 1% yeast and 10% curd and the nutritional value was compared with the control. Only one sample from each of the treatments was used as the preliminary analysis. Proximate analysis, Antioxidant activity by DPPH assay, total polyphenol content by Folin Ciocaltue method, and Gama-amino butyric acid (GABA) levels were monitored. The crude protein levels were in the range of 10.72% to 15.32%. High crude protein was observed in the samples fermented with curd and yeast. The protein content increased from 11.56% (raw) to 14.22 % (fermented with yeast) and 11.56 % (raw) to 14.5 % (fermented with curd). The protein content of the parboiled sample increased from 10.8% (parboiled) to 14.72 % (fermented with yeast) and from 10.8 % to 15. 32% (fermented with curd). The GABA concentration varied from 162.03 $\mu$g/ml to 305 $\mu$g/ml. Fermentation increased the GABA levels in both raw and parboiled millet samples. Further, fermentation did not affect the levels of polyphenols and the antioxidant capacity of raw and parboiled millet samples. Fermentation and parboil treatment may upgrade the dietary benefits and effectiveness of this underutilized millet which tends to be efficiently utilized for the development of new functional food products.

**Keywords:** Crude protein, Fermentation, Parboiling, *Setaria italica,*

# Characterization and Determination of Antimicrobial Susceptibility in the *Staphylococcus* Species Isolated from Caprine Mastitis Milk from Kandy District

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Caprine mastitis is one of the most common diseases among caprine dairy herds widespread throughout the world. It causes huge economic losses due to the reduction of milk yield, changes of milk quality and culling of affected non-responsive animals. *Staphylococcus* species are the most common pathogen causing mastitis in goats. They can cause severe economic losses through the induction of severe diseases and are unresponsive to treatment and carry multidrug resistance. Therefore, the current study aims to isolate *Staphylococcus* species from caprine mastitis milk and determine their antimicrobial susceptibility. Thus, a total of 35 samples were collected from goats with clinical and subclinical mastitis cases presented to the Large Animal Hospital, Department of Farm Animal Production and Health, Faculty of Veterinary Medicine and Animal Science. The mastitic milk samples were cultured on sheep blood agar using the quadrant streaking method. Presumptive identification of the isolates was carried out with Gram stain and biochemical tests such as catalase, oxidase and coagulase test. The Kirby Bauer disk diffusion method was used to test antibiotic susceptibility of all the *Staphylococcus* isolates against five antibiotics that are commonly used in small ruminant practice according to the clinical and laboratory standards institute (CLIS) guidelines. Accordingly, 67% of isolates were Gram positive cocci, while other bacteria were Gram negatives and Gram-positive rods respectively. Interestingly 70% Gram positive cocci were *Staphylococci*. Further, AST results showed that, 78.81% of the isolates were resistant to Amoxicillin/clavulanic acid while 57.4% were resistant to Amoxicillin and 35.71% were resistant to Oxytetracycline. Neomycin and Enrofloxacin showed the least resistance with 14.28% and 7.4% respectively. Out of fourteen isolated *Staphylococcus* samples, two showed multi-drug resistance. Our study shows the existence of MDR *Staphylococci* among does with mastitis. Further, it shows a significant trend in the development of resistance to current antibiotics such as Oxytetracycline, Neomycin and Enrofloxacin. Therefore, this study warrants the necessity in continuation of further research to identify the mechanism of resistance, to identify different staphylococcal species that show antimicrobial resistance and MDR and and strictly implement controlling programs to prevent irresponsible antibiotic using.

**Keywords:** Antibiotics, Caprine, Control, Mastitis, Resistance

# Veterinary Education and Extension
# Session – I

# A Comparative Analysis of Emotional Intelligence (EI) and Intelligence Quotient (IQ) on the Academic Performance of Veterinary Undergraduates in Sri Lanka

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Emotional Intelligence (EI) and Intelligence Quotient (IQ) are closely intertwined with the Academic Performance (AP) of university students. This study investigated the intricate relationship between EI and IQ toward academic performance among undergraduate veterinary students of the Faculty of Veterinary Medicine and Animal Science (FVMAS), University of Peradeniya, Sri Lanka, aiming to discern the most influential intelligence factor. Robust quantitative research methods were employed to gather EI, IQ, and AP metrics data from a selected cohort of 100 veterinary students. Descriptive statistics, ANOVA, Pearson's product-moment correlation, and multiple linear regressions were employed to analyse data. Strong positive correlations were found between academic performance and both emotional intelligence ($r = 0.687$, $p < 0.01$) and intelligence quotient ($r = 0.667$, $p < 0.01$), where heightened levels of EI and IQ demonstrate a significant association with superior academic performance. Furthermore, the regression analysis confirms the pivotal role of both EI and IQ in predicting AP. The above results showed that the intelligence quotient and emotional intelligence have unstandardized coefficients of .009 and .148, respectively. Based on the obtained results, the regression equation ($Y = 3.738 + (0.009)\ X1 + (0.148)\ X2$) was derived to predict academic performance (Y) from IQ (X1) and EI (X2). These outcomes underscore the paramount importance of addressing both cognitive and non-cognitive proficiencies in veterinary education. By seamlessly integrating comprehensive emotional intelligence training within the curriculum, FVMAS can effectively enrich students' communicative acumen, foster empathy, and cultivate adeptness in building meaningful relationships. Such educational initiatives are poised to holistically prepare veterinary students for success in their future professional pursuits.

**Keywords:** Academic, Emotional, Intelligence, Performance, Quotient

# A Questionnaire Based Survey on Impact of Economic Downturn on Livestock Farmers in Nanattan Divisional Secretariat Division of Mannar District

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The recent economic downturn negatively impacts all ventures of Sri Lanka including the livestock sector. An economic crisis could have impacts on all inputs, outputs and supply chain of farm operation. While Sri Lanka is reeling through an economic crisis, a pre-tested questionnaire was administered to document the constraints faced and the remedies taken by 70 livestock farmers from Nanattan Divisional Secretariat of the Mannar District. This study showed that the expenditures related to the feed, veterinary drugs, electricity, water and the total cost per month significantly increased after the economic crisis ($P < 0.05$). However, the amount of milk production and profit per month had not changed ($P > 0.05$), despite the market price for animal products significantly ($P < 0.05$) increasing after the crisis. Forty-five farmers (64.2%) reduced or abandoned the total number of livestock in the farms after crisis. The commonest constraint faced by all farmers was decreased consumer's purchasing power and difficulty in distributing farm products. A total of 68 farmers (97.14%) opted for self-medication or traditional healing methods instead of seeking veterinary services. Nevertheless, 69 out of 70 farmers followed proper deworming practices. Difficulty in obtaining adequate feed at the appropriate time was experienced by 68 farmers (97.14%). About 36 farmers (51.4%) stated that they had shifted to alternative feeding sources like cut and fed type (48.57%) and kitchen waste (2.85%) after the crisis while the rest still followed (48.57%) conventional feeding. In conclusion, farmers in the study area are affected by the current economic downturn and they need economical support to boost the productivity and profitability of their farming operations. This study highlights the importance of declaring the livestock related activities as an essential service in Sri Lanka in order to get economical support from the Government. Awareness must be created among consumers about malnutrition, and they should be encouraged to consume animal products. In this context, veterinarians, public health officers, researchers and field extension officers must work together under the umbrella of the one health concept to overcome malnutrition among public at this time of the economic downturn.

**Keywords:** Constraints, Economic downturn, Livestock farmers, Mannar district, Questionnaire survey

# Knowledge, Skills and Attitudes of Final Year Veterinary Undergraduates towards Neutering of Dogs

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Neutering: ovariohysterectomy or castration, is a common surgery performed on dogs. Therefore, this study was conducted to evaluate the knowledge, skills and attitudes towards neutering of dogs among the final year veterinary undergraduates in Sri Lanka. An online questionnaire survey was conducted with the participation of final year veterinary undergraduates of the University of Peradeniya in June 2023. The response rate was 87.5% (70/80). All respondents stated that they have received the education on neutering dogs during their study program. The majority stated that they are familiar with the process and techniques involved in neutering surgeries. Nearly 87% (95% CI; 77.6-98.6%) is interested in performing neutering surgeries in the future. Further, there is an association between the gender and the interest towards performing neutering. Female students are significantly interested ($\chi^2 = 16.44$; df = 1; $p < 0.05$) compared to male students. Eighty percent (95% CI: 67.3-92.7%) of students are confident in discussing the benefits and risks of neutering dogs with pet owners. Population control, reducing the incidence of certain tumours and reducing aggressiveness were the commonly identified benefits whereas major risks identified were pain and ethical considerations. In this study, 95.7% stated that they are familiar with the process of neutering surgery in general. However, 40% (95% CI; 28.2-53.1%) had not got the opportunity to receive hands-on experience in performing neutering surgeries. This may be due to the low number of surgical appointments during the economic crisis which should be further investigated. More than 90% agreed that it would be beneficial to have additional training to enhance knowledge and skills related to neutering dogs. This study shows that veterinary undergraduates are well aware of the importance of neutering. However, to improve practical competence there is a great requirement to take necessary measures to improve the hands-on exposure in neutering dogs during the Veterinary undergraduate degree programme.

**Keywords:** Attitudes, Dog, Neutering, Skills, Veterinary Undergraduates

# Evaluation of the Effectiveness of the 2020 Curriculum in Developing Knowledge Related to Communication Skills in Veterinary Degree Program at University of Peradeniya

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Client communication (CC) is an essential competence of veterinarians. Veterinary curricula play a significant role in developing this competence. The BVSc. curriculum of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya was recently revised and 2018/19 batch was the 1st cohort of the new 2020-BVSc curriculum. At present, 2017/18 batch, the immediate senior batch to the first cohort, is following the old 2000-BVSc curriculum. A Google form-based survey was conducted to evaluate the effectiveness of the new curriculum in developing knowledge related to CC skills. Batch 2017/18 was considered as a comparison group. Response rates of 2017/18 batch and 2018/19 were 79.1% (57/72) and 53.9% (48/89) respectively. Majority of the 2018/19 batch agreed that formal learning on CC including the Calgary-Cambridge Guide was available within their curriculum (Mean: 97.9%; 95% CI: 88.9- 98.9 %;) whereas that of the batch 2017/18 was comparatively low (Mean: 40.4%; 95% CI: 27.6-54.4%). According to students' view, Mann-Whitney U test showed that their level of knowledge in identifying clients' non-verbal clues, techniques in history taking, communication during decision making and identifying angry clients were higher in the 2018/19 batch than the senior batch ($p<0.05$). More than 90% of students of the 2018/19 batch and 79.05% of students of 2017/18 batch stated that there is a difference between empathy and sympathy. However, the ability of 2018/19 batch to correctly identify empathy and sympathy was higher than the other batch ($p<0.001$). Both groups' level of confidence in communicating diagnostic plans, treatment options and explaining discharge instructions was relatively poor (<55%). However, the confidence in getting accurate history, handling emotional clients, reading client's body language and explaining euthanasia is higher in batch 2018/19 ($p<0.05$). In conclusion, in the students' opinion, the 2020-BVSc. curriculum has effectively contributed to improve knowledge related to CC skills. Although the 2017/18 batch has not completed the clinical year, they suggested in the survey, that "teaching CC from the beginning of the study programme" would further improve their knowledge in CC whereas the suggestion made by the batch 2018/19 was the need to get "exposed to clinics from the beginning" of the curriculum.

**Keywords:** Client communication, Curriculum, Evaluation, Veterinary Degree

# A Study of Students' Perception of Effectiveness of Mentoring Programs for Veterinary Undergraduates

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This research paper explores the students' perception of the effectiveness of mentoring programs for veterinary undergraduates. The aim of the study was to assess the students' awareness, experience and the benefits of students' academic and professional development within the field of veterinary medicine and to identify weaknesses of the existing mentoring program. A questionnaire survey was developed using google forms to gather comprehensive insights regarding the faculty mentoring program. The questionnaire consisted of 6 sections and a number of questions under each and every section. The questionnaire was distributed among 180 students in the faculty and 106 of them responded to the questionnaire. Thus, the percentage of respondents was 59%. The findings revealed that the mentoring programs play a crucial role in enhancing students' educational experience, providing valuable guidance, support, and career advice. Participants reported improved academic performance, increased self-confidence, and a better understanding of professional expectations in the veterinary field. The qualitative analysis highlighted the significance of mentor-mentee relationships, emphasizing the importance of effective communication, mutual trust, and shared experiences. However, certain challenges and limitations within the mentoring programs were identified, including limited resources and varying levels of mentor commitment. These findings contribute to the growing body of knowledge on mentoring programs in veterinary education and provide practical implications for the development and improvement of such programs. The research underscores the need for ongoing evaluation and refinement to optimize the impact of mentoring on veterinary undergraduates' educational journey and future careers.

**Keywords:** Academic development, Guidance, Mentoring, University students, Veterinary students

# Veterinary Education and Extension Session-II

# Preliminary Survey on Antimicrobial Prescription Behaviors of Recently Graduated Veterinarians in Companion Animal Practice in Sri Lanka

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This study aimed to assess the antimicrobial prescription behaviors of recently graduated veterinarians in companion animal practice in Sri Lanka through a web-based questionnaire comprising six case scenarios including acute gastroenteritis, urinary tract infection, acute bacterial respiratory tract infection, acute viral respiratory tract infection, open wound fracture, and superficial pyoderma. The pretested questionnaire was sent to 100 participants and 58 responded. Most veterinarians were from clinics with more than one veterinarian, primarily treating dogs and cats. A considerable number of respondents i.e., out of 26%, and 33%, respectively indicated bacterial culture and ABST are more important than empirical treatment. 41% of respondents indicated that they would prioritize clinical signs over owners' compliance when selecting antibiotics. 60% of veterinarians weighed dogs before performing treatments, while 52% indicated that they weighed cats. Most veterinarians often relied on notes from their veterinary degree program rather than prescription guidelines and, therefore, did not consult current recommendations. In all six case scenarios, the antibiotic choices of the respondents did not agree with the recommendations in published guidelines. There were major deviations for four scenarios. No less than 12.5% of respondents indicated that the 3$^{rd}$ and 4$^{th}$ generation cephalosporins for all case scenarios while these drug classes are not recommended for any of the scenarios given. Similarly, the 1$^{st}$ and 2$^{nd}$ generation cephalosporins were selected by 18% of the participants to treat all case scenarios, despite not always being recommended. For instance, 77.6% of the participants indicated that they would prescribe at least one antimicrobial for uncomplicated acute gastroenteritis, despite no antibiotics being recommended. Similarly, for urinary tract infections, 25% of participants indicated that they would prescribe fluoroquinolones as the first choice in contrary to recommended antibiotics such as amoxicillin or trimethoprim. For acute viral respiratory infection in cats, antibiotics including the 1$^{st}$ and 2$^{nd}$ generation cephalosporins and amoxicillin-clavulanate were often prescribed at 37%, and 30% respectively despite having a secondary bacterial infection. For superficial pyodermas, 3$^{rd}$ and 4$^{th}$ generation cephalosporins were frequently used (12.5%) instead of Clindamycin which is recommended. The findings suggest a potential gap in knowledge and awareness among veterinary practitioners regarding antibiotic prescription guidelines.

**Keywords:** ABST, Acute Gastroenteritis, Acute Bacterial Respiratory Infection, Superficial Pyoderma.
*Acknowledgments: University Research Grant (URG/V/2019-60)*

# Impact of Covid-19 Restriction and the Economic Backlash Due to Political Instability on the Welfare of the Captive Elephants and Hardships Faced by their Owners and Caretakers

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Captive elephants play a major role in showcasing the cultural heritage of Sri Lanka. Elephant population of Sri Lanka was estimated to be 5879 in 2011 and the captive population was 189 in 2003. Maintaining an elephant in captivity is a great responsibility and it demands many resources. The COVID-19 pandemic and the economic backlash which followed due to the political instability effected a major negative impact on the day today life. Even though everyone is aware that there is also a negative impact on the welfare and general status of captive elephants, knowledge on the areas of impact and magnitude was lacking. This study aims to understand the ways and extent of the negative impact of the economic breakdown due to the impact of COVID-19 pandemic and the economic backlash of Sri Lanka on the captive elephant population. Relevant data regarding these aspects were collected through a structured single questionnaire-based surveys directed towards elephant caretakers and owners. Details regarding the status of the elephants and differences in their management before and after the economic crisis were also collected through these questionnaires. The data collection process during the study was challenging as the surveys were carried out during the normal routine of elephant caretakers. Results from the study showed that the economic downfall was felt to lesser degree by the captive elephant population themselves when compared to caretakers and the owners. This is regarding the diet composition, supplement utilization, health management, food availability and the body condition of elephants before and after COVID-19 and the economic downfall that followed. Only 10% of the elephants in the study population were affected negatively except in relation to forage availability, where 20% were negatively affected. It was concluded that caretakers and owners were relatively more negatively affected. The increased cost for feed, difficulty in sourcing transport services, demand decreases and decreases in food availability are some of the challenges faced by about 30% (12/40) of them. Among the caretakers, 60% agreed their quality of life has been reduced, showing the greater impact on the life of people. This is mostly likely attributable to the owners' dedication and consideration that taking good care of the elephants under their care is a huge responsibility. This may lead to the relatively less impact seen on the captive elephants than on the caretakers. Further extensive studies are necessary to find the solutions for the problems faced by the captive elephants and associated people.

**Keywords**: Covid-19, Economic crisis, Elephant welfare, Elephant caretakers

## Survey on Veterinary Students' Attitude and Knowledge towards Animal Euthanasia

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Animal euthanasia is a complex issue in veterinary care when dealing with suffering animals and raises ethical concerns among veterinarians. This study aimed to investigate veterinary students' attitudes and knowledge about animal euthanasia in Sri Lanka. The study involved 378 undergraduate students from all batches of the Faculty of Veterinary Medicine and Animal Science at the University of Peradeniya, who responded to a printed questionnaire on the subject. Data analysis was conducted using 337 valid responses. Gender and academic semester were chosen as predictor variables to assess their associations with students' knowledge and attitude toward animal euthanasia among other factors such as the batch, academic year, age, and religion, using univariable binary logistic regression models. Academic year was omitted due to its strong association with a semester in predictor variable assessment using Cramer's V test for model-building. Age and religion were excluded due to their limited variability among participants. The findings revealed that 62.6% of students were willing to euthanize animals when no other options existed. Gender significantly influenced attitudes on euthanasia, with males 3.3 times more likely to positively consider it than females (OR 3.3, 95% CI 1.8-6.1, $P < 0.001$). Academic progression was also associated with euthanasia attitudes ($P = 0.02$), demonstrating that as students advanced through the semesters (3, 4, 6, and 8), they became more accepting of euthanasia compared to Semester 1 students. Regarding knowledge on euthanasia, 42% viewed it as the best option for relieving animal suffering, while 58% disagreed. Interestingly, gender had no significant association with euthanasia knowledge, suggesting equal educational exposure across genders ($P > 0.05$). Findings of this study offer valuable insights into the attitudes and knowledge of veterinary undergraduates regarding animal euthanasia in Sri Lanka. Results suggest that early integration of euthanasia education into the veterinary undergraduate programme positively influences students' attitudes toward animal euthanasia. Furthermore, it highlights the need for future research to explore the underlying causes of gender-related biases in attitudes towards euthanasia, including cultural, religious, social, and emotional factors, with potential implications for promoting an attitude change through veterinary education in the future.

**Keywords:** Attitudes, Education, Euthanasia, Gender, Veterinary Students

# The Empathy Level Among Veterinary Students in Sri Lanka

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Empathy is a critical attribute for veterinarians, facilitating meaningful connections with clients and fostering trust in delivering optimal care for animal patients. This research investigates the dynamics of empathy development among veterinary students at the University of Peradeniya using a cross sectional study design. The study assesses empathy levels across four academic years to uncover potential shifts in empathetic tendencies and their implications for future veterinary practice. Empathy levels were measured using the Davis Interpersonal Reactivity Index (IRI), which evaluates four dimensions: Perspective Taking, Fantasy, Empathic Concern, and Personal Distress leading to calculation of mean empathetic score (MES). Data were collected from the first, second, third, and final-year students through a comprehensive Google Form survey. Descriptive statistics, Welch Two Sample T-test and ANOVA were employed to analyse data. A total of 197 veterinary undergraduates including 143 female and 54 males responded to the questionnaire. The study found that overall empathy levels among veterinary students at the University of Peradeniya remain high throughout their academic journey. Overall, female students have a high empathy level (MEI = 91.23 ± 7.786) than male students (MES = 88.13 ± 6.171) and the difference in the empathy level between females and males is significant($p>0.05$). This highlights gender-based empathy differences, emphasizing the need for empathy development in males. However, there was no significant difference of MES with respect to academic year or the religion though literature suggests that there is a declining empathy level as a veterinary undergraduate advances in their academic program. Integrating empathetic training into the curriculum could enhance future veterinarians' ability to provide high-quality care and foster better client-veterinarian relationships. This research provides valuable insights into veterinary education and the importance of empathy in shaping compassionate and competent professionals.

**Keywords:** Empathy, Veterinary undergraduates, Sri Lanka

# Evaluation of Veterinary Students Perception on their Day-one Competencies at the Completion of the BVSc Degree of University of Peradeniya

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The successful transition from veterinary education to professional practice requires veterinary students to acquire the necessary competencies to provide high-quality service on day one of their careers. This questionnaire survey was conducted with the aim of evaluating the perception of veterinary undergraduates regarding the achievement of day-one competencies at the level of completion of the final BVSc. examination of the University of Peradeniya. The survey was conducted with the participation of volunteer participants via a Google form in 2023. The response rate was 87.5% (70/80). Most of the respondents are confident in their level of achievement of most of the competencies tested in this survey. However, there were significant disparities among the level of confidence in achieving competencies based on the animal species handled. Friedman and the post hoc Wilcoxon tests showed that confidence in restraining, physical examination, and developing appropriate treatment protocols of dogs, cats, and cattle were significantly higher than that of poultry and pigs ($p<0.003$). Similarly, the confidence in the knowledge of conducting modern reproductive biological approaches was significantly lower in poultry and pigs ($p<0.003$). Only 33% of respondents expressed confidence about their knowledge of legislation relevant to veterinary practice. The majority are unsure about their knowledge of restraining wild, zoo, and aquatic animals. For the statement, "Overall, I am satisfied with achieving Day 1 competencies at the completion of the BVSc degree program", 11.4% of students stated that they strongly agree (CI: 6-22.7%) and 50% of respondents have responded as agree (CI: 37.2-61.4), and 31% (CI: .5-43.1%) have responded as neutral and 7.1% (CI 2.3-15.7) have responded as disagree. In conclusion, this study provides insight for discussion and improvements in specific areas of the curriculum on achieving day-one competencies to enhance the preparedness of day-one graduates to practice as veterinary professionals.

**Keywords:** Day-1 competencies, Undergraduates, Veterinary Medicine

# Wildlife and Aquaculture

# Session-I

# Can Captive Elephants Be Left without Chains and Will They Obey Commands Given with a Blunt Elephant-Friendly Ankush?

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Captive elephant welfare has become a matter of global concern, and the unique cultural role of Sri Lankan captive elephants demands special attention. This study investigates the feasibility and impact of allowing captive adult elephants to roam without chains for two hours daily, accompanied by the use of an elephant-friendly ankush (bullhook) made blunt with rubber knobs. The study examines the perceptions of 16 tourists all over the country, five elephant owners, and 11 mahouts on these two practices. The findings reveal that 80% of tourists, particularly those who had observed Thai elephants, expressed a strong preference for seeing elephants without chains. They suggested using belts instead, which do not harm the sensitive skin of elephants. Tourists highly favored the use of a traditional ankush made blunt with rubber knobs and suggested its removal when not required. Out of elephant owners who were interviewed, 60% of them conveyed the importance of chaining elephants at night, particularly during musth in males, but supported the use of a blunt ankush with detachable rubber knobs for control and promoting chain-free concepts. Proper training and certification for mahouts were emphasized. Responses from mahouts were mixed, with some considering the blunt ankush is effective, while others had doubts. Mahouts stressed the significance of building trust and communication with elephants to minimize the reliance on ankush as a controlling tool. The study concludes that there is a growing concern for captive elephant welfare among tourists, and 80% of respondents supports abandoning chains and 93% of respondents supports using elephant-friendly ankush with blunt ends in favor of more humane practices. Elephant owners show varying responses, with some promoting chain-free concepts and the use of an elephant-friendly ankush. Mahouts' opinions on ankush usage differ, highlighting the need for more comprehensive training and alternative methods of control. The research underscores the importance of compassionate and ethical practices in captive elephant management. Stakeholders in the elephant tourism industry should consider these findings to ensure the well-being of captive elephants, while preserving cultural traditions and providing meaningful experiences for tourists. By prioritizing the welfare of these majestic creatures, we can contribute to a more sustainable and compassionate future for captive elephants in Sri Lanka and beyond.

**Keywords:** Ankush, Captive Elephants, Chains

# The Impact of Anthropogenic Noise on Predator Awareness in the Yellow Billed Babbler *(Argya affinis)*

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Anthropogenic noise has a major impact on the communication of birds. Since their life cycle depends on vocal communication, increased anthropogenic noise in the novel urban environment directly affects their survival and fitness by impacting their mating behaviour, reproductive success, foraging behaviour, parent-offspring communication, and predator awareness. I used the yellow billed babbler (*Argya affinis*) as a model to determine whether there is an impact on predator awareness of birds by traffic noise. I also studied whether they are able to identify alarm calls of predators when there is anthropogenic noise and whether there is a difference in their responses to predators due to anthropogenic noise. I used the shikra (*Accipiter badius*) as the predator because it is one of the most common predators they encounter, and the spotted dove (*Spilopelia chinensis*) as the control. I conducted 41 playback experiments at the University of Peradeniya premises and selected areas of the Galigamuwa Divisional Secretariat in May - June 2023, using recorded soundtracks of shikra calls, shikra calls combined with traffic noise, and spotted dove calls. Responses of the babblers were recorded and analysed using analyses of variance. Results showed that there is a significant ($p < 0.0001$) increase in response time when the shikra calls are combined with traffic noise. Further, in the presence of traffic noise, there is a significant ($p < 0.0001$) increase in the time taken to become alert to predator calls. This study provides evidence that there is an impact of traffic noise on the awareness and responses of the yellow billed babbler to predators. In conclusion, anthropogenic noise reduces predator awareness and therefore it may reduce the fitness and survival of birds.

**Keywords:** Anthropogenic noise, Birds, Predator awareness, Traffic noise, Yellow billed babbler.

# Behaviour of Young Asian Elephants (*Elephas maximus*) at the Elephant Transit Home, Udawalawe

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The Elephant Transit Home (ETH) at Udawalawe rehabilitates orphaned elephant calves in order to reintroduce them to the wild. Elephants are kept with minimal human contact except during short public-viewing sessions during which they are fed with milk. The prevalence of stereotypic behaviours in elephants at the ETH can be considered an indicator of the stress they are undergoing during rehabilitation. We hypothesized that a higher prevalence of stereotypic behaviour reflects higher stress levels and that elephants will have higher stress while being exposed to the public. We conducted this study to identify stereotypic behaviours and their frequency in elephant calves during milk-feeding sessions. Therefore, we compared the behaviour of elephants at their waiting station prior to public view with their behaviour at the feeding station while being exposed to the public. Scan and focal animal sampling were conducted in both areas. Elephants were categorized as infants (< 2 years), juvenile class-1 (2 - 3.5 years) and juvenile class-2 (> 4 years). Animals from both sexes representing all age groups were sampled. None of the observed behaviours could be classified as stereotypic, but differences were identified in other types of behaviour. Analysis of variance (ANOVA) showed a significant effect of age ($p = 0.008$) and the interaction between age and sex ($p = 0.01$) on the duration of running at the feeding station. Similarly, ANOVA showed significant effects of age ($p = 0.01$) and sex ($p = 0.02$) on the duration of walking in the waiting area. Generalized linear models showed a significant effect of the place of observation ($p < 0.0001$), time ($p = 0.046$) and their interaction ($p = 0.02$) on the frequency of standing observed during scan sampling. There was only a significant effect of the time of observation ($p = 0.025$) on the frequency of walking. Thus, our results indicate that the major factors that affect behaviour are the age and sex of the elephants. The findings of this study suggest that public viewing sessions during the rehabilitation programme cause minimal stress to elephants at the ETH.

**Keywords:** Feeding. Orphaned elephants, Rehabilitation, Stereotypic behaviour, Stress

# A Study on Leptospiral Serogroups Circulating among Captive Elephants in Sri Lanka

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Leptospirosis, caused by spirochetes of the genus *Leptospira,* is a widespread bacterial notifiable zoonotic disease affecting mammals, including humans, livestock, and wildlife in Sri Lanka. Despite its prevalence, there is limited information on leptospirosis in elephants in Sri Lanka (*Elephas maximus).* This study aimed to determine the seropositivity of leptospirosis and urine excrete of pathogenic *Leptospira* in captive elephants, participating in the Kandy Esala Perahera August-2022, Sri Lanka, by analyzing blood and urine samples from a subset of elephants. Among thirteen conveniently selected elephants, 10 (76.9%) were found to harbor anti-leptospiral agglutination antibodies, indicating seroactivity at a titre of 1:100 Microscopic Agglutination Test. These antibodies reacted to various serogroups, including Terrasoui, Canicola, Panama, Javanica, Hebdomadis, Hardjo, Patoc, and Grippotyphosa, implying exposure to a diverse serovars of *Leptospira* species. Further analysis revealed that six out of the 13 elephant serum samples (46.2%) were positive; with individual elephants showing higher titres (> 1:400) against specific serogroups including Canicola; 1/13 (7.7%), Panama; 2 /13 (15.4%), Grippotyphosa;1/13 (7.7%) and Patoc 2/13 (15.4%). However, the urine samples yielded negative results for the detection of pathogenic leptospiral DNA. Nonetheless, it is essential to note that the intermittent excretion of pathogenic *Leptospira* via urine emphasizes the importance of continuous screening in elephants. These animals had agglutination antibodies against different serogroups which implies that they have been exposed to many carriers in their living environment, given that captive elephants in Sri Lanka are used for cultural events and tourism, closely interacting with the public, there is a high potential risk of transmitting leptospirosis to humans. Thus, this study underscores the need for ongoing surveillance of leptospirosis in elephants and highlights the significance of collaborative efforts between veterinary health specialists and public health agencies. Such endeavors are crucial to mitigate the risk of transmission and protect both animal and human populations from this zoonotic disease.

**Keywords:** Agglutination antibodies, Captive Elephants, Leptospirosis, Serology, Zoonosis

# Occurrence and Aetiology for Pododermatitis in Privately Owned Elephants (*Elephas maximus*) in Sri Lanka

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Pododermatitis, commonly known as the foot or hoof disease, poses a significant health challenge for elephants. This condition entails inflammation, ulceration, and infection of the foot pads, resulting in pain, lameness, and compromised mobility. Despite its prevalence, limited information is currently available on this health concern, both in captive and wild elephant populations. Understanding the causes and symptoms of pododermatitis is crucial to enhancing the welfare and conservation of these majestic creatures. In the context of the Kandy Esala Perahera 2022, forty-two elephants were examined, and it was found that 14 elephants (33%) exhibited foot lesions in at least one foot. Samples from specific lesions were collected following standard microbiological procedures. Subsequent biochemical tests led to the identification of five distinct types of microorganisms: *Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter cloacae*. In the current study, new bacterial genera namely *Pseudomonas* and *Enterobacte*r were discovered in elephant foot lesions. Additionally, specific species such as *Staphylococcus epidermidis*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* were identified as new bacteria associated with the condition. Interestingly, prior investigations did not detect any specific species within the *Klebsiella* genus. Nevertheless, in this study, *Klebsiella pneumoniae* was found to be present in the elephant foot lesion, shedding new light on the microbial composition of this ailment. The findings indicate that multiple pathogens contribute to pododermatitis in captive elephants. Accurate identification of the causative agent is essential for effective treatment, as misdiagnosis may result in severe complications such as disease exacerbation, antibiotic resistance, and prolonged suffering for the affected animals. This research underscores the importance of swift and precise diagnostic measures to guarantee the well-being and enduring conservation of elephants afflicted with pododermatitis.

**Keywords:** Biochemical Tests, Elephants, Pododermatitis

# A Preliminary Study on Conservation of Wild Animal Genome with Cryopreserved Primary Cell Cultures

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Biobanking of primary fibroblast cell culture is an effective tool for the conservation of wild animal genomes with multiple benefits for a longer period. Those can be used as the reference materials of the species, for research purposes and as a preventive measure for the extinction of these species etc. The objective of the study was to establish a primary cell culture bank as a conservation strategy for wild animals in Sri Lanka. In this study, ear skin biopsies were used to establish primary somatic cell cultures of wild mammals. Feather follicles were used as samples for avian species. The ear skin biopsies were taken from 10 mammalian species: Giant Squirrel, Wild Boar, Barking Deer, Indian Palm Civet, Toque Monkey, Flying Squirrel, Flying Fox, Indian Brown Mongoose, Small Civet and Black-Naped Hare. The cell pulp of the calumus in the feather follicles were collected from one avian species (White Throated Kingfisher). Then cells were isolated by using the enzymatic explantation method and cultured in DMEM with antibiotic solution and 20% foetal bovine serum at 37 ℃ in 5% $CO_2$. Established cell cultures were maintained up to 14-16 days and the medium was replaced every 3-4 days. Cell cultures were evaluated every 3-5 days, considering the morphology and growth of cells, adherence of the explant, and absence of microbial contamination of the cultures. All the mammalian culture samples (n=10) started their cell growth within 3-5 days after culture establishment. But one mammalian sample was observed with microbial contamination and was discarded immediately. The avain sample did not show possible cell growth. All remaining nine mammalian samples achieved 80%-90% cellular confluency within 12-14 days after culture establishment. When cell cultures achieved 80%- 90% confluent, those were harvested by trypsinization and cryopreserved in liquid nitrogen at -196 ºC. DMSO is used as a cryopreservation agent. The samples were cryopreserved for 3 weeks to 6 months and checked for their re-culture ability. To check the re-culture ability, cryopreserved cells were thawed and cultured in DMEM with an antibiotic solution and 10% foetal bovine serum. This study underscore the potential of primary cell culture for cryopreservation of wild animal genome under local conditions in Sri Lanka.

**Keywords:** Cryopreservation, Genome preservation, Primary cell culture, Wild animal,

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# Wildlife and Aquaculture

# Session-II

# Molecular Detection of *Megalocytivirus* in Live Bearing Tropical Fresh Water Ornamental Fish

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The global ornamental fish industry, valued at over $10 billion annually, is primarily driven by the demand for vibrant, captive-bred freshwater species as aquatic pets. Sri Lanka has emerged as a prominent hub for ornamental fish farming and exports; however, the industry faces a significant threat due to disease outbreaks. *Megalocytivirus*, a virus belonging to the family *Iridoviridae*, causes mass mortalities in both marine and freshwater ornamental fish, particularly in Asian countries. Despite documented cases of *Megalocytivirus* infection in ornamental fish cultured in Sri Lanka as well as exported to Australia, the burden of *Megalocytivirus* in live-bearing ornamental fish in the country remains inadequately understood. Thus, the objective of the present study was to investigate the presence of *Megalocytivirus* in live-bearing freshwater ornamental fish species in the Western Province (WP) of Sri Lanka. A total of 144 live-bearing freshwater ornamental fish (two fish from each of the four different species of fish; guppies, platys, swordtails, and mollies from each aquarium) were collected from 18 aquaria located in the WP. DNA extracted from pooled gill samples collected from all the fish from each aquarium was subjected to polymerase chain reaction (PCR) using universal PCR primers for all Megalocytiviruses. Surprisingly, none of the samples amplified the expected target of 777 bp, indicating either a low (undetectable) viral load or the absence of active infections at the time of sampling. Potential factors contributing to these results include seasonal variations, inhibitory substances, or genetic variations within the target gene, affecting primer binding. The study's limitations, such as small sample size and short duration, emphasize the necessity for future research with larger sample sizes and longer study periods to enhance statistical power and overall representativeness. The absence of *Megalocytivirus* in apparently healthy ornamental fish species is promising for the industry's sustainability and trade. Nonetheless, integrating alternative detection methods alongside PCR can offer comprehensive insights into *Megalocytivirus* prevalence, facilitating more effective disease management and prevention strategies to safeguard the long-term health of the ornamental fish industry.

**Keywords:** Aquarium fish, Live-bearing, Megalocytivirus, Ornamental fish

*Acknowledgements: University Research Grant (URG/2022/66/V)*

# A Study of Co-Infection of *Enterocytozoon Hepatopenaei* (EHP) and *Vibrio* Species in *Litopenaeus Vannamei* Cultured in Puttalam District of Sri Lanka

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Hepatopancreatic microsporidiosis is a parasitic infection caused by the parasite *Enterocytozoon hepatopenaei* (EHP). EHP infection has been emerged in *Litopenaeus vannamei*, a shrimp species which has been introduced to Sri Lanka recently and cultured in the Northwestern Province of Sri Lanka. Growth retardation and white faecal strands are the major manifestations of EHP. *Vibrio* spp. are well known pathogens of shrimp. Co-infection of *Vibrio* in EHP infected shrimp has been reported in other countries. Therefore, the objectives of the present study were to isolate and characterize *Vibrio* species from EHP infected *L. vannamei* shrimp in Sri Lanka, showing growth retardation, and determine their antimicrobial susceptibility. A total of 11 farms (8 *L. vannamei* farms and three *P. monodon* farms) located in Chilaw, Puttalam District, that had stocked post-larvae produced either from imported specific pathogen free (SPF) broodstock of *L. vannamei* or *P. monodon* and that had reported slow growth in their shrimp, were visited. Five moribund shrimp were collected from each farm and were subjected to external examination, parasitological examination for EHP spores, histopathological examination and bacteriological examination. A total of 10 *Vibrio* spp. were isolated from the hemolymph and hepatopancreas of shrimp from 10 farms, and were identified as *V. fluvialis, V. parahemolyticus, V. harveyi, V. alginolyticus, V. vulnificus* and *V. damsela*. According to histopathological and parasitological examinations, six farms were considered positive for EHP due to the presence of spores suggestive of EHP in faecal smears and hepatopancreas, and the presence of EHP spores and plasmodia in histopathological sections of hepatopancreatic tissues. *Vibrio* spp. were isolated from five out of six EHP infected farms (83%). The results of this preliminary study revealed that co-infection of EHP and *Vibrio* species in *L. vannamei* suffering from slow growth, is possible. It was surprising to observe the absence of EHP spores in *Penaeus monodon* though they were suffering from slow growth and infected with *Vibrio* spp. While there is little concrete proof that these pathogens can co-infect *Litopenaeus vannamei* shrimp and cause specific diseases, their co-existence raises possible consequences for shrimp health and aquaculture productivity.

**Keywords**: EHP, *Litopenaeus vannamei*, *Vibrio* Species

# Detection of Megalocytivirus in Gourami Fish in Gampaha District

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Megalocytivirus is a viral pathogen that belongs to the family *Iridoviridae*. It can affect ornamental gourami (members of family *Osphronemidae*), causing significant health issues and mortality in infected individuals. The virus is histologically characterized by the presence of large intranuclear inclusions and intracytoplasmic inclusions within infected cells, leading to various clinical signs such as lethargy, loss of appetite, abnormal swimming behaviour, and external lesions. Transmission of the virus primarily occurs through direct contact between infected and susceptible fish, as well as through contaminated water and equipment. The impact of Megalocytivirus on the gourami fish population is considerable, resulting in severe economic losses for the ornamental fish industry. This study was conducted to screen the presence of megalocytivirus in gourami fish samples collected from Gampaha district of Sri Lanka during May-June 2023, which belongs to the rainy season of that area, owing to the South-West monsoon. The research involved the collection of 42 random healthy fish samples from 20 different aquaria located in the region. The investigation utilized polymerase chain reaction (PCR), to screen the collected samples for the presence of megalocytivirus. Surprisingly, none of the fish samples analysed tested positive for megalocytivirus infection. This outcome suggests that gourami fish in the Gampaha district during the rainy season were not affected by this particular viral pathogen. Interestingly, the literature review revealed that most fish diseases, including megalocytivirus, commonly occur during the hot summer season. Thus, the timing of this study during the rainy season was expected to yield a lesser probability of detecting megalocytivirus infection in gourami fish. However, the absence of positive samples indicates a potential absence or low prevalence of megalocytivirus in the studied population during this specific time frame. The findings of this research provide valuable insights into the health status of gourami fish in the Gampaha district, highlighting the absence of megalocytivirus during the rainy season. Future studies should explore the prevalence and occurrence of megalocytivirus in gourami fish during a different season and across various regions to obtain a comprehensive understanding of its distribution and impact on fish populations.

**Keywords:** Gampaha district, Gourami fish, Megalocytivirus, Polymerase Chain Reaction (PCR), Prevalence, Rainy season

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# An Investigation of Potential Routes of Transmission of Microsporidian Parasite, *Enterocytozoon hepatopenaei* (EHP) in *Litopenaeus vannamei* Cultured in the Northwestern Province of Sri Lanka

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*Enterocytozoon hepatopenaei* (EHP) is a microsporidian parasite that causes hepato-pancreatic microsporidiosis (HPM) in penaeid shrimp. EHP has emerged as a significant threat to the global shrimp aquaculture industry, including whiteleg shrimp (*Litopenaeus vannamei*) cultured in Sri Lanka. To determine the potential routes of EHP transmission, samples were collected from 11 shrimp hatcheries [broodstock feces, post-larvae (PL), maturation tank water, polychaetes used as live feed (Specific Pathogen Free (SPF) and wild-caught)] and 2 farms (other aquatic animals) located in the Northwestern Province (NWP) of Sri Lanka. Smears prepared from selected samples [PL (n=8), broodstock faeces (n=6), water (n=6)] were subjected to EHP spore examination with trichrome staining under a light microscope. Varying spore numbers were observed in different samples, with some showing a very high number [PL (n=6), feces (n=3), water (n=3)], several displaying a relatively less number [PL (n=1), faeces (n=2), water (n=3) and a few showing no spores [PL (n=1), faeces (n=1)]. Molecular detection of EHP using a nested PCR targeting SSU of *16S rRNA* of EHP revealed that all tested samples (n=21) of broodstock faeces (n=6), PL (n=8), polychaetes (3 wild-caught, 2 SPF), crab (n=1), and snails (n=1) were negative for EHP in the first step. However, in the subsequent nested step, a total of 18 gave positive results that included one SPF polychaete sample (50%), five broodstock fecal samples (83%), two wild-caught polychaete samples (67%), all the PL samples (100%) collected from hatcheries, and both the snail and crab samples collected from farms. Our findings revealed that the shrimp broodstock faeces, PL and water in several hatcheries investigated are contaminated with EHP favouring the horizontal transmission of the disease. Further, polychaetes and other aquatic animals were found to harbor EHP confirming their potential carrier status. These findings shed light on the transmission dynamics of EHP within the hatchery environment and among different marine species. The presence of EHP in various sampling sources raises important concerns about the potential EHP spread within the shrimp farming areas in NWP. This study highlights the urgent need for comprehensive surveillance and targeted control measures to mitigate the impact of EHP on shrimp aquaculture.

**Keywords:** EHP, HPM, *Litopenaeus vannamei*, PCR, Shrimp

# Morphological Identification of *Hirudinella*-Like Species from Bigeye Tuna and Skipjack Tuna from Galle in Sri Lanka

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The *Hirudinella* genus comprises a diverse group of robust hemiuroid digeneans, known as large "fleshy worms," that have an elongated cylindrical body with two significant external suckers at the anterior end of the body which parasitize the stomach of pelagic marine fishes. The parasite causes stomach ulcers, severe wasting disease and even death of infected fish. This study aimed to investigate the morphology and molecular characteristics of *Hirudinella* species infecting tuna fish species, including Bigeye (*Thunnus obesus*) and Skipjack (*Katsuwonus pelamis*). A total of 20 adult and young *Hirudinella* parasites were collected from fish caught in deep sea fishing harbors. External and internal morphological features were examined and measured using vernier caliper, while molecular analysis was conducted using PCR amplification and gel electrophoresis. The prevalence of *Hirudinella* like parasites in Bigeye tuna was 40% while in Skipjack tuna it was 50%. The morphological analysis revealed specific characteristics of *Hirudinella* species, including elongated, cylindrical bodies with external suckers located at the anterior end and the middle of the body. The parasites exhibited an excretory duct, a protective cuticle with surface structures, and hermaphroditic reproductive organs. The digestive system consisted of a mouth, pharynx, and intestines. Usually, two testes and one ovary were identified in all parasites isolated. PCR was performed for specific target DigL2 gene amplification which showed a PCR product of 534 bp amplicon. The amplified products were visualized through gel electrophoresis, confirming the presence of the Hirudinella DigL2 gene. This study provides detailed morphological and molecular data on *Hirudinella* species infecting tuna fish in deep sea environments. The findings contribute to the understanding of the taxonomy and biology of these parasites and can aid in their identification in the region of Indian Ocean. Further studies will be conducted for analyzing phylogenetic relationships of *Hirudinella* parasite identified in Sri Lankan deep sea marine water tuna fishes with the parasites isolated from fishes of Pacific and Atlantic Oceans.

**Keywords:** Bigeye, Hemiuroid digeneans, *Hirudinella*, PCR, Skipjack, Trematode

# Evaluation of the Daily Nutritional Demands of Domesticated Asiatic Elephants Brought to the Kandy Esala Perahara and the Nutritional Composition of Common Feedstuff that are Used for Feeding Them

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The objective of the study was to examine the suitability of the commonest feedstuff offered to elephants at Kandy Esala Perahara, in terms of their nutritional composition. The average feed intake by an adult elephant was calculated by studying five elephants randomly selected out of the twenty elephants that were studied. The study was based on four main feedstuff namely, Kithul tree trunk (*Caryota urens*), Kithul tree leaves, Jack tree leaves (*Artocarpus heterophyllus*), and Sugar cane trunk (*Saccharum officinarum L.*). Minor feed types such as coconut (*Cocos nucifera*), Banana (*Musa* spp) and, Watermelon (*Citrullus lanatus*), were not accounted for, due to their irregularity in supply volume and frequency. The average amount of each feed type consumed by the elephant was estimated systematically. The average feed intake, with respect to the four feed types, was then calculated for all twenty elephants by extrapolating the data from the five elephants studied. The representative samples of feedstuff were taken from the elephants' sheds, and their nutritional compositions were estimated by proximate analysis. Using this data, the average daily nutritional intake of the elephants included in this study was calculated and was compared with standard nutritional criteria in the feeding process for elephants from previous studies. According to the results, an elephant on average consumes 4.48% of its body weight on fresh matter basis. Furthermore, 40.4 % of the diet consists of kithul trunk (KT), 22 % kithul leaves (KL), 23.25 % jack tree leaves (JL) and 14.25 % sugar cane (SC). The calculations indicated that an elephant consumes 3.49% Crude Protein (CP), 2.76% Crude Fat (CF), 45.74% Crude Fiber (CFi) as compared to the standard daily nutritional demand of, 8-10% Crude Protein (CP), 1.2-1.8% Crude Fat (CF), 33.52% Crude fiber (CFi), and 3.06 MJ/Kg (On Dry matter basis) of Metabolizable Energy. Thus, the study revealed that the crude protein level that an elephant consumes (3.49%) on a daily basis is significantly lower than the average requirement (8-10%) and close to the critical lower margin of 2.5%. All other nutritional criteria that were studied were fulfilled by the diet given. Thus, the addition of alternative affordable protein rich feedstuff and a dietary supplementation with protein sources are highly recommended.

**Keywords:** Crude fat, Crude fiber, Crude protein, Metabolizable energy

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