## **Proceedings**

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### Volume I



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

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

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#### Message from the Vice Chancellor, University of Peradeniya



It is with great pleasure that I pen this message to mark the first Undergraduate Research Symposium organized by the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya.

The Faculty of Veterinary Medicine and Animal Science (FVMAS) has a unique and special place within the University system of Sri Lanka as it

is the only institute in Sri Lanka that offers the Bachelor of Veterinary Science (BVSc.) degree. It continues to play a vital role in producing professionals in the field of Veterinary Medicine and Animal Science and have been instrumental in the recent developments in the Livestock and Animal Health sectors. From its inception in 1948 and after its shift to Peradeniya from Colombo in 1952, the FVMAS has created a diverse set of Professionals that play critical roles in companion animal, livestock, poultry, aquatic, zoo and wild animal sectors. Veterinarians are responsible for not only animal healthcare, welfare and economical production, but also public health by having direct roles on controlling zoonotic diseases, food safety and antimicrobial resistance. As such, Veterinarians engage in a broad scope of research activities that are highly beneficial to the entire animal health and livestock sectors. In preparation to this mammoth task that lies ahead of them, it is very important that undergraduates are exposed to this wide array of research enabling them to broaden their understanding about the real-world challenges that lie before them. It is imperative that these blossoming veterinarians are exposed to the field of research early in their carriers. This forum provides the perfect platform for these students to present their findings in front of a distinguished scientific community and will hopefully act as a foundation in engaging in productive research and academic activities in time to come. It will also provide them with a space to interact with various distinguished personalities of the veterinary profession and allied sectors

I take this opportunity to congratulate the Dean, the staff of the FVMAS and the members of the organizing committee for organizing the first undergraduate research symposium of the FVMAS.

Professor Upul B. Dissanayake Vice Chancellor

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



I take much pleasure in writing this message for the Proceedings of the Undergraduate Research Symposium 2020. This is the first time that the Faculty of Veterinary Medicine and Animal Science (FVMAS) is organising a symposium for students to present findings of their 'Animal Production & Health Research Projects'. Although the original plan was to host the event as a standard symposium with a physical gathering, the circumstances surrounding the COVID-19 pandemic pushed it on to a

virtual platform. A 'Virtual Symposium' is definitely a *first* for the FVMAS; I believe that it is a *first* for University of Peradeniya too. My sincere gratitude goes to the members of the Veterinary Medical Education Unit (VMEU) for taking on the challenge of organising this novel endeavour.

My heartiest congratulations go out to the sixteen students of Batch 2013/ 2014 who were adjudged to have conducted the best research projects. The faculty has long identified the importance of training students on research methodology, and for this purpose, introduced the Animal Production and Health Project as part of the BVSc. degree in 1993, to expose undergraduates to the basics of literature review, planning and conducting research, analysis and interpretation of results. This is also a good opportunity for students to gain skills in critical thinking, time management and communication.

The need of a platform to recognize the achievements of students as well as to disseminate their research findings has been fulfilled by this Undergraduate Research Symposium. I would like to express my gratitude to Dr. A. Arulkanthan, Dr. D.R.A. Dissanayake, and Dr. N.K. Jayasekera for volunteering for the time-consuming task of initially judging all the projects of Batch 2013/ 2014, to enable the sixteen best projects to be selected for oral presentation today. All other students will present digital posters, thus ensuring that all students have active participation in the symposium.

I greatly appreciate the encouragement given by the Vice Chancellor, Prof. Upul B. Dissanayake towards this event. I would also like to extend my thanks to the committee members of the Veterinary Medical Education Unit for organising the symposium and for their editorial support; the supervisors of student projects; judges of the symposium; and all those who gave their assistance in several ways to make this event a success. I would especially like to thank Prof. Preeni Abeynayake and Dr. I. D. V. L. Dharmawardhana for generously sponsoring the awards for the best presenters. Finally, I take this opportunity to congratulate all presenters and wish them all success in their future careers.

Dr. D.M.S. Munasinghe Dean/ Faculty of Veterinary Medicine and Animal Science

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# ORAL PRESENTATIONS

#### A Preliminary Study on Ophthalmic Lesions of Dogs with Chronic Renal Failure

Ekanayake E. M. D. M. K.<sup>1</sup>, Palkumbura P. G. A. S.<sup>2</sup>, Wijesundera R. R. M. K. K.<sup>2\*</sup>

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Ocular complications associated with chronic renal failure are common in humans. Although similar conditions have been recorded in dogs the exact pathogenesis and relation is not identified. This preliminary study performs a comparative and qualitative analysis of ophthalmic changes in relation to severity of renal pathology in dogs with chronic renal failure. Twelve dogs (n=12) with chronic renal failure were classified according to the International Renal Interest Society (IRIS). Renal and ophthalmic lesions were identified adopting Hematoxylin and Eosin (HE) and Masson's Trichrome staining. Distribution and severity of cortical scarring, medullary scarring, tubular degeneration and atrophy, interstitial inflammation and glomerulosclerosis in kidneys were compared along with IRIS stages. Accordingly, the severity of renal pathology was categorized as mild, moderate, severe and very severe stages. Ocular lesions identified in choroid and retina were categorized and compared along with the progressive severity of renal pathology. Gross ocular pathology was limited to lens opacity (9% of cases). Histopathology revealed prominent retinal and choroidal changes. Ninety percent (90%) of cases had mild to severe choroidal fibrosis with neovascularization and fibrosis around choroidal vasculature. Eighty percent (80%) of the cases had retinal detachment in which seventy percent (70%) had inner retinal degeneration whereas thirty percent (30%) had mild to severe retinal atrophy. Histopathology of lens was less evident as most of the lens specimens were trimmed off during microtome processing due its hard nature. Therefore, lens opacities were identified as cataracts based on the clinical history and gross pathology. Comparative analysis of renal pathology revealed the progressive severity of lesions along with IRIS stages of chronic renal failure. In addition, there was an occurrence of vision threatening ocular pathologies mainly in choroid and retina along with the ascending severity of renal pathology in chronic Therefore, there might be an extra renal impact of uremia, renal failure of dogs. hypertension and serum osmolarity changes in chronic renal failure on the development of vision threatening ocular pathologies in dogs. Thus, it is important to focus on diagnosis, treatment and control of ocular lesions while managing chronic renal failure in dogs.

#### Prevalence of Ecto-Parasites in Three Fresh Water Fish Species (Tilapia, Rohu and Silver Carp) Obtained from a State Fish Breeding Centre in Sri Lanka

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The culture based fisheries (CBF) involves in stocking of fish seeds in fresh water bodies with the aim of increasing the production of food fish in Sri Lanka. The success of CBF depends on the production of healthy fish seeds in the hatcheries. As the ecto-parasites are known to cause significant morbidity and mortality in juvenile fish, this study was aimed at determining the prevalence of ecto-parasites by examining skin scrapings, gills and caudal fin snips of 154 juvenile fish (47 tilapia, 60 rohu, and 47 silver carp) obtained during August 2019 from a state fish breeding station that supplies fish seeds for the purpose of CBF. The overall prevalence for at least one ecto-parasitic infestation in the sampled population was 98.7% (152/154). Seven genera of ecto-parasites were found in tilapia {*Trichodina* spp. (100%), Apiosoma spp. (66%), Cryptobia spp. (74.4%), Ichthyobodo spp. (74.4%), Dactylogyrus spp. (76.5%), Gyrodactylus spp. (2.1%) and Centrocestus spp. (2.1%)}, and five genera were found on rohu {*Trichodina* spp. (80%), *Chilodonella* spp. (21.6%), Tetrahymena spp. (13.3%), Apiosoma spp. (3.3%) and Centrocestus spp. (71.6%). Further, silver carps had six genera, namely Trichodina spp. (91.5%), Chilodonella spp. (2.1%), Ichthyopthirius multifilis (100%), Tetrahymena spp. (6.3%), Dactylogyrus spp. (2.1%), *Centrocestus* spp. (93.6%). When comparing the prevalence of ecto-parasites between the species of fish, *Chilodonella* spp. was significantly high (P < 0.05) in rohu (21.6%), while tilapia had significantly high percentage (P < 0.05) of Dactylogyrus spp. (76.5%) and Apiosoma spp. (66%). Interestingly, the prevalence of *Centrocestus* spp. (2.1%) was very low (P < 0.001) in tilapia compared to other species of fish sampled. There was no significant correlation observed between the abundance of parasitism and the Fulton's condition factor of fish. In conclusion, this study demonstrated a total of ten genera of ecto-parasites with varying degree of prevalence and abundance in the sampled fish population. This data implies the importance of regular monitoring of parasitism in all stages of fish in the breeding station in order to supply healthy seeds for CBF.

#### Development of Teaching and Learning Material for the Veterinary Skills Laboratory Using Locally Available Resources

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Veterinary students need to develop competence in clinical skills in preparation for the provision of entry-level care which depends on amount of sufficient hands-on experience. The number of students is increasing and using live animals for practicing is not realistic nor ethical. Many veterinary teaching institutes faced challenges when they tried to train their students with traditional methods. Models and simulators housed in a Clinical Skills Laboratory (CSL) are increasingly used to help to overcome the challenges. This type of practice allows students to perform procedures repeatedly in a safe and stress-free environment. In addition, CSL can be used to evaluate the student's clinical skills and to inspect the effectiveness in the learning process. Experts suggest that this can be used for the practical examinations as well. Providing open access and supporting self-directed learning are some of the major advantages of CSL. In the present study, we have designed simulatory models using locally available, low cost material with the aim of assisting other veterinary educators to consider opening or expanding their own facility. Sixteen practical stations were designed with simulators and stationed them in a new CSL. In addition we have developed the student practical guide for using each model in the CSL.

Acknowledgements: Faculty of Veterinary Medicine & Animal Science (Dean's Fund)

#### Preliminary Study on Kidney and Liver Lesions of Cattle from CKDu-Prevalent North Central Province of Sri Lanka

Rodrigo S. A. C. H.<sup>1</sup>, Palkumbura P. G. A. S.<sup>2</sup>, Wijesundera R. R. M. K. K.<sup>2\*</sup>

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Chronic Kidney Disease of unknown etiology (CKDu) has currently become one of the most burning environment-related health issues in Sri Lanka. CKDu is mostly prevalent in few provinces, namely North Central Province (NCP), North western province, Eastern Province, and Uva Province. Currently, NCP accounts for the highest incidence and prevalence of CKDu. However, the exact pathogenesis of the disease is yet to be found. Due to its mysterious nature, a considerable number of studies have been undertaken to establish the most probable causative factor from the proposed array of different environmental exposures. The renal morphology of CKDu patients in Sri Lanka has been reported as a tubulointerstitial nephritis/ tubulointerstitial disease, but significant glomerular lesions such as, glomerular ischemia and focal glomerulosclerosis have also been reported. However, a study on kidney and liver lesions of animals in CKDu-prevalent geographical areas has not performed yet, globally or locally. Therefore, this study was performed to establish the gross and histopathological morphology of renal and hepatic tissues of the cattle from CKDuprevalent NCP. Twenty (n=20) bovine kidney and liver samples of cattle from NCP were collected mainly from abattoirs. Tissues were fixed in 10% neutral buffered formalin, processed, wax embedded and were stained with hematoxylin and eosin (HE), and Masson's Trichrome. Gross pathological lesions of the kidneys were limited to multifocal petechial hemorrhages. However, gross lesions were not observed in the livers. The main histopathological lesions observed in the renal tissues were tubular degeneration, lymphocytic inflammatory cell infiltration, interstitial fibrosis, and mild glomerulosclerosis. The liver sections showed multifocal and diffuse lymphocytic infiltration along with centrilobular necrosis. The initial stages of interstitial fibrosis in renal parenchyma were also observed. Interestingly, the renal lesions of cattle showed high similarity with that of humans. Therefore, this study is highly suggestive of a common etiological factor which affects both humans and animals live in the same areas of NCP. Furthermore, it is imperative of that, this common source affects the kidneys of animals in the same way it affects that of humans.

#### Prevalence of Gastrointestinal, Ecto and Haemo Parasites in Goats in Selected Areas in Mannar District

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Goats can thrive on poor quality forage without much input on supplementary feeding. Due to this advantage, goat farming is popular in Mannar district, where the availability of forages is limited. Although the parasitism is an economic concern in goat industry, the information on this aspect is scant in the above district. Therefore, this study was carried out to determine the occurrence of gastro-intestinal, ecto- and haemo- parasites among 78 goats (from 13 semi-intensively managed farms) during August 2019. The faecal samples were subjected to McMaster technique while stained blood smears were examined for blood parasites. Morphological features were used to identify the ecto-parasites. The overall prevalence of helminth eggs and coccidian oocysts in the faeces were 88.4% (69/78) and 100% (78/78), respectively. Four types of helminth eggs {Strongyle (84.7%), Strongyloid (23.0%), Trichuris (6.0%) and Moneizia (21.7%) were identified. The EPG (eggs per gram of faeces) did not differ (P>0.05) between males (740±178) and females (777±134).Although not significant, the EPG was slightly higher in young (6-12 months:  $771\pm231$ ) and adult goats (>12 months:  $924\pm178$ ) than the kids (<6 months:  $538\pm148$ ). The OPG (oocysts per gram of faeces) was higher in males (12950±4784) than females (5915±1140), although the difference was not significant. Further, the OPG was significantly higher (P < 0.05) in kids (15933±5581) compared to other age groups (young: 3365±680, adults: 5943±1461). Two genera of haemoparasites, namely Anaplasma spp. (66.7%) and *Theileria* spp. (57.5%) were encountered, albeit the mean parasitaemia was very low (Anaplasma spp.:  $0.017\pm0.024\%$  and Theileria spp.:  $0.014\pm0.020\%$ ). The parasitaemia of Theileria spp. and Anaplasma spp. did not differ between the sex of goats (P>0.05), while the kids had higher parasitaemia of Anaplasma spp. (P<0.05) and Theileria spp. (P>0.05) compared to other age categories. In addition, six genera of ectoparasites, namely Haemophysalis spp. (98.7%), Hyaloma spp (6.4%), Rhipicephalus spp. (3.8%), Ctenocephalids felis (19.2%), Bovicola spp (1.2%) and Linognathus spp. (2.5%), were identified. This cross sectional study indicates that the parasitism in goats in the study area is diverse, and necessitates longitudinal studies to understand the epidemiology of parasitism in goats in the study area.

#### Occurrence and Associated Antimicrobial Resistance of E. coli and Salmonella in Commercial and Backyard Chicken in Millaniya and Bandaragama Veterinary Divisions

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Overuse and misuse of antimicrobials in veterinary and human medicine have been blamed for the emergence of antimicrobial resistance. Antimicrobial resistance in foodborne bacteria has become a global crisis. E. coli and Salmonella are two zoonotic bacteria that readily acquire antimicrobial resistance and frequently contaminate poultry products. This study attempted to investigate the prevalence of antimicrobial resistance E. coli and Salmonella in the backyard and commercial layer chickens and thereby to predict the potential public health risk. Twenty-five backyard chicken farms and four commercial layer farms in Bandaragama and Millaniya veterinary divisions were investigated. Cloacal swabs from five randomly selected chickens were pooled to represent the farm. Isolation, identification and antimicrobial susceptibility testing of the isolates were conducted according to standard protocols. A parallel questionnaire survey was conducted to gather information on backyard farm management. No Salmonella was isolated from cloacal swabs however, 126 E. coli isolates (108 from backyard and 18 from commercial farms) were recovered. E. coli isolates from the backyard system exhibited a considerable level of resistance against ampicillin (24%), tetracycline (23%) and sulfamethoxazole-trimethoprim (18.5%). Further, a low level of resistance reported against each ciprofloxacin and nalidixic acid (9.3%), streptomycin (6.48%) and imipenem (1%). E. coli isolates from commercial layers had a substantial level of resistance against sulfamethoxazole-trimethoprim (72.2%), tetracycline (61%) and ampicillin (50%) whereas a moderate level of resistance against each ciprofloxacin and nalidixic acid (17%) as well as streptomycin and chloramphenicol (11%). Two proportion analyses revealed a significantly higher resistance among E. coli from commercial layers against Tetracycline and sulfamethoxazole-trimethoprim. Multiple drug resistance (criterion; resistance  $\geq 2$  antimicrobial classes) was observed in 23 (18.2%) E. coli isolates (13 from backyard and 10 from commercial farms). The questionnaire survey indicated that the antimicrobial usage in backyard farms with or without veterinary interventions is 24%. Thus irrespective of the management system resistance has emerged among E. coli against widely used antimicrobials. Substantial level of multidrug resistance calls immediate attention to conduct comprehensive studies to understand the mechanisms and transmissibility of resistance and to enforce regulations to control the use of antimicrobials in the poultry industry.

#### Detection of Clinically Inapparent Dengue Infection among Patients Who were Presented to the Kandy Virology Laboratory for Infective Screening

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Dengue is an infectious mosquito-borne disease that has been transmitting rapidly since the middle of the  $20^{\text{th}}$  century in the tropics and the subtropics of the world. The virus which causes dengue belongs to the Flaviviridae family. Expanding urbanization and human population growth around the world have produced particularly favourable conditions for the transmission of dengue. The majority of such cases of dengue fever (DF) are asymptomatic. Signs and symptoms include high levels of fever, myalgia, headache and appetite loss. While dengue fever is a severe, debilitating condition, it is not usually fatal and a minority of people diagnosed with dengue virus experience dengue haemorrhagic fever and the dengue shock syndrome. Asymptomatic dengue infection is described as not having any clinical signs or symptoms of illness. Sri Lanka being a tropical country with two monsoon seasons, two dengue disease peaks in each monsoon could be observed. Various studies have shown that in endemic countries; particularly in South Asia with cyclic dengue epidemics have high rate of asymptomatic dengue infection. The current study was conducted to determine the proportion of afebrile patients among the people who were presented to the virology laboratory of the Kandy Teaching Hospital for infective screening. In total, 100 consecutive serum samples were collected which were received by the Kandy virology department within a period of two months. NS1 antigen was detected using a onestep sandwich-format microplate enzyme immunoassay to confirm infected afebrile patients. Among those patients, three of them were positive for NS1 antigen. Seroprevelance of asymptomatic dengue was 3% at the beginning of dengue outbreak among people who were afebrile at the presentation. We found a substantial number of asymptomatic dengue infection among the people who are supposed to undergo surgical treatment and other medical interventions. These clinically inapparent dengue infected people play an important role in dengue transmission by acting as reservoirs. In fact it is very important to detect and quantify asymptomatic dengue infection and in turn to control the fatal dengue infection. Frequent surveillance in general population for detection of asymptomatic infection is recommended to understand disease burden and transmission dynamics.

Acknowledgements: Staff of Virology Laboratory of Kandy Teaching Hospital

#### Detection of Megalocytivirus from Freshwater Angelfish (Pterophyllum scalare)

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Genus Megalocytivirus of the family Iridoviridae has been associated with systemic disease accompanied by mass mortalities in a wide range of marine and fresh water fish species, causing significant economic losses to the aquaculture industry. In the present study, we examined sixteen pairs of apparently healthy freshwater angelfish collected from sixteen different aquaria located in Kandy, Kegalle and Matale. After external examination, each fish was subjected to necropsy and the kidney samples were cultured aseptically on trypticase soy agar for the isolation of bacteria. Isolated bacteria were identified upto the genus level using biochemical characteristics. Liver samples from each fish were processed for routine histopathology. From each pair of fish, pooled tissue samples were collected separately from liver, spleen, kidney, gills, muscle, skin and brain. DNA was extracted from pooled tissue samples using a commercial DNA extraction kit. Conventional Polymerase Chain Reaction (PCR) was used to detect the presence of Megalocytivirus using universal PCR primers for all megalocytiviruses. PCR amplicon (777 bp) was subjected to direct nucleotide sequencing. No behavioral or gross pathological abnormalities detected in sampled fish. Out of 16 kidney samples cultured, only 7 (43.7%) yielded bacterial growth and the major bacterial species found were Aeromonas ssp. and Pseudomonas spp. Out of 112 DNA samples (7 pooled tissue DNA samples from each pair of angel fish, 16 pairs), positive PCR results were observed only in one angelfish sample. Among the seven DNA samples, the expected fragment of 777 bp was amplified only in liver, kidney, spleen, gills and muscles. Histopathological examination revealed the presence of hypertrophic cells, single cell necrosis and lymphocytic aggregations in the liver tissue of PCR positive sample. Sequence analysis showed a maximum similarity to infectious spleen and kidney necrosis virus (ISKNV) major capsid protein gene. According to our findings, 6.25% of angelfish sampled in the present study were found to carry Megalocytivirus in their tissues. Our study confirms that apparently healthy angelfish can harbour Megalocytivirus. Therefore, further studies are needed to understand the true prevalence and host range of this economically important viral pathogen in Sri Lankan ornamental fish stocks.

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#### Metabolic Profile Testing of Cattle Fed on Sugarcane

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Recently established dairy farms are facing up with shortage of quality forage-base year around for feeding their cattle. In this context farmers have experiment of feeding discarded vegetables, banana stems, and even pumpkin for their cows. Metabolic profile test can be used to detect the qualitative and quantitative adequacy of the feed given to dairy cows to achieve optimum milk production and good fertility postpartum. Early diagnosis of nutritional deficiency or metabolic disease is very important to optimize the livestock production and to prevent common diseases of early lactation. Range of analytes/ metabolites can be used to assess energy, protein and mineral status of peri-parturient dairy cows. In this study population metabolic profile testing was done in a dairy herd fed with sugarcane as the main feed ingredient. Three groups of cattle were selected as for metabolic profile testing and consisted of dry, early lactation and late lactation cows. The main feed ingredient of all these groups were sugarcane. Ten cows from each group was sampled for this study. Serum samples were tested for glucose, total protein (TP), Albumin, BHB, urea and blood urea nitrogen (BUN). The mean values of tested parameters were compared between groups using One-way ANOVA (Minitab) and compared with reference values. According to the statistical analysis, there was a significant difference between the mean values of PCV, BHB, urea and BUN between groups. Most blood parameters of late lactation group was in normal ranges when compared with the other two groups. None of the ration was able to provide adequate energy and protein requirement of the cows. Ration can be further adjusted based on metabolic findings.

#### Comparison of Microbiological Quality of Table Eggs Collected from Backyard and Commercial Poultry

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Chicken eggs are a popular source of good quality nutrition in human diets. Both backyard and commercial poultry contribute to the overall egg production of Sri Lanka. Although many developed countries have their standards to evaluate microbiological quality of table eggs, in Sri Lanka such standards have not been established yet. Therefore objectives of this study were to evaluate and compare the microbiological quality of fresh table eggs collected from backyard and commercial poultry farms. Along with these, other qualities like egg weight, egg volk volume and egg white volume and the cleanliness of the egg shells were also evaluated. Commercial layer farms located in Kurunegala district of Sri Lanka were selected for the study. The eggs were collected from 15 backyard poultry farms and 10 commercial farms. The aerobic plate counts (APC) of the egg shell wash, egg white and egg yolk were tested using spread plate method as described in the standard SLS 516:Part 1 :1991. The mean APC on egg shells from backyard poultry and commercial poultry were  $4.42\log_{10}$ CFU/ml and  $5.161\log_{10}$ CFU/ml respectively. Therefore there was a significantly higher number of APC present on commercial poultry egg shells compared to backyard poultry egg shells (P value = 0.010). Aerobic bacteria were found in each egg white and egg yolk of eggs from both backyard poultry and commercial poultry but the counts were not significantly different between each from two different sources. Further the egg volumes (egg white and egg volk) were also measured and found that there was no significant difference (P value > 0.05) between the egg white and yolk volumes in eggs from two production systems. However, the mean egg weight (49.08g) was significantly higher in eggs from commercial poultry than that (44.46g) of the eggs from backyard poultry (P value = 0.014442). Since this pilot study indicates the levels of bacterial contamination is higher in table eggs produced in commercial poultry, we propose a detailed, extensive study to evaluate the microbiological quality of table eggs produced in the country and identify contamination points to take measures for reducing contaminations.

#### Preliminary Investigation of Caprine Sub-clinical Mastitis in the Western Province of Sri Lanka

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Mastitis causes significant economic losses in dairy goat industry. Despite its significance, very scanty information is available on the prevalence and antimicrobial sensitivity of bacterial pathogens causing mastitis in goats in Sri Lanka. Therefore, the present study was carried out to identify the bacterial organisms causing subclinical mastitis in goats in selected farms in Western Province, Sri Lanka and to assess the antimicrobial sensitivity of the isolate bacteria. Occurrence of subclinical mastitis was detected using California mastitis test (CMT) in five farms comprising 111 milking animals. Bacterial pathogens were isolated and identified using conventional bacterial culture techniques. Antimicrobial sensitivity testing was carried out using CLSI guidelines. Total 29 (26%) samples were CMT positive and 82 (74%) were negative. All CMT positive milk samples were positive for bacterial growth. Among those positive samples, 22 (76%) yielded one bacterial species, whereas 7 (24%) samples had mixed bacterial isolates. Altogether 4 bacterial species were isolated. Coagulase negative staphylococci (CNS) had the highest occurrence (56%) followed by Staphylococcus aureus (19%) Klebsiella species (14%), Pseudomonas species (6%) and Streptococcus species (5%). All CNS isolates were sensitive to tetracycline, cefuroxime and ciprofloxacin. Most of the CNS isolates (95%) were sensitive to cotrimoxazole followed by ampicillin (85%), gentamicin (80%), amoxicillin clavulanate (65%), cefotaxime (60%) and ceftazidime (35%). In contrast, all S. aureus isolates were resistant to ampicillin and ceftazidime. Amoxicillin-clavulanate and cefotaxime sensitivity was 12.5% each. Further, 57.1% of the S. aureus isolates were identified as methicillin resistant S. aureus. However, 85.7% of S. aureus isolates were sensitive to cotrimoxazole, followed by cefuroxime (71.4%). All Klebsiella and Pseudomonas isolates were resistant to ampicillin while sensitive to gentamicin, tetracycline, cefuroxime and ciprofloxacin. Additionally, all Pseudomonas and 60% of Klebsiella isolates were resistant to amoxycillin clavulanate. All Klebsiella isolates and 50% of *Pseudomonas* isolates were sensitive to cotrimoxazole while 50% each of Klebsiella and Pseudomonas isolates were sensitive to cefotaxime. All Streptococcus isolates were sensitive to ampicillin, amoxicillin clavulanate, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin and cotrimoxazole while 50% of the isolates were sensitive to gentamicin and tetracycline.

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#### Morphological and Molecular Identification of Hemoparasites in Captive Snakes in the Serpentarium of University of Peradeniya

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Snakes are commonly found reptiles in Sri Lanka and originating from wild, harbor different types of pathogens which affected on their health. Phylum Apicomplexa: Haemogregarinidae are the most commonly reported hemoparasites from snakes. Of over 300 Hepatozoon species identified, more than 120 were described from snakes. However, recent genetic assessments have found Hepatozoon lineages recovered from both prey and predators, indicating that diet may play an important role in transmission of the infection of final vertebrate hosts. High number of reptiles exchange between countries and within the country for many purposes such as anti-venom production, exhibitions, raring as pets, etc. At present there is no requirement for health screening or quarantine of reptiles, and so there is a real risk of the simultaneous importation of human and animal pathogens along with the vectors necessarily for their dissemination. Enhancing the knowledge base relating to the health status and zoonotic potential of non-domesticated companion animals such as snakes are very important. This study has been carried out on hemoparasites of captive wild-caught medically imported snakes which is used to research purposes at Udaperadeniya. Those snakes were collected from different geographical areas in Sri Lanka, especially districts of Colombo and Kandy. Twenty seven snakes of six species were selected and the Blood smear examination was done using Leishman staining method. Infection positive samples were used for molecular identification. Twenty seven snakes of six species were screened and twelve of them were positive for hemoparasites. All positive samples were infected with same hemoparasite according to morphological identification. Snakes that are captive for long period had low parasitemia than recently captured snakes. Statistical analysis proved negative correlation between captive duration and parasitemia. PCR results confirmed that these hemoparasites are belonging to phylum Apicomplexa. Further study is needed to identify the species of above found hemoparasites.

#### Prevalence of Antibodies against *Babesia bigemina* in Unvaccinated Animals in Three Veterinary Ranges in Kurunagala District

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Tick fever in cattle is an acute lethal disease caused by the hemoparasites of *Babesia* spp (mainly Babesia bovis and Babesia bigemina) transmitted by ticks. It has a great economic impact on the farmers in terms of milk yield and fertility issues. In endemic areas cattle showing the clinical signs are highly suggestive of the disease. To determine the immune status of the animal, detection of antibodies is performed using serological means such as Enzyme Linked Immunosorbent Assays (ELISA). Evaluating the immune status in an area prior to vaccination is crucial. Therefore, this study was conducted in order to evaluate the immune status of the herds in three veterinary ranges in Sri Lanka. Altogether 29 blood samples were collected from unvaccinated animals of different herds, ages and both genders. Microscopic examination of the smears gave negative results for all samples. The SVANOVIR®B. bigemina-Ab test kit was used to determine the presence of antibodies against the organism using the serum samples. Out of 27 samples, 08 samples gave positive results. The prevalence of antibodies in each range was calculated and graphed to compare the immune status of the animals and determine whether they have formed a natural resistance to the disease by developing a herd immunity. It revealed although a pre immune state is achieved without vaccination (either by natural exposure or passive transfer of antibodies from the mother), it is not sufficient to protect the herd from the disease. To identify the strength of pre immunity by detecting the antibody titre, a serial dilution was prepared using the positive samples. The antibody titre was then correlated with the age of the animals used for sampling. It revealed that the calves (<1 year) were more resistant to the disease compared to older animals and the old animals also harbor antibodies against babesiosis which may imply a lifelong immunity achieved by continuous exposure to reinfections. It was confirmed that without vaccination, herds in these three veterinary ranges have developed pre immune state, but not complete herd immunity, which suggests that these areas require vaccination as a prophylactic measure.

#### Changes of Fecundity in Annual Reproductive Cycle of Sucker Mouth Armored Cat Fish (*Pterygoplichthys disjunctivus*) from the Polgolla Reservoir, Sri Lanka

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Sucker mouth armored catfish (Pterygoplichthys disjunctivus and Pterygoplichthys paradalis) are two of the invasive alien fish species introduced to Sri Lanka. Invasive alien species can have very destructive effects on aquatic environment. These populations have established successfully in Polgolla reservoir as well as many of other fresh water reservoirs in Sri Lanka. The population of these species have grown substantially and cause a major problem for commercial fish industry by number of ways. The studies of reproductive parameters of invasive sucker mouth armored cat fish is very important to understand population growth and to control their effects on water bodies. This report provides the details on body weight, ovary weight and fecundity and relations among these parameters within a one year time period of *P. disjunctivus* living in Polgolla reservoir. The samples were collected from the Polgolla reservoir from August 2018 to July 2019. The average body weight (BW) was 532.25±186.17g, whereas, the weight of the largest fish reported was 1048gwhile the smallest was 100g. The average ovary weight (OW) was 27.01±14.65g (range 1.95g to 66.84g). The average fecundity (F) was 2273.42±1355.08. The highest fecundity reported was 8704.85 while the lowest fecundity reported was 186.94. This fish has shown the highest mean body weight in January and high fecundity in January and February. There were no changes in mean ovary weight of mature cat fish throughout the year. It was seen that there was a highly significant relationship between the ovary weight and body weight (P<0.001, R-sq=22.5%). There was also a significant relationship (P=0.001, R-sg=13.2%) between fecundity (F) and body weight (BW). Furthermore, the relationship between fecundity (F) and ovary weight (OW) was also highly significant (P< 0.001, R-sq= 20.09%). Our results show that the sucker mouth armored cat fish in Polgolla reservoir breeds throughout the year with increased fecundity in January and February ensuring preferable environmental conditions for the new born. These observations will be important in future programs intended for population control of this fish.

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#### Sero Surveillance of Antibodies for *Anaplasma marginale* in Dry Cows of the Ridiyagama Dairy Farm, Hambantota

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Bovine anaplasmosis is an infectious non-contagious disease caused by Anaplasma marginale and constitutes a major constraint to dairy and beef industry. Cattle of any age can be susceptible to the infection with A. marginale, but the severity of disease increases with age. Once cattle become infected, will remain persistently infected carriers for life. Yet there is no any effective global cure due to the variability and diversity showed by different strains of A. marginale distributed worldwide. The stained blood smears can be used to identify clinically infected animals during the acute phase of the disease, but not to detect the infection in pre-exposed or carrier animals. The aim of the present study was to identify the carriers of A. marginale in the Ridiyagama Farm, Sri Lanka. Forty-seven clinically healthy animals in a free grazing dry herd in their mid-gestation period were selected randomly to investigate the prevalence of antibodies against A. marginale by serologic demonstration using Enzyme Linked Immunosorbent Assay(ELISA) along with conventional of microscopic examination. SVANOVIR A. marginale-Ab ELISA kit was used to analyze the samples. Results revealed 9 of the 47 samples were positive for antibodies against A. marginale. Then serial dilutions were performed to determine the antibody titers of each of the positive sample. A prevalence of a high antibody titers for A. marginale due to frequent exposure was not observed. The comparison of antibody titers separately, with the breed and parity of animal showed that although the parity plays a role in developing antibodies against A. marginale breed does not. In order to obtain a broader view if would be beneficial to screen all the animals in the farm as this study can be further extended to a development of a vaccine against A. marginale.

#### Poultry Production Distribution and Backyard Chicken Production in Colombo District

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To meet the increasing demand for the poultry products in Sri Lanka a considerable number of entrepreneurs have invested in the commercial and the backyard poultry industry over the last few decades. The popularity of backyard eggs and the ability to get a good source of income with a minimum input have attracted the low-income families to engage in the backyard poultry industry. However, the nature of the backyard poultry operation poses a great health risk to the public as well as to the commercial poultry industry in Sri Lanka and lack of reliable information aggravates the scenario. Hence, the current study attempted to gather information on the nature and distribution of backyard poultry rearing systems in Colombo, the most densely populated district in Sri Lanka. Data that was available in the government veterinary offices (n=8) was collected and a questionnaire survey was conducted among 32 backyard poultry farms in three selected veterinary ranges. Collected data was analyzed to reveal the numbers of backyard rearing systems, relative distribution, management practices, biosecurity measures, and the associated risks. This study revealed that there are considerable numbers of duck, quail, turkey, pigeon and game bird rearing systems operating in Colombo district other than chicken. Of these, pigeon and game birds rearing were quite popular not as products but as pets presenting in shows. Poor biosecurity and unacceptable management practices such as indiscriminate waste disposal and antimicrobial use were observed and these may increase the risk to public health. Although the input is little most farmers were satisfied with the productivity however the role of the government veterinarian is currently insufficient for the sustainable development of the backyard poultry. Thus, the present study revealed important information on the hidden aspects of the backyard poultry production systems in the Colombo district. The results generated in this study emphasize the necessity of conducting a broader survey to cover the other densely poultry populated districts of Sri Lanka. Availability of such information will be valuable in quality assurance of poultry products and control of zoonotic diseases in an event of an outbreak.

# POSTER PRESENTATIONS

#### Occurrence of Ecto-Parasites and Haemoparasites in Dogs and Cats Presented to a Private Veterinary Clinic in Veyangoda

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This study was carried out in a private veterinary clinic in Veyangoda area to determine the occurrence of haemoparasites and ectoparasites in dogs (n=57) and cats (n=13) during August to September 2019. Of the dogs sampled, 50.9% (29/57) were positive for either Babesia gibsoni and/or Dirofilaria repens microfilaria. The prevalence of B. gibsoni, D. repens microfilaria and co-infection of the above two parasites in dogs were 45.6% (26/57), 10.5% (6/57) and 5.3% (3/57), respectively. The prevalence of *B. gibsoni* was significantly higher (Chi square test, P < 0.05) in males (22/40: 55%) than females (4/17: 23.5%). Although not significant (Chi square test, P > 0.05), the dogs less than 24 months old had higher prevalence (15/28: 53.6%) of B. gibsoni compared to the animals more than 24 months old (11/29: 37.93%). Further, an inverse correlation was observed between the B. gibsoni parasitaemia and the age of dogs, while a positive correlation was found between the body temperature and parasitaemia. Further, 30.8% (4/13) of cats were positive for an intra-erythrocytic piroplasm that morphologically resembled B. felis. A total of 21 dogs (36.8%) were positive for at least one genera of ecto-parasites, which were *Ctenocephalides* canis (3/57: 26%), Rhipichephalus spp. (20/57: 35.1%), Heterodoxus spiniger (3/57: 5.26%) and Trichodectes canis (3/57: 5.3%). One genus of tick (Rhipichephalus spp.) was found in 46.2% (6/13) of the cats sampled. This study revealed that the haemoparasites were common in dogs and cats in the study area, and the declining trend observed in the prevalence and intensity of *B. gibsoni* infection with increasing age of dogs might be due to the development of age related immunity.

#### Evaluation of Anti-cancer Effect of Flueggea leucopyrus (Katupila)

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*Flueggea leucopyrus* (Katupila) is a herbal plant which could be found in many parts of Sri Lanka. The related medicinal properties it contains include deobstruent, diuretic, blood purifier, anthelmintic and purgative. Recently traditional medical professionals in Sri Lanka have become increasingly interested in using this plant as a treatment for various cancers due to potent anticancer effect with least side effects. The current study was carried out to investigate anticancer effect of F. leucopyrus especially in human colonic cancers. The whole plant of F. leucopyrus was collected and chopped into smaller pieces using a herbal grinder. Aqueous extract and boiled water extract was obtained from the chopped whole plant of F. leucopyrus. DLD1 human colon cancer cell line was cultured in RPMI cell culture media supplemented with foetal calf serum and antibiotics. The aqueous extract and the boiled water extracts were added to the cell culture and investigated the cell viability using MTT assay following 24h treatment. L929 normal cell line was also cultured and treated with F. leucopyrus aqueous extract and boiled water extract for 24h. L929 normal cells were also subjected to MTT assay. The results clearly confirmed that the F. leucopyrus aqueous extract and boiled water extract have a significant inhibitory effect on DLD1 human colon cancer cell line. L929 cells were used to determine cytotoxic effects of F. leucopyrus against normal cells. Results of MTT assay for L929 cell line suggested comparatively very low cytotoxic effect on normal cells. Thus it provides supportive evidence for the use of both aqueous and the boiled water extract of this plant as an alternative anticancer treatment.

# Establishment of Broth-micro Dilution Test to Detect Tetracycline Resistance in *E. coli* Isolates

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Antimicrobial resistance (AMR) has become one of the major public health problems in the world. Nowadays scientists are trying hard to develop and establish accurate AMR detection methods, understand AMR mechanisms and invent new antibiotics. In-vitro antimicrobial susceptibility testing (AST) or AMR testing can be performed using either qualitative or quantitative methods even though each has its advantages and disadvantages. However, an AST testing laboratory should have both methods developed, to provide a better service in deciding treatments. Aims of this study were to establish broth-micro (BM) dilution test (quantitative method) to detect AMR and determine its accuracy by comparing with disk diffusion (DD) test (quantitative method). For the present study, previously identified five E. coli isolates were selected and BM dilution test was done in sterile 96 well plates for tetracycline. First, overnight (O/N) E. coli broths were prepared by incubating in a shaking incubator. Then, the O/N broths were washed twice in PBS and cell suspensions were adjusted to obtain  $10^5 E$ . coli/ml broth solution. Thereafter, 400 µl of E. coli broth was mixed with 100 µl tetracycline solution in wells of 96 well plates to obtain the final tetracycline concentrations in mixtures starting from 64 µg/ml to 0.5 µg/ml (a two-fold dilution series of tetracycline). For each tetracycline concentration, four replicates were done. Then plate was incubated for 18hrs at 37°Cand absorptions were measured at 620nm wavelength using spectrophotometer. Based on the 620nm wavelength values, minimum inhibitory concentration<sub>50</sub> (MIC<sub>50</sub>) were calculated. In addition, the same *E. coli* isolated were tested for their AST patterns using DD method and zone diameters were obtained. The results obtained through BM and DD methods were interpreted based on the breakpoints given by the Clinical Laboratory Standard Institute. The ATCC 29522 -E. coli strain was used as the quality control strain in all procedures. The interpreted results obtained through BM method and DD method were compatible for quality control strain as well as for *E. coli* test isolates. Therefore, BM method to detect AMR is successfully established and can be used in detecting AMR in future studies.

### Detection of Hemotrophic Mycoplasma in Imported Boer Goats in Sri Lanka

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Hemotropic mycoplasmosis is a common disease in goat and sheep worldwide and implicates substantial economic losses to the industry. It has not been reported in Sri Lanka previously. Mycoplasmosis in goat and sheep is caused by Mycoplasma ovis and Candidatus Mycoplasma haemovis, which are the same organism with two different copies of the 16S RNA. Hemotropic mycoplasma is associated with the plasma membrane of erythrocytes and commonly cause subclinical infection in small ruminants. In acute infection, it causes haemolytic anaemia, reduced appetite, poor weight gain, icterus, lethargy and infertility. In this study blood samples were collected randomly from 20 healthy Boar goats in goat breeding Centre belonged to the Department of Animal Production and Health located in Imbulandanda, Matale. Blood smears were prepared from all samples collected and stained with Giemsa and Diff Quick stains and examined under 1000 x magnification using light microscope. Presence of hemotropic mycoplasma in blood smears were scored according to a previously published scheme. DNA was extracted from a 75µl of whole blood from each sample by using commercial DNA extraction kit (QIAGEN®DNeasy Blood and tissue kit) according to manufacturer's instructions. The PCR was performed for on extracted DNA samples for amplification of the 16S rRNA gene using primers specific for Mycoplasma ovis and Candidatus Mycoplasma haemovis according to previously published protocols. Geimsa stain was superior to Diff Quick stain and five samples were identified as highly positive by light microscopy while two samples were identified as slight to moderate positive, three samples were identified as suspected and ten samples were identified as negative. PCR revealed 15% (3/20) of the tested samples were positive for Mycoplasma ovis/ Candidatus Mycoplasma haemovis. Three PCR positive samples were identified as highly positive in light microscopy. This study revealed the occurrence of Mycoplasma ovis/ Candidatus Mycoplasma haemovis in study population.

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# Age Determination and Development of Age Classes for Asian Elephant Skulls Using Mandibular Dentition and Mandibular Morphometry

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Asian elephants are currently listed as an endangered species by the International Union for Conservation of Nature (IUCN). The Sri Lankan elephants, *Elephas maximus* is distributed mainly in the dry zone of Sri Lanka with a population of 3400-5400. With the rapid increase of human population in the island elephant habitats are decreasing and getting fragmented and led to human-elephant conflict and increased orphan elephants, which are reared at Elephant Transit home (ETH), Udawalawe National Park. Age determination of both dead and live animals are essential for the conservation and management of animals. Elephants have unique features like horizontal development of dental plates which grow through out of their life and are used as a tool to determine the age. Number of studies have been conducted on the dentition of African elephant for determination of age. However, studies on age determination of Asian elephants using skeletal remains and dentition are very limited. Previous studies propose six age classes for Asian elephants, i.e. 'Calf', 'Juvenile', 'Sub-adult', 'Adult', 'Mature-adult' and 'Geriatric. This study was carried out using a collection of maxillae and mandibles of elephants available at ETH, with the objective of using these skeletal remains to determine age in a reliable manner by dental ontology according to previously developed age determination protocols. In our study, the dentition of twenty mandibles were used to assign an age class to each Asian elephant skull. In addition to dentition, other measurements associated with age of the skull including morphometry and weight were also collected. Skull morphometry was obtained through two mandibular measurements (length and width). This study found that skull morphometry and weight were highly positively correlated with age ( $R^2$  for length and age = 0.866, width and age = 0.778, weight and age = 0.737, p = 0.000). These correlations can be used effectively for age determination of unknown specimens. However, further refinement of these correlations can be achieved by using morphometric and dentition data from animals of known age.

# Preliminary Investigation of Respiratory *Mycoplasma* Infections in Sri Lankan Goats

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Respiratory diseases are a major cause of death in goat kids and decreased productivity in older goats. Several *Mycoplasma* species cause respiratory diseases in goats. Respiratory Mycoplasma infections have not been reported in Sri Lankan goats. However, previous studies have reported relatively high prevalence of respiratory *Mycoplasma* infection among goats in neighboring countries. The highest incidence was reported in Pakistan (20%) followed by India and Bangladesh (8%). PCR is a rapid and simple method of detection and identification of the Mycoplasma species. The present study investigated the occurrence of Mycoplasma infection in goats using randomly selected lung samples obtained from Colombo a municipal slaughterhouse using PCR. A total of 36 lung samples obtained from the slaughterhouse along with one sample from a goat that died of atypical pneumonia were used in this study. The DNA was extracted using DNeasy blood and tissue DNA extraction kit (Qiagen) according to manufacturer's instructions and PCR was performed using genusspecific primers for Mycoplasma and species-specific primers for Mycoplasma arginini, Mycoplasma ovipneumoniae and Mycoplasma mycoides subspecies capri according to previously described protocols. It was observed that 63.8% of the samples were positive for *Mycoplasma* genus. Additionally, the clinical case was also positive for *Mycoplasma* genus. This study has provided preliminary evidence for the occurrence of respiratory Mycoplasma infection in goats in Sri Lanka and further studies are necessary to identify specific pathogenic *Mycoplasma* causing respiratory infections in Sri Lankan goats.

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# A Preliminary Study to Detect Antibiotic Residues in Fresh Milk Produced in Selected Dairy Farms around Peradeniya Area

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Dairy industry of Sri Lanka has been developed throughout last few years. There is an increased demand for milk and dairy products due to the higher nutritive value of milk. Antibiotics are used to treat dairy cows but inappropriate usage can lead to appearance of their residues in milk. Antibiotic residues in milk can cause adverse health effects in humans. These residues can also interfere with starter cultures causing economic losses in fermented dairy food production. It is essential to monitor antimicrobial residues in milk and introduction of appropriate measures to reduce this risk at both national and international levels. The objective of this study was to determine the presence of antimicrobial residues in fresh cow milk in Peradeniya area using the acidification test. Thirty three (33) milk samples were collected randomly from veterinary teaching farm, medium scale dairy farms, and small scale dairy farms in the Peradeniya area. The samples were transported in ice to the laboratory and tested immediately using acidification test which is a bioassay. The results were interpreted visually by the colour change of the pH indicator present in the test medium. When the sample is contaminated with antibiotic residues, indicator colour persists as it is and if not contaminated, indicator colour changes from purple to yellow after incubation due to the production of lactic acid by starter culture in the test medium. Among 30 individual milk samples (except bulk milk samples), 25 samples (83.33%) were negative and 4 samples (13.33%) were positive for antibiotic residues while only one (3.33%) sample was suspicious. According to the results, there are occurrences of antibiotic residues in cow milk in Peradeniya area. That could be due to lack of knowledge of farmers and not adhering to withholding period due to not having a proper recording system. Acidification test is a rapid and a cheaper screening method which can be used to detect antibiotic residues in milk at the farm level. In order to confirm and quantify the antibiotics involved in milk, confirmatory methods such as GC-MS (Gas Chromatography - Mass Spectrometry) and HPLC (High Performance Liquid Chromatography) are needed.

## Mitochondrial D-Loop Variations of Three Indigenous Cattle Populations of Sri Lanka

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Mitochondrial D-loop sequence variation has led to recognition of two main haplo groups "T" and "I" corresponding to taurine (*Bos taurus*) and indicine (*Bos indicus*) respectively among the cattle breeds of the world. Haplo group "T" has been further categorized into five sub-haplo groups (T1, T2, T3, T4 and T5) and haplo group "I" into two sub-haplo groups (I1 and I2) subsequently. Though several distinct indigenous zebu cattle populations are recognized based on phenotype and management system in Sri Lanka, information on their population structure, genetic diversity and Mitochondrial D-loop sub-haplo group is lacking at present. The overall objective of this study is to unravel the population structure and genetic diversity among three distinct indigenous cattle populations of Sri Lanka by identification of the mitochondrial D-loop haplo groups and determining population diversity parameters. A data set of 181 mitochondrial D-loop sequences of cattle (n=181) inclusive of 56 reference sequences of both taurine and indicine obtained from the NCBI (National Centre for Biotechnology Information) and 125 sequences of indigenous zebu cattle including 51 sequences of White cattle/ Thamankauwa breed, 49 sequences of Lanka cattle/ Batuharaka and 25 sequences of Thawalam cattle were analyzed. Molecular evolutionary genetic analysis revealed that the data set followed the HKY: Hasegawa-Kishino-Yano as the best fit nucleotide substitution model with the lowest Bayesian Information Criterion (BIC score of 8621. 492). The Phylogenetic analyses were done constructing the maximum likelihood tree as implemented in Mega X software and Median Joining Network with PopART software. Results of both analyses revealed that the individuals of the three indigenous populations are clustered within both indicine sub-haplo groups I1 and I2, indicating that none of the indigenous populations are reciprocally monophyletic. Analysis of Molecular Variance (AMOVA) results of the three indigenous populations using Arlequin version 3.1 indicates that the percentage of variance within the populations (96.72) is higher than the percentage of variance among populations (3.28) and the Fixation Index (F<sub>ST</sub>) is 0.03283. Population specific F<sub>ST</sub> indices; SWC=0.03587, SBH=0.03047 and TWH=0.03316, show some evidence of inbreeding. It can be concluded that none of the indigenous zebu populations studied are reciprocally monophyletic and both indicine sub haplo groups (I1 and I2) are found in all three studied populations. Most of genetic variation is present within populations among individuals compared to among populations while all three populations studied are genetically similar with only 3.28% of population variation among them.

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# Nutrient and Mineral Profiles of Sucker mouth Armoured Catfish (*Pterygoplichthys Spp*) Collected from Water Bodies in Sri Lanka

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Sucker mouth armoured catfish (*Pterygoplichthys spp*) is an invasive fish species causing massive threat to the inland fisheries in Sri Lanka. The control of the spread of this species is timely important. Feasibility to consume as a human food is one of the ways to control this species. Therefore the present study was intended to determine the basic nutrient (protein, fat, ash, and crud fibre) and mineral content of sucker mouth armoured catfish in Sri Lanka. For mineral analysis, 25 fish were collected from five reservoirs in Sri Lanka namely, Polgolla, Kalawawa, Trincomalee, Udawalawa and Victoria. Proximate analysis was conducted for finding the nutrient profile by using nine fish samples collected from Polgolla, categorizing in three weight groups. Proximate analysis indicated that the mean crud protein level, fat, crud fibre, ash and moisture were 61.1±6.3 g.kg<sup>-1</sup>, 13.4±5.7g.kg<sup>-1</sup>,  $4.1\pm1.3$ g.kg<sup>-1</sup>,  $22.9\pm5.6$  and  $73.0\pm2.5$ g.kg<sup>-1</sup> respectively. Furthermore, there were no significant (P>0.05) relatioship bewteen the weight of the fish with the any of the nutrients. Mineral analysis revealed that mean levels of Ca. Mg. P. Cu. Zn. Mn. Co. Na. and K were 5.7±0.6 g.kg<sup>-1</sup>, 11.0±0.6, 1.0±0.1, 0.02±0.003, 11.5±0.5, 10.1±0.8, 3.4±0.1 and 5.7±0.1 g.kg<sup>-1</sup> respectively. Only the Cu content in the fish samples had significant (P < 0.05) relationship with the weight of fish. Furthermore, the content of Ca, Mg, Cu, Mn, Na and K in the muscle tissue of the collected fish samples had no significant relationship with the location where fish sample were collected. However, phosphorus value was significantly less in the sample collected from Trincomalee and Udawalawe than other three locations, whereas Zn content in the muscle tissue of armoured cat fish collected from Victoria reservoir was significantly lower than that of other four locations. The nutrient and mineral profile of the sucker mouth armoured cat fish analysed in the present study revealed that the sucker mouth armoured cat fish could be used for human consumption. Further studies may focus on the toxic levels and heavy metal contents in the tissue of this fish type.

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#### Measurement of Tibial Plateau Angle in Normal Crossbred Dogs

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The main objective of this study was to measure tibial plateau angle of clinically healthy crossbred dogs which can be used as a reference value in the case of dynamic stabilization of cranial cruciate ligament rupture. For a successful application of this surgical procedure. the tibial plateau angle measurement is crucial. Forty stifle joints of 20 clients-owned dog carcasses which were free of diseases were included in this study. Mediolateral radiographs with 90-degree flexion of the stifle and tarsal joint were obtained. Age, sex, body weight, and tibial plateau angle were recorded for each dog. Mean values of tibial plateau angle were calculated. The mean tibial plateau angle was  $23.120 \pm 3.460$ . Repeated-measures ANOVA identified non-significant difference (p>0.05) in tibial plateau angle between left and right limbs (left:  $23.570 \pm 4.180$ ; right:  $22.680 \pm 3.380$ ). No significant difference (p>0.05) in tibial plateau angle was identified between joints of male and female dogs (male:  $23.690 \pm 2.830$ ; female:  $22.660 \pm 3.990$ ). The finding of mean tibial plateau angle values of crossbred dogs in Sri Lanka could be used to diagnose cranial cruciate ligament rupture, which is one of the common causes of rear-leg lameness in dogs and a major cause of degenerative joint disease (progressive and permanent deterioration of joint cartilage). Further this finding of tibial plateau angle value can be used in surgical correction of cranial cruciate ligament rupture by tibial plateau levelling osteotomy and tibial tuberosity advancement. In the future, tibial plateau angle measurements may be used to screen dogs suspected of being susceptible to cranial cruciate ligament rupture injury.

# Molecular Characterization and Phylogenetic Analysis of the Chicken Anemia Virus in Sri Lanka

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Chicken Anemia Virus (CAV) has a great economic impact on the poultry industry all around the world. Chicken Infectious Aneamia is caused by CAV and has characteristic symptoms such as anemic condition, thymic atrophy, bone marrow aplasia and hemorrhages. Immune suppression is the major problem caused by CAV which leads to secondary infections that can reduce the productivity and suppress vaccine induced immunity. The objectives of the present study were to detect CAV using a molecular method using Polymerase Chain Reaction (PCR), conduct the molecular characterization and phylogenetic analysis based on the partial sequence of viral protein (VP1) of CAV strains isolated from Sri Lanka. Tissue samples from 19 clinically suspected cases collected from commercial farms in Wariyapola and Avissawella area were used for this study. The PCR was conducted using primers that amplify fragment of VP1 specific genome sequence of CAV. DNA extracted from live vaccine Nobilis<sup>®</sup> CAV P4 was used as positive control. Out of 11 suspected samples collected from Wariyapola, two samples namely FVMAS BVS CAV: 004/19 and 005/19 were positve for PCR. The 8 samples collected from commercial broiler farms in Avissawella area FVMAS BVS CAV:001/19, 002/19 and 003/19 gave three postive results. After sequencing the PCR products, phylogenetic analysis was performed. The phylogenetic analysis revealed that FVMAS BVS CAV 001/19 and 003/19 isolated from Avissawella and FVMAS BVS CAV 005/19 virus strains isolated from Wariyapola area showed the highest percentage identity Nobilis®P4 vaccine strain and phylogenetic analysis reconfirmed the results obtained from pairwise sequence comparison. The lowest identity between FVMAS BVS 005/19 isolated from Wariyapola area and FVMAS BVS CAV 002/19 strain isolated from Avissawella area were observed while FVMAS BVS CAV 002/19 showed the lowest identity to the Nobilis P4<sup>®</sup> vaccine strain. In conclusion, this study was able to optimize PCR-based molecular method to diagnose CAV in Sri Lanka. Findings of this study warrant further molecular characterization and phylogenetic analysis of CAV with more available VP1 gene sequences to understand the molecular epidemiology of the disease condition in Sri Lanka.

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# Microbiological Quality of Fish Meal Used as Raw Material in Poultry Feed Sold in Wholesale Shops of Selected Areas in the Kurunegala District

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Quality of feed is important as it directly affects the production and health of the poultry. The poultry feed industry has substantially developed with its counterpart; the poultry industry over the last few decades however, Sri Lankan feed manufacturers are often focused on the nutritional quality rather than the microbiological quality. As animal origin feed ingredients are more liable for microbial contamination and feed can act as a source of pathogens to poultry and industry workers, the present study was conducted to assess the microbiological quality of fishmeal; one of the main animal origin feed ingredient used in formulating poultry feed in Sri Lanka. The study was conducted in Kurunegala, the district with the highest poultry population. Convenient sampling was done to collect 26 fish meal samples (each weighing approximately 150g) from 13 main poultry feed wholesale shops scattered in the study area. Fish meal samples were analyzed to determine total viable bacterial count, Enterobacteriaceae count and the presence of Salmonella. As Sri Lankan standard for microbiological quality of poultry feed/ingredients is currently unavailable European standard for feed /feed materials derived from animal by-products was used to interpret the acquired data. This investigation revealed that the total viable bacterial count in Sri Lankan fish meal would vary between  $0.2 \times 10^3$  and  $1.1 \times 10^6$  colony-forming units per milliliter. The genus Enterobacteriaceae was detected in 15/26 (57.7%) samples and of these 3 (11.5%) samples had *Enterobacteriaceae* counts above the European acceptable limits. Salmonella contamination was detected in 3/26 (11.5%) samples when European standard highlights any Salmonella contamination is unacceptable. Therefore, 5/26 (19.2%) fish meal samples tested in this study were below the European standard for feed/feed materials derived from animal by-products. Tropical environment, handling, and the local storage practices may have contributed to the higher microbial counts observed in fish meal available in Sri Lanka. As the microbiological quality of the final product may vary depending on various local factors; raw materials, manufacturing, handling, packing, storage etc. this study highlights the necessity of the establishment of Sri Lankan standard for microbiological quality of poultry feed/feed ingredients.

# Determination of Viability of Lactic Acid Bacteria in Commercially Available Yoghurt in Peradeniya City Area

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Yoghurt is one of the oldest fermented milk products, popular all around the world. It is produced by the process of fermentation by lactic acid bacteria including Streptococcus thermophilus and Lactobacillus bulgaricus. These organisms belong to the group of bacteria collectively called as "probiotics". Health benefits of yoghurt are mainly derived from the probiotics. Therefore, the viability of probiotics is important in order to provide health benefits. International Dairy Federation propose that lower limit of the probiotic count should be 10<sup>6</sup>CFU/g. Different factors such as storage time, quality of starter culture, storage temperature may affect the viability of the probiotics. The objective of this study was to determine the viability of lactic acid bacteria in commercially available voghurt in Peradeniya city area. Twenty-two yoghurt samples were bought randomly from different shops in Peradeniya area during the period of three months from August to October. The samples were transported in ice to the laboratory and the microbial viable cell counts in voghurts were determined immediately. Enumeration of bacteria was done according to the standard spread plate method and incubated at 37°C for 24-48hours. The pH of each sample was measured using a pH meter. According to the results, 22.72% samples had less than 10<sup>6</sup>CFU/g and 72.72% samples had more than 10<sup>6</sup>CFU/g of viable count of bacteria. This study reveals that majority of yoghurt samples (72.2%) contain probiotic bacterial counts above the recommended level of  $10^{6}$ CFU/g. The pH of the samples at the time of culturing were within the normal range (4.01-4.66 pH). The lower level of probiotic count could be due to improper storage temperature, longer transportation time, insufficient level of inoculation of probiotics etc. Therefore, this study can be expanded to determine viability of probiotics in the samples from other areas of Sri Lanka as well as to determine the factors affecting the viability of probiotics. This will help to improve the health benefits of yoghurt by increasing the awareness among the yoghurt producers.

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# Nocturnal Behavior of Free-Ranging Orphaned Asian Elephant Calves in the Udawalawe Elephant Transit Home

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Studies on nocturnal behaviors of elephants are rare and even lesser for elephant calves. Studying of nocturnal behavior is important for understand fully the activity pattern of the elephant calves. The objective of the present study was to build a nocturnal activity budget of the orphaned elephant calves at elephant transit home, Udawalawe. The calves used were approximately 6 months to 6 years old and observations were made for a period of 104hrs in 13 days. The observations on their behavior was performed in 10min intervals and reported in three time blocks, i.e. from 19.00 to 22.00 hrs, 22.00 to 2.00 hrs and 2.00 to 5.00hrs. Data were collected via direct observation by using instantaneous group scan sampling on 64 calves including 37 males and 27 females. The results showed that the calves spent the highest percentage of scans in feeding (45.5%) and lying resting (32.8%). Calves exhibited both standing and lying rest, and they spend more time lying rest (32.8%) than standing rest (11.9%). The percentage of scans spent in lying resting varied significantly between the time blocks and it occurred mostly in 2.00-5.00hrs time block. The percentage of scans spent on feeding also varied significantly between the time blocks. We found that calves spent a higher percentage of scans in feeding during early hours of the night (19.00-22.00hrs). Some of our observations are similar to the observations made in the previous studies while some other observations are different to what reported for captive and wild elephants. The observations made on this study will be useful in management and welfare of captive elephants.

# Determination of the Level of Antioxidants Released from Curry Leaves (Murraya koenigii) when Heated for Different Time Intervals

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The research is focused on studying the level of Antioxidants, total phenol and total flavonoids extracted of *Murraya koenigii* leaves when heated for different time intervals in an aqueous media at 90°C. Both compound leaves and leaflets were tested separately. Leaves were also tested as whole dried leaves and dried powder form. Freeze dried water extracts from 1g of curry leaves were used to perform DPPH (2, 2-diphenyl1-1-picrylhydrazyl-hydrate) assay, Folin-ciocalteu colorimetric assay and modified aluminum chloride colorimetric assay. Antioxidant potential was evaluated using the  $IC_{50}$  values. Total phenol levels were evaluated using the galic acid standard curve and GAE (galic acid equivalent) values. Total flavonoid levels were evaluated using the Ouercetin standard curve and QE (quercetin equivalent) values. The aqueous extracts of dried leaves showed an increase in the level of antioxidant activity (compound leaves - by 43.75%, leaflets - by 84.09%, considering IC<sub>50</sub> values) after 60 minutes of heating time. However, the aqueous extracts of dried powder showed a decrease in antioxidant activity (compound leaves – by 14.03%, leaflets – by 86.44%, considering IC<sub>50</sub> values) after 60 minutes of heating time. The unheated samples of dried powder gave higher antioxidant activity than the unheated samples of dried leaves. To get maximum antioxidant potential it is recommended to use powdered Murraya koenigii leaves without heating or to heat 10 min at 90 °C, and if dried leaves are used, the maximum antioxidant activity can be obtained within 45 to 60 min at 90 °C. Compound leaves of Murraya koenigii showed higher antioxidant activity than leaflets.

# *In-vitro* Investigation on Anti-coccidial Activity of Different Plant Aqueous Extracts as Alternatives for Coccidiosis Control in Poultry

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Avian coccidiosis is one of the most important diseases of poultry since it is a major cause of mortalities in poultry farming worldwide. The performance and productivity of poultry are also adversely affected by the coccidial infection. Use of anti-coccidials and vaccines are the only available options for its control with numerous drawbacks hence the control of coccidiosis is a challenge. Main disadvantages of using anti-coccidials (coccidiostats and coccidiocidals) are development of drug resistance and consumer safety concerns over drug residues in poultry products. Therefore herbal preparations could be an alternative for treatment and control of coccidiosis in chickens. This study was conducted to evaluate anticoccidial effect of different medicinal plants Areca catechu fruit, Vernonia cinerea leaves, Ananas comosus leaves, Tithonia diversifolia leaves, Carica papaya leaves, Cyperus rotundus leaves and Momordica charantia seeds in-vitro and to determine their ability in controlling the coccidiosis in chickens. The results were compared with Moringa oleifera leaves which are a previously known herbal anti-coccidial. The results indicated that, aqueous extraction of Areca catechu immature fruit has remarkable anti coccidial effect while V. cinerea, A. comosus, T. diversifolia, C. papaya, C. rotundus and M. charantia have no or little anticoccidial effect comparable to the positive control *M. oleifera* leaves extract. At the dose rate of 400mg/ml Areca catechu can be an effective alternative in the field to control avian coccidiosis and further in vivo investigations should be conducted to investigate the potential therapeutic use of this plant.

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## Investigation of a Chronic Visceral Granulomatous Condition in Pearlscale Goldfish (*Carassius auratus*)

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Chronic visceral granulomatous inflammation is occasionally seen in food fish as well as in ornamental fish. Certain bacterial species such as *Mycobacterium* spp and FLO (*Francisella* like organisms) have been reported to cause this condition. They are associated with focal, multifocal or diffuse granulomatous lesions with non-specific clinical signs such as abdominal distension and exophthalmos. The objective of this study was to investigate a chronic visceral granulomatous condition in a group of Pearlscale goldfish brood stock. A total of four goldfish showing signs of abdominal distension, scale protrusion, haemorrhages and chronic, non-healing ulcers on the skin were necropsied. Accumulation of ascitic fluid in the abdominal cavity and agglomeration of internal organs into a single mass due to extensive adhesions were observed at necropsy in all four fish. One fish had multiple, white colour nodules/granulomas in the internal organs. Examination of external parasites, observation of squash tissue smears for the presence of acid fast rods, culture isolation of non-fastidious bacteria and mycobacteria from internal organs, and histopathological investigation of internal organs were attempted on all four fish. Furthermore, DNA extracted from kidney tissue of each fish were used in polymerase chain reaction (PCR) assays to detect the presence of *Mycobacterium* spp, *Francisella* spp. and Red Sea Bream Iridovirus. Isolated mycobacteria were further confirmed by acid fast staining and PCR. Culture isolation findings confirmed that all four fish harbor mycobacteria at least in two or more of the internal organs (kidney, liver, spleen and intestine). Mycobacterium PCR using kidney tissue was positive only in one fish. This fish had histopathological lesions suggestive of early granuloma formation and was also positive for *Francisella* like bacteria by PCR. Three fish were positive for RSIV by PCR. Even though the results are inconclusive, a possible involvement of mycobacteria in the visceral granulomatous inflammation in pearlscale goldfish was confirmed. However, further studies are needed using more infected goldfish to understand the multiple etiologies that possibly be associated with this condition. Mycobacterioisis is a zoonotic disease and depopulation of infected fish followed by disinfection is the only choice to control this chronic infection in ornamental fish.

# Determination of Age, Causes and Patterns of Death in Dogs of Police Kennels Division in Sri Lanka over a Decade

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There was a concern regarding the higher death rates among the dogs of Sri Lanka Police Kennels (SLPK) by the authorities concerned thus, this study was undertaken to determine the causes of death, age and the patterns of deaths among the SLPK dogs. Records of 227 dogs that died during the period from 2009 to 2018 were scrutinized to ascertain the primary cause of death by reviewing the necropsy report of each dog. Causes of deaths were categorized under 12 identified disease categories. With an average annual death percentage of 9.3, the mean age at death of a SLPK dog was 5.41 years. The mean age at death was 6.05 years when the deaths of pups (less than 1 year) were excluded. Leading causes of deaths were renal diseases (25.11%), geriatric conditions (18.5%), cardiac diseases (14.98%), viral diseases (11.45%) and neoplastic conditions (7.93%). Explosive detection dogs had higher risk of death associated with renal diseases, hepatic diseases and neurological diseases in comparison with the narcotic detection and criminal tracking dogs. Predominant cause of deaths in narcotic category dogs was cardiac diseases. No significant relationship was found between geographical location of the dog and the prevalence of renal diseases. As it was evident that the present record keeping method has certain drawbacks, it would be highly suggestive to maintain an advanced record keeping system in the SLPK to monitor the intensiveness of activities, regular health check-ups etc. of dogs, which enable quick retrieval of data and taking appropriate corrective measures promptly. It is recommended to implement training and working hours depending on geographical locations of animals, and early intervention when behavioral changes or signs of health deterioration are noticed in order to reduce deaths. Further research should be initiated to identify the factors leading to deaths, which will help reduce the occurrence of diseases and increase life span of valuable dogs in SLPK.

# Accuracy of Fine Needle Aspiration Cytology in Diagnosis of Internal and External Tumors in Dogs

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Cytopathology carried out using fine needle aspiration biopsy (FNAB) is the fastest way to diagnosis of a variety of internal and external tumors in dogs. It is popular among the clinicians as it is less invasive, rapid, cost effective and patient friendly method. However, few studies have conducted to compare the accuracy of cytopathology with that of histopathology which is considered as the gold standard of the diagnosis of the tumors. Further different staining techniques are used to identify the cells in cytopathology to enhance the accuracy of diagnosis. Therefore, the objective of this study is to compare the accuracy of diagnosis of cytopathology carried out using Leishman and Diff Quick stains with that of histopathology of tumors. Ten dogs presented to the Veterinary Teaching Hospital, University of Peradeniya for a six months period with abnormal internal or external tissue masses which referred to surgical excision were included in this study. Cytopathological smears were prepared using FNAB method from surgical excised tumors and stained with Diff Quick and Lishman stains. Same surgical biopsies were used to prepare histopathological sections and stained with Hematoxylin and Eosin. Diagnosis were done at three levels of higher order as stated by Cowell and Tyler (1999) using ten clinicians. A 100% accuracy was observed in differentiation of neoplasia from inflammatory lesion using cytopathological smears prepared using both stains. However, the accuracy of diagnosis of the type of neoplastic lesion was dropped to 79% when used smears stained with Leishman and 80% in smears stained with Diff Quick. The lowest accuracy was reported for the identification of Mixed and grannulosa cell tumors and level of accuracy dropped 20 and 10% respectively. This is mainly due to inability of the two stains to discernible the nuclear changes which is important in accurate diagnosis of these two tumor types. Further, there was no significant difference was observed between the two staining techniques used. The results of this study confirmed the possibility of using cytopathology as a reliable, useful and rapid diagnostic tool to evaluate some types of internal and external tumors in small animal practice.

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# Ultrasonographic and Microbiological Evaluation of the Prostate Gland and the Urinary Bladder of Adult Male Dogs with Concurrent Urinary Tract and Prostatic Infections

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Prostatic diseases are a common clinical condition in intact, male dogs. There is a progressive increase in prostatic diseases with advancing age. Bacterial prostatitis could occur secondary to lower urinary tract diseases or diseases of the prostate. Once the organisms establish in the prostate gland, organism may re-infect the urinary tract. The present study investigated the association between recurrent urinary tract infections and prostatic abnormalities of adult male dogs. A total of sixteen male dogs presented with a history and clinical signs suggestive of prostatitis and/or urinary tract infections were subjected to general clinical examinations. Out of that ten dogs who were positive for concurrent UTI and prostatic diseases were selected as the study group. Ultrasonographic evaluation of prostate was performed using two-dimensional trans abdominal sonograms with a phased-array transducer at a frequency of 7.5 Mhz. Images of both prostate and urinary bladder were obtained and the ultrasonographical findings of each dogs were recorded. Urine samples were collected aseptically by catheterization and prostatic wash samples were obtained via ultrasound guided prostatic massage. Both urine and prostatic fluid samples were subjected to microbiological culture and biochemical tests to identify the organisms causing the infections. Prostatic disorders detected ultrasonographycally in the patients include, abscess, cysts, tumor, hyperplasia. Abscesses appeared as anechoic or hypoechoic spherical collections of echogenic fluid with poorly defined borders and whereas cysts appeared as hyperechoic fluid filled areas. Eight of the 10 patients studied contained bacteria in the prostatic wash. In all eight patients, same organisms were isolated from the urine samples. Two prostatic washes did not yield any bacterial growth. Organisms isolated from the samples included Klebsiella spp., Streptococcus spp., Proteus spp. *Escherichia coli* and *Staphylococcus spp.* It is evident from our study that the patients with prostatic diseases could develop lower urinary tract infections and most often those infections are recurrent. Patients with different prostatic diseases could develop bacterial prostatis as the normal defense of the prostate gland is disturbed. When the bacteria established in the prostate gland it is difficult to eliminate due to presence of blood prostate barrier. Therefore these organisms may cause recurrent infections.

# Identification of Common Marine Fishing Practices and Marine Food Fish Species Caught in Fisheries District, Negombo

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Capture fisheries production and aquaculture production are primary sectors contributing to the whole fisheries production in the world. Capture fisheries sector has two sub-sectors as marine capture fisheries and inland capture fisheries. Capturing fish species that are naturally living in the sea is known as marine capture fisheries. While fish is an essential part of a healthy diet, out of the world's protein requirement, 16% is supplied by fish resources. The marine fisheries sector plays a major role in the economic and social life in Sri Lankans. Sri Lanka has several fisheries districts where we can witness a culture associated with marine fisheries. Negombo is one such fisheries districts which annually supplies a considerable amount of fish production. The Negombo fish market which is the second largest fish market in the country, supplies an excellent atmosphere to thousands of fish vendors and fish consumers in selling and buying of fish. Although fish is a significant part of a day to day meal in Sri Lankans, a very few people have better knowledge of identifying fish species. Creating a guide for the purpose of easily identifying food fish species was one of the objectives of the present study. Another aim was to identify significant fishing practices in the Negombo area. As a result of this study, details about marine food fish species caught in the Negombo area along with their common names and identification features is presented as a soft copy (CD) with this report. Further, it was found that the most commonly harvested and mostly demanded fish species were goldstripe sardinella (සාලයා) and travelly species (පරාවා) respectively. In total, 80 marine food fish varieties were found during the survey. Further, the fish species most commonly caught by fishermen had less demand among customers. According to the results, fishermen in the Negombo area have involved in two types of fishing operations like coastal fisheries and offshore/deep-sea fisheries. Negombo marine fisheries industry can be developed vastly by introducing new technologies for fishing and by introducing good hygienic practices while handling, processing and selling of marine food fish species.

# Effect of Feeding Bitter Gourd Seed Powder on Egg Yolk Color and Production Parameters in Hy-Line Strain, White Leghorn Laying Hens

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This study was carried out to evaluate the effect of adding bitter gourd (Momordica *charantia*) seed powder into the ration on egg yolk color and production parameters in layer hens. For this purpose, 25 weeks old hy-line strain white leghorn laying hens were randomly selected from six pens with deep litter closed house management system. Ninety birds were allotted in completely random design (CRD) with three groups (n=30 per group) and each group consisted of 03 replicates (n=10 per replicate). The three groups were, control; a ration was prepared without adding of any extra fat source to regular layer ration which was available commercially (C), treatment 1; the regular layer ration was added with 1.5% (w/w) desiccated coconut(T1) and treatment 2; the regular later ration was added with 1.5% (w/w) bitter gourd seed powder (T2). Hens were fed with each diet with an amount of 115g/hen/day and study period was 12 weeks. 8 eggs were collected randomly twice a week from each replicate. Egg yolk color was determined using DSM yolk color fan having tabs from 1 to 15. The ANOVA was used to compare production parameters and egg yolk color. The egg production and the body weights of the birds were comparable among treatment groups. The egg volk colors were significantly different among the treatment groups. Egg weight was reduced with the advancement of trial period. Interestingly, the egg yolk color in T2 group was significantly increased (p < 0.05) as compared with other groups. Thus, adding of a small dose of crushed bitter gourd seeds into the ration significantly enhanced the egg yolk color in layers. However, feeding bitter gourd seeds did not exert any negative effect on production performances of birds. This color enhancement may have been caused by carotenoids present in bitter gourd seeds or the alteration in retinoid metabolism in birds.

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# **Efficacy of a Newly Developed Veterinary Topical Product** to Treat Mange, Fungal and Bacterial Infections in Dogs

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A newly developed veterinary topical product for dogs and cats was evaluated for efficacy against demodectic and sarcopic mange mites, bacterial, and fungal infections in naturally infested dogs. Thirty dogs were treated topically with the product at the proposed minimum dosage as once in two days apart till recovery or end of the observation. Mite numbers, bacterial counts and fungal counts were estimated from skin scrapings just before and after the treatment (day 0, 21 and 42). Clinical signs of mange, bacterial and fungal infections on each dog were assessed by analyzing the extent of lesions in the skin scrapings. Efficacy of the treatment was evaluated based on a reduction in mite numbers, fungal count, bacterial count and an assessment of the clinical signs associated with canine demodicosis, sarcoptic mange and fungal, bacterial infections. Statistical analysis were done for the each infection type using nonparametric Wilcoxon signed rank test and the significance was evaluated. Mange mite count before treatment was not significantly (p>0.05) different with that of after treatment. However, the clinical signs of mange were markedly reduced in four cases out of nine cases of the mange infection. The bacterial and fungal count after treatment was significantly (p<0.05) reduced when compared with that before treatment. Therefore, the newly developed veterinary topical product may not be effective for the mange infection. However, the product could be effective for the treatment of the bacterial and fungal infections in canine species. Although the complete recovery of the fungal and bacterial infection was observed even within 3 weeks in some cases, some other cases took more than seven weeks for the full recovery. The results of the study would recommend to use antiparasitic agent along with this new product, otherwise this newly developed product should include a strong anti-parasitic agent as one of the raw material to make effective for mange infection. Further research should be done to evaluate the side effects of the product in long term application.

# Verification of the Performance of an Introduced Commercially Available ELISA NS1 Kit for Early Diagnosis of Dengue Fever

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Enzyme-Linked Immunosorbent Assay (ELISA), is a method for detecting and quantifying substance such as peptide, protein, antibodies, antigens and hormones. ELISA involves the use of enzymes and the specific binding of antibody and antigens. It can be divided into four major types: direct, indirect, sandwich and competitive. There are commercially available ELISA kits to check or test for many diseases in human and animals. When an ELISA kit is adopted to analyze a sample, the most reliable kit should be selected from many commercially available ELISA kits. Before using ELISA kits for diagnosis, it must be validated and verified. The sensitivity, specificity, and accuracy of commercially available ELISA kits should be evaluated during validation and validation of the kit. If a new test or test method is introduced in the General Diagnostic Laboratory, verification or validation must be performed. In addition, any further modification of the existing test procedures will require further validation work. ISO 15189 demands that verification or validation of each investigation procedure to verify both the correct application and correct performance of a diagnostic test is essential. This study is to verify the performance of the commercially available ELISA NS1 kit. For this study, samples were taken by 20 patients' serum samples that were already tested and diagnosis was confirmed previously by using PCR and/or Viral isolation and/or IgM seroconversion. Introduced new ELISA NS1 antigen kit showed 100% sensitivity, specificity and accuracy respectively. Thus, sensitivity, specificity and accuracy of the ELISA NS1 antigen kit is satisfied the manufacture's claim. The new ELISA NS1 kit which was validated and verified would be very useful for early detection of dengue infection in human and in turn the management and treatment of dengue patients.

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# Antimicrobial Susceptibility Profile of *Aeromonas* spp. Isolated from Fresh Water Aquarium Biofilms

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Biofilms are commonly observed in freshwater aquatic ecosystems and harbor a wide range of microorganisms including antibiotic resistant bacteria. Therefore, aquarium biofilms provide an ideal setting for the acquisition and dissemination of antimicrobial resistance. Aeromonads are Gram negative bacteria ubiquitous in fresh water environments and are capable of forming biofilms both on biotic and abiotic surfaces. Therefore, Aeromonas spp. can be used as indicator organisms to assess antimicrobial resistance in biofilms. In the present study, we isolated motile *Aeromonas* spp. from biofilms collected from freshwater ornamental fish aquaria and assessed their susceptibility against five commonly used antimicrobials in aquaculture. A total of 15 aquarium biofilm samples were collected from 15 different aquaria located in Kandy district. Primary isolation was done on trypticase soy agar (TSA) and incubated under room temperature for 24 hours. All isolates that were Gramnegative, motile rods were subjected to a series of biochemical tests. Based on the results, a total of 22 presumptive, motile Aeromonas spp. were identified. Aeromonas spp. was isolated from 93% of the biofilm samples cultured. Aeromonas spp isolated in the present study showed different levels of susceptibility for the five anti-microbial agents. All the isolates were resistant to amoxicillin and metronidazole while 50% and 45.45% were resistant to tetracycline and erythromycin respectively. Hundred percent (100%) susceptibility was observed for ciprofloxacin. A significant number of isolates (63.63%) were multiple drug resistant (MDR). Multiple antibiotic resistance (MAR) index in Aeromonas spp. isolated in the present study ranged from 0.4 - 0.8. Bacteria having MAR index  $\geq 0.2$  usually originate from a high risk source of contamination. Our study confirms that Aeromonas is one of the commonly occurring bacteria in biofilms present in tropical freshwater aquaria and these biofilms can be a source of MDR Aeromonas spp. Presence of these multiple antibiotic resistant bacteria in aquatic biofilms represent a potential public health risk. Moreover, establishment of biofilms containing MDR Aeromonas strains in aquaculture environments will facilitate persistent infections in fish that are not easily treatable. Misuse and overuse of antimicrobials in ornamental fish industry might have led to emergence of antibiotic resistant aeromonads.

# Screening of Poultry Meat Obtained from Retail Outlets in Peradeniya City Area for Antimicrobial Residues

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Poultry industry is the fastest growing component of meat demand in the world as well as in Sri Lanka. People prefer broiler meat because it is an excellent source of protein and micronutrients which are essential for growth and development of human body. Occurrence of antibiotic residues is one of the major public health concerns in modern food industry due to the adverse health effects to the consumers. Therefore the Veterinarians and livestock producers are mainly responsible to avoid antimicrobial residues by monitoring and adhering to withdrawal periods after administration of drugs to the poultry. The objective of this study was to screen commercially available broiler meat in Peradeniya city area for antibiotic residues. To achieve this objective, 35 broiler meat samples obtained from retail outlets in Peradeniya city area were screened by Six Plate Test (SPT). The samples included randomly selected breast muscles (8), thigh muscles (12) and livers (15). SPT is a bio assay technique and a useful tool in preliminary identification of antibiotic residues in animal originated food. Detectable inhibitory zone around the sample was considered as a positive sample whereas no inhibitory zone around the sample was considered as a negative sample. Out of the 35 samples, 7 (20%) had given positive results while others showed negative results. Out of the 7 positive samples, there were 4(23%) positive liver samples while there were 2 (16.6%) positive thigh muscles. There was only 1 (12.5%) positive breast muscle sample. SPT is a screening test. Therefore, it is necessary to confirm the presence of antibiotics by confirmatory methods such as HPLC (High Performance Liquid Chromatography), TLC (Thin Layer Chromatography) and GC-MS (Gas Chromatography coupled with Mass Spectrometry). It is also important to increase the awareness among broiler meat producers to avoid antibiotic residues thereby assure the consumer safety.

# A Preliminary Study on Prevalence of Bacterial Contamination of Wounds in Dogs in the Kandy Area

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Skin wounds are a common complication with dogs. The proper knowledge on physiology of wound healing process and application of appropriate therapeutic intervention is essential in successful treatment and management of wounds in dogs. Many of these wounds are colonized by bacteria. Some wounds may show signs of infections. Wound infection can delay wound healing and increase the healthcare cost. Therefore, a prospective study was performed on dogs with different types of wounds to identify the bacteria present in these wounds. A total of 27 wound samples were taken from dogs presented to The Government Veterinary Hospital, Gatambe during the period of August-September, 2019. Samples were obtained using cotton swabs and c ultured on blood agar and MacConkey agar. Of these 27 samples only one sample was negative. The most common type of wound was dog bite wounds with occurrence of 12 out of the 27 cases (44.44%). Other wound types were laceration (18.51%), Pyoderma (11.11%), ruptured abscess by scratching (7.40%), dermatitis (7.40%), post-surgical complication (3.70%), and road traffic accident (3.70%). Staphylococcus species (74.07%) was the most frequently isolated bacterium whereas other isolated bacteria were Streptococcus species (22.50%), E. coli (12.5%), Pseudomonas species (11.25%), Pasteurella species (7.5%), Klebsiella species (6.25%), and Proteus species (6.25%). Less frequently isolated bacteria were Gram positive Coccobacilli (3.75%), Enterobacter species (2.5%), Bacillus species (1.25%) and other Gram-positive rods (1.25%). According to our study, it was revealed that all most all wounds had a mixture of bacteria. However, Gram positive bacteria were isolated in 25 wounds out of 27 whereas 23 wounds had Gram negative bacteria. Only one type of wound comprised of only Grampositive bacteria. Current study is further extended to analyze the antimicrobial susceptibility test on commonly isolated genera.

# Use of Urine Protein, Creatinine Ratio in the Reassessment of Renal Function in a Group of Working Dogs at the Police Kennels, Kandy

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Proteinuria is the presence of abnormal quantity of protein in urine or abnormal excretion of protein in to the urine. Proteinuria is an independent risk factor for renal diseases and measuring proteinuria is a sensitive and specific screening tool in patients who are at risk of developing renal disease. Very small amount of protein can be present in urine of healthy dogs but the elevation of protein levels in urine is indicative of kidney damage. Proteinuria can be measured by several tests. Urinalysis is a remarkable tool that can reveal many of the diseases; it is readily available and inexpensive tool for the diagnosis of renal disease. The urine dipstick colorimetric test is the usual first line semi quantitative screening test for the detection of proteinuria in dogs. However, false positive reactions are common. Many studies have revealed that urine protein, creatinine (UPC) ratio is the gold standard method in measuring proteinuria. This is one of the commonly used inexpensive laboratory test. Hence in this study we used UPC ratio as a quantifying measurement of proteinuria in a group of working dogs of police kennels Sri Lanka, to compare their UPC ratios before and after commencing their formal training and duty. In the working group, mean UPC ratio in explosive group was 1.57±1.45, narcotic group was 2.29±1.28 and tracking group was 0.760±0.105. According to the renal staging system used by the International Renal Interest Society, it was concluded that, this group of dogs had elevated UPC ratios after the training and duty for one year. When compared with a similar study carried out several years ago, we noticed that in narcotic, explosive and tracking groups of this study were having higher UPC ratios. In his study, we have successfully used UPC ratio to identify the working dogs that are vulnerable to renal disease.

# Prevalence and Antibiotic Resistance of *Staphylococcus spp.* Isolated from Raw Milk and Milk Products in Pannala Veterinary Division

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Staphylococcus spp. is a commensal or opportunistic pathogen which found asymptomatically on the human body. However, Staphylococcus is a significant cause of foodborne diseases in the world. The objectives of this study were to determine the prevalence and antimicrobial resistance of Staphylococcus spp isolated from raw milk and milk products in the Pannala Veterinary Division. A total of 66 samples including 30 raw milk samples from 10 Milk Collecting Centers and 36 dairy products were collected. Samples were analyzed by the standard microbiological methods to isolate and identification of *Staphylococcus spp*. *Staphylococcus spp* was isolated from raw milk (93%), ice cream (83%), flavored milk (92%) and UHT milk (8%). A total of 39 isolates were possible to revive and those were subjected to antimicrobial susceptibility testing using 09 antimicrobial impregnated disks namely; Amoxycillin (AMX), Ampicillin (AMP), Cefalexin (CN), Ceftazidime/ Clavulanic acid (CAC), Chloramphenicol (C), Ciprofloxacin (CIP), Erythromycin (E), Gentamycin (GEN) and Tetracycline (TE). The highest number of isolates were resistant to AMP (76.92%), CN (71.79%) and CAC (71.79%). The highest sensitivity was observed for GEN (87.17%), C (87.17%) and CIP (76.92%). The MAR index was measured and isolates exhibited 5 different antibiotic-resistant patterns with MAR index ranging from 0.1 to 0.5. MAR index revealed 37.5% of the isolates were with MAR index greater than 0.2. Eleven different antimicrobial patterns were observed and the highest MAR index of 0.5 showed CAC, AMX, E, CN, AMP resistant pattern. Meanwhile, lowest MAR index of 0.1 was demonstrated CAC, CN or E resistant pattern. The present study indicates the potential public health risk associated with the milk and milk products in Pannala Veterinary Division. Therefore, stringent surveillance, regulatory measures and appropriate interventions are required to minimize S. aureus contamination and prudent antibiotic usage that can ensure consumer safety.

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#### **Evaluation of Field Sterilization Program of Dogs**

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Quality assessment of field sterilization programs of dogs includes economic, animal welfare, social, and epidemiological evaluations. This study mainly focuses on animal welfare assessment related to the field sterilization program. Animal welfare assessment consists of an evaluation of health, behavior and physiology. This study illustrates on animal health and physiology aspects of animal welfare. The field sterilization program used in Sri Lanka is known as the Capture Neuter Return program. Capture Neuter Return program has seven significant steps namely; capturing, transporting, kenneling, preoperative care, surgery, post-operative care and release. Details related to all these steps such as capturing method, capturing time, transporting method, transporting time, premedication, surgery time, post-operative care, recovery time were collected using a structured questionnaire. In addition, death rates and post-operative complications were taken in to consideration. Details of both domestic and stray dogs were collected in Thanamalwila MOH area. Three hundred seven total samples were collected and among them 259 were owned and 48 were stray animals. Stray dogs were captured and transported to the clinical site, while owned dogs were brought by its owner to the program site. In this study, surgery time and recovery time was compared between these two dog populations to observe any significant effect on capture and transport methods on recovery time. The correlation between surgery time and recovery time were analyzed for both stray and domestic dogs. All the data was analyzed using Minitab statistical software. Two sample t-test was performed to identify significant difference between surgery time of domestic and stray dogs and significant difference between recovery time of stray and domestic dogs. Then correlation function was performed on surgery time and recovery time to find out significant correlation between two variables. There was no significant difference of surgery time and recovery time between two dog populations and there was no significant correlation between surgery time and recovery time. On this basis, it can be concluded that there are no significant effects on handling and transporting stressors to the post-surgical recovery time.

# Characterization of Aerobic Microflora in Wounds of Elephants at the National Zoological Gardens, Dehiwela and Pinnawala Orphanage

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The Sri Lankan elephant is one of three recognized subspecies of the Asian elephant. Chronic wounds are one of the most common health issues in captive elephants in Sri Lanka. Objective of this study was to identify the organisms associated with wounds of elephants in Pinnawela Orphanage and Dehiwela Zoological Gardens and determine antibiotic sensitivity of the isolated organisms. Samples from the wounds of 16 elephants were collected using sterile swabs in transport medium. Samples were cultured on blood agar and incubated at 37°C for 24 hrs. Gram staining was done for selected colonies and sub cultured on blood agar and pure isolates were obtained and isolates were identified by using conventional biochemical tests. Antimicrobial sensitivity tests were performed according to the CLSI guidelines to represent several classes of antimicrobial drugs. Total 18 isolates were recovered and identified. Out of which 11 were Gram negative rods, 6 were Gram positive cocci and the remaining isolate was a fungus. Most of the Gram-negative rods were Proteus isolates (45.45%), followed by Klebsiella spp (36.36%) and Pasteurella spp (18.18%). Among 11 Gram-negative rods, 72.7% were resistant to amoxicillin, 9.09% to amoxicillin clavulanate, 18.18% to cefuroxime, 45.45% to cefotaxime, 27.27% to ciprofloxacin, 4.09% to cotrimoxazole, 9.09% to gentamicin, 45.45% to ceftazidime and 45.45% to tetracycline. Among six Gram-positive cocci four isolates were staphylococci while two were streptococci. Among the staphylococci, 71.42% were resistant to amoxicillin, 14.28% to amoxicillin clavulanate, 14.28% to cefuroxime, 14.18% to cefotaxime, 14.28% to ciprofloxacin, 14.28% to gentamicin, 42.85% to ceftazidime and 14.28% to tetracycline. All streptococci are susceptible to all antibiotics. From this study, it can be concluded that the elephant population in Zoological Garden Dehiwala and Elephant Orphanage Pinnawala are susceptible to most antibiotics which were used in this study.

# A Web-based Information System of Veterinary Services in Gampaha District of Sri Lanka

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Companion animal health care has become a field of importance all over the world along with the one health concept. One of the major problems faced by the health care community throughout the last decade was uneven geographical distribution of resources and services. Moreover, lack of availability of information on such service providers has enhanced the problems faced by the pet owners. In the modern world, internet is the most frequently used source of dissemination of health care related information. Thus, the objectives of this study were to assess the current status of web-based information on veterinary services available in Gampaha district and to develop a web-based information system for veterinary service for that area. Two questionnaire-based surveys were conducted separately for hundred and fifty pet owners and fifty veterinarians who were randomly selected via social media from the western province to assess the current status of available veterinary service information. The data was analysed with the use of Microsoft Excel software. Based on the results of the pet owners' survey, the most common method of finding a veterinarian was from a friend (41%) while the second common way was through the internet (34%). Response given by the pet owners for the method used for searching veterinary service by internet search was 65% and 35% through social media and search engines respectively. More than 80% of the respondents were not satisfied regarding the current online veterinary services and the responses for type of expected information were regarding veterinary practices, awareness on diseases, information of animals and others as 34%, 33%, 32% and 1% respectively. In the veterinarians' perspective, more than 90% stated that advertising of veterinary service was good and 78%' s veterinary practice has been advertised. Highest proportion of the sample (49%) used the internet as the advertising medium while 18%, 9% and 5% of the sample used printed, electronic and other types of media respectively. Furthermore, 84% of the surveyed veterinarians stated that, it would be good to have a web site as a successful mode of advertising. Consequently, as a pilot project, a web site for the veterinary services available in Gampaha district was designed using a Wordpress theme. The site can be accessed using the URL: http://petdoctor.lk/ and will be further improved.

# Detection of Bovine Viral Diarrhoea Virus (BVDV) Antigen in Three Large Scale Farms in the Upcountry Region

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Reproductive problems causing pregnancy failure in dairy farms should be investigated to find out the exact cause so that appropriate control measures can be implemented. Reproductive failures in heifers are real concern for farm management. Diagnosis of exact cause/s for pregnancy failure is extremely difficult. This study was carried out to detect bovine viral diarrhoea in the heifers and cows that had a history of abortion in the period of early pregnancy. Detection of BVD infection (viral antigen) was attempted in cows deemed to have early embryonic mortality (EEM) in three cluster farms in Upcountry using ear notch samples. Thirty animals from Farm A, 12 animals from Farm B and 26 animals from Farm C were selected at first round of testing. Once prevalence rate of BVD infection of three farms were established based on preliminary testing, a larger study was planned to estimate the incidence of BVD infection in three farms. The detection of the BVD infection was carried out by performing IDEXXBVDV antigen point of care test. The results of the study reveal that none of the animals tested in first round was positive for BVDV antigen indicating that EEM in these farms were caused by some other than BVD infection. Therefore, testing was further expanded to include some more animals from the three farms (n = 82) this time targeting breedable heifers. All these cows were also negative for BVDV. IDEXXBVDV antigen test kit is known as a fast, easy and accurate testing which has a high sensitivity and specificity to detect BVDV. This test kit is considered as fast and easy diagnostic method since it consists of a positive control. However, the specificity and sensitivity of the diagnostic test should be further studied to ensure the accuracy of the test.

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### **Embryo Production in Sow Using a Superovulatory Protocol**

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The objective of the study was to harvest embryos from a superovulated sow. Considering the litter size and number of litters per year, a lactating sow at Veterinary Teaching Farm, Udaperadeniya was used for this study and the protocol was selected based on the previous studies. The selected sow was injected with 1,500 IU pregnant mare serum gonadotrophin (PMSG) at an average of 25 days postpartum. After ninety-six hours of the PMSG injection, 1,000 IU of human chorionic gonadotrophin (hCG) was injected to the sow. The heat signs of sow were detected and after 24 hours of post hCG, the sow was kept for 24 hours with the boar for natural mating. The mating was visually observed. Embryo flushing from the sow was carried out by surgical method. To sedate the animal, Acepromazine 0.2mg/kg (IM route) was used. After 30 minutes Ketamin15mg/kg (IM route) was administered to anaesthetize the sow. Surgical collection of embryos was performed through a paramedian incision. At the level of bifurcation of the uterine horn, a ballooned tip foley catheter was inserted to uterine horn lumen at the dorsal aspect of uterine horn. Embryo flushing catheter was inserted at the other side of uterine horn. One litre of embryo flushing media which was thawed in 37°C in a water bath was slowly infused into the uterine horns, 500ml for each horn. While massaging the uterine horns gently, the embryo flushing media and uterine contents were collected to a sterile container. The collected embryo flushing media with uterine contents were observed under stereo microscope. The results revealed that there were no embryos observed in the flushing media and only the epithelial cells were observed. The results of the study highlighted that the positive results were not acquired with the protocol and procedure used in this experiment. However, further studies are needed to obtain the favourable results.

### Pregnancy Rate of Repeat Breeder Cows Inseminated with Chilled Semen

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Cows that are cycling normally, with no clinical abnormalities, which have failed to conceive after at least three successive inseminations is considered as repeat breeding. This condition is one of the common problems in large scale dairy farms in Sri Lanka which causes substantial economic loss to the farm. The objective of this study was to evaluate the success rate of repeat breeder cows inseminated with chilled semen. For the study, 28 repeat breeder cows were selected from two different farms (Mawathagama and Matale). Cows were evaluated for their Body Condition Score (1-5 scale), health status and the activity of the ovaries. All the selected cows were at the cycling stage of ovaries, non-diseased, and the average body condition score was 2.5. The synchronization of ovulation was carried out using a modified Ovsynch protocol. The protocol was carried out by insertion of a Cue-Mate device for 7 days. An injection of 0.1 mg of GnRH was given I/M on day 0. On day 7 of the protocol, 0.5 mg of PGF2a was injected and the cue mate was removed on day 8. Another GnRH injection was given on day 9. Timed Artificial Insemination (TAI) was carried out using chilled semen after 16-20 hours of the last GnRH injection. Chilled semen was prepared at the Central Artificial Insemination Centre and stored at 4<sup>o</sup>C until used in the Artificial Insemination. Pregnancy diagnosis was carried out 45 days post AI using an Ultra Sound Scanning machine. All the animals showed estrus at the time of AI. Four animals (n=4) got pregnant out of 13 repeat breeder cows in the Mawathagama farm and the success rate was 30.76%. Nine animals (n=9) were become pregnant out of 15 repeat breeder cows in the Matale farm and the rate was 60%. The average pregnancy rate of using chilled semen was 46.43%. In conclusion, the results showed that chilled semen can be used in repeat breeder cows to obtain high pregnancy rates.

# Antimicrobial Resistance in *Aeromonas* Species Isolated from the Effluent Water of Fresh Water Ornamental Fish Farms and Aquaria in Kandy District

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Antimicrobial agents are used widely in modern ornamental fish farming both for therapeutic and prophylactic purposes. Antibiotics are administered to ornamental fish either in water as baths or in feed. As many farms and aquaria directly discharge effluent water to the environment, undissolved and unconsumed antimicrobials in feed and water are released into the natural water bodies. Aeromonads are Gram-negative bacteria widespread in aquatic environments and it is the major bacterial genus found in tropical fresh water. Moreover, mesophilic, motile Aeromonas spp. are a major pathogen in fresh water fish and an emerging food-borne pathogen in humans. The objective of the present study was to isolate Aeromonas spp. from effluent water samples collected from fresh water ornamental fish farms and aquaria and to determine antimicrobial susceptibility of isolated Aeromonas spp. against five antibiotics commonly used in aquaculture. Ten aquaria and ten breeding farms located in Kandy district were visited and one effluent water sample was collected from the main effluent canal of each farm/aquarium. Centrifuged sediment of effluent water was cultured in Trypticase Soy Agar (TSA). Bacterial isolates that were motile Gramnegative rods showing positive results for oxidase, catalase, fermentative in oxidation fermentation test and resistant to Vibriostat O/129 were identified as presumptive motile Aeromonas spp. A total of twenty two presumptive motile Aeromonas spp. were isolated. Those were subjected to antimicrobial susceptibility test using disk diffusion method. Results revealed that all isolates were resistant to amoxicillin and metronidazole while 72.7% of the isolates were resistant to enrofloxacin, 63.6% were resistant to erythromycin and 54.5% were resistant to tetracycline. Multiple antibiotic Resistance (MAR) index in Aeromonas spp. isolated in the present study ranged from 0.4 -1. MAR index values greater than 0.2 indicate high risk source of contamination. Our study shows a high prevalence of multiple antibiotic resistant Aeromonas spp. in fresh water ornamental aquaculture effluent water. Therefore, effluent water can be a source of drug resistant bacteria.

### Average Weaning Age of Dogs Presented to Veterinary Teaching Hospital

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In Sri Lanka, dog breeding has become a lucrative business, like everywhere else and the number of pet animal clinics also has increased. In parallel to this, current situation related to dog breeding must be studied, compared with national and global standards and proper advice must be given to such breeders in order to have a healthy dog population and to uplift professional standards of small animal practitioners. A questionnaire was prepared to be administered during personnel interviews after obtaining the consent of clients in this regard. Only clients who brought dogs below 2 years for vaccination to the Veterinary Teaching Hospital (VTH) during August to September in 2019 were used in the study and only the animal that was presented was used for analysis. A total of 110 clients with their dogs were used to collected information. A total of 77.27% (n=85) of puppies had been weaned earlier than recommended age which is 6 to 8 weeks. The median weaning age of the dogs was 4 weeks which is much earlier than the recommended. Most frequently mentioned reason (n=58, 52.7%) for early weaning was to sell the pups earlier. Most respondent clients (n=71, 64.5%) had fed their pups only with home-made food while a few of them (n=33, 30%) fed pups with a mixture of commercial and home-made food. Nearly 79 (71.81%) pups had a proper deworming history. Only 10 (9.1%) respondent clients answered the questions regarding the mother of their own pup because a majority had bought their pups from breeders and they were either unaware of such information or such information was of no interest to them. Only 14 breeders have informed the clients that the mothers of their pups did not have sufficient milk and therefore they had to be weaned earlier. A total of 12 clients had obtained their dogs from the street stray group. Mostly Rottweiler (n=18, 16.36%) and Labrador (n=10, 9.1%) pups have been weaned earlier than 6 weeks. No health condition attributed to early weaning was observed in any of the dogs during the visit anyway. A detailed study in this regard, a good publication and information to be circulated among breeders and small animal practitioners are recommended.

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# Survey to Identify Meat and Meat Products Available in Some Selected Markets Situated in the Western Province

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Meat industry has become a well-developed industry in the world. Nowadays, meat and meat products are essential components in the diets of many people in developed and developing countries. Meat is a rich source of protein, energy, fat, vitamin B12, sodium, phosphorus, iron and zinc that fulfils major nutritional requirements of humans. The objectives of this survey were to identify meat and meat products available in some selected supermarkets and markets in the Western Province and to identify qualities and characteristics of meat and meat products along with the consumer demands for those products. This survey-based study was carried out in ten supermarkets and five small markets situated in Western province of Sri Lanka. Data were collected interviewing managers and workers attached to the markets and taking photographs of the products including their labels. From our survey we found, mainly six types of meat and meat products such as chicken, beef, pork, mutton, lamb and turkey are available and of which chicken and chicken products were the only type available in all the supermarkets and small markets. In addition to raw meat, there were pre-cooked, further processed and canned meat products. Among different products, pre-cooked products were found from chicken, beef and mutton. Canned products were found from chicken, beef, mutton and turkey. Further processed products were found only from chicken. Among the total number of meat and their products, 69%, 15%, 7%, 5%, 2% and 2% were, chicken, pork, beef, button, lamb and turkey respectively. When considering chicken, 52% (35/67), 33% (22/67), 9% (6/67) and 6% (4/67) of pre-cooked, raw, further processed and canned products were found respectively. Depending on the availability and selling rates of those different products, it could be stated that chicken and chicken products are the most popular among local consumers. Low cost, wide availability, tasty, low religious connections and many products holding quality certifications could be the reasons for this high popularity of chicken and chicken products among local consumers.

# Pregnancy Rate of Repeat Breeding Cows Transported from Warmer Climate to Cooler Climate

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Heat stress has been recorded to cause repeat breeding in Bos taurus cattle when reared in hot-humid climates without a controlled environment. A repeat breeding problem in a large farm at Mawathagama, in Kurunegala district was reported the large animal clinic of the University. There were 15 cows with very long post-partum open period of  $420 \pm 26.29$ days due to repeat breeding. All the animals showed severe heat stress signs and their body temperatures reached 41- 42°C during the day times with severe salivation and increased breathing. The animals had Body Condition Score (BCS) ranging from 2.5 to 3. Considering all these factors and palpation of the reproductive tract of the cows the problem was diagnosed as repeat breeding due to heat stress. Therefore, the aim of the study was to determine the pregnancy rate of repeat breeding cows after they were transported to cooler climate. The cows were transported to Veterinary Teaching farm at Udaperadeniya in Kandy District. Cows were allowed to adapt to the cooler climate for two weeks and estrus synchronization program was started and cows were artificially inseminated (AI). Pregnancy diagnosis was conducted 45 days post AI and pregnancy was confirmed by ultrasound. Pregnancy results revealed that 5 cows were pregnant and the pregnancy rate was (45%). The non-pregnant animals were subjected to the second estrus synchronization program and inseminated accordingly. For the second round of insemination 7 cows were found to be pregnant from 10 inseminated cows (pregnancy rate 70%). The total pregnancy rate was 80% (12/15) from the whole 15 sample. In conclusion this study showed that repeat breeding due to heat stress can be overcome by providing them a suitable cooler climate.

#### Collection and Cryopreservation of Semen from Jaffna Local Sheep

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Jaffna local sheep has been identified as an endangered farm animal species found in the Jaffna Peninsula of Sri Lanka, and strategies are needed to conserve this valuable species. The aim of the study was to collect and cryopreserve their semen under field conditions. For this study, seven samples were obtained from two Jaffna local sheep brought to the Veterinary Teaching Farm, Udaperadeniya. Semen collection was carried out using electro ejaculation method with proper restraint of the animal and the sample evaluation was carried out macroscopically and microscopically before processing the sample to produce frozen semen. The semen extender consisted of egg yolk, glycerol (7%), citrate, fructose, distilled water and an antibiotic (Penicillin and Streptomycin). After adding the extender, semen was loaded into mini straws (0.25ml) under room temperature and stored at 4°C for 4 hours and frozen at -196°C. The mean percentage of live spermatozoa in samples was  $72.1\pm2.1\%$ before freezing. After processing, the mean percentage of live spermatozoa in the samples was  $69.1 \pm 2.7\%$ . Semen was examined for motility, live to dead ratio and morphological abnormalities. The mean percentage of post thawing motility showed a considerable decrease. The mean percentage of post-thawing motility in the samples was  $18.5 \pm 1.5\%$ . The results of this study revealed that methods of Jaffna local sheep semen collection and cryopreservation should be further improved using different protocols.

# Prevalence and Morphological Identification of Intestinal Parasites in Little Tuna (*Euthynnus affinis*) Collected from Fish Market, Kandy

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Marine food fish industry in the country is expanding at an exponential rate becoming the major protein source among people. The export market of the marine food fish products is also expanding since fish derived proteins are considered high quality and a healthy option. In order to ensure food safety among the marine food fish consumers, it is essential to study the internal parasites which could be found in them. One of the most common marine food fish in Sri Lanka, Euthynnus affinis was used to examine the internal parasites. The intestinal specimens of 40 Euthynnus affinis fish have been collected from the fish market, Kandy. They have been examined thoroughly for the presence of adult parasites and faecal samples collected from the rectum was used to identify the parasitic eggs. Direct wet mounts have been used for examination of adult parasites. Individual adult worms collected were mounted in glycerol gel following lactophenol treatment for 24 hours in order to observe precise morphological features. Salt floatation and faecal sedimentation techniques were used to examine parasitic eggs. In the faecal examination Acanthocephala eggs, Ascaroid type eggs and Strongyle eggs were identified. Parasite prevalence as measured by the presence of eggs in the faecal samples was 45%. Parasite prevalence as measured by the presence of adult parasites in the intestinal specimens was 82.5%. All adult worms collected belonged to Acanthocephala group with morphological features similar to Family Rhadinorhynchidae. Overall internal parasite prevalence was very high (82.5%) in Euthynnus affinis indicating possible food fish contamination with parasites and their products. In order to ensure safety consumption and quality control of marine food fish, further studies are highly warranted in precise identifying of parasite species and their zoonotic potential.

# Prevalence of Haemoparasites in Dogs from Selected Locations in Galle and Kandy Districts

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Vector borne parasites are common in dogs in many tropical and sub-tropical countries including Sri Lanka. Despite the health concerns, there are several gaps in the knowledge on the geographical distribution of the above parasites in dogs in Sri Lanka. Therefore, this study was carried out to determine the prevalence of haemoparasites in dogs from selected locations in Galle and Kandy districts. Capillary blood samples were collected from a total of 88 dogs (Galle: n=43 and Kandy: n=45) during September to October 2019, and the parasites were identified by examining Leishman's stained blood smears. Two species of haemoparasites were encountered in this study, namely Babesia gibsoni (15/88: 17.1%) and Hepatozoon canis (1/88: 1.1%). The prevalence of B. gibsoni did not differ (Chi square test, P > 0.05) between the sampling locations, history of ectoparasitic treatment, and the age and sex of dogs. The influence of breeds on the prevalence of B. gibsoni was not analyzed due to the inadequate sample size in each breed category. Further, the parasitaemia of B. gibsoni did not differ (two sample t-test: P > 0.05) between sex, sampling locations and history of ecto-parasitic treatment. However, an age related decline of the parasitaemia of B. gibsoni was observed. The lack of significant difference in the prevalence of *B. gibsoni* between the districts sampled might be related to inadequate sample size.

# Some Observations on Post-Musth in Captive Asian Elephants (*Elephas maximus*)

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Musth manipulation in captive elephants using hormones and vaccines has been documented for better management of elephants. Accordingly, these findings were to monitor manageability of captive adult male elephants after administration of GnRH intramuscular injections. Three adult male elephants, Saliya, Mangala and Bibila were visited once in 2 weeks, 3 times during their post musth. During each visit, 4ml of GnRH (1.6 mg per dose, BOPRIVA® bovine immune castration vaccine, Zoetis GMS Australia) vaccine was intramuscularly administered. In addition, Jana Raja, a tusker was also blood sampled to determine whether he was in true post-musth. Testosterone and cortisol levels in blood samples was measured using cobasE411 electrochemiluminescence method in a private human laboratory that had modified the test for elephants. Saliya, did not show elevated testosterone corresponding to post musth and was tied 20m away from Jana raja, who was also in extended post-musth. GnRH vaccine did not have an impact on testosterone levels of Saliva while cortisol levels kept fluctuating. After the 3<sup>rd</sup> injection, Saliva became obedient, was removed from his must stable and tied 800m away from Jana raia. Both Mangala and Bibila were managed in solitude 40km away from Saliya and both had elevated serum testosterone levels corresponding to post-musth. With three GnRH vaccine injections, both testosterone and cortisol, which was initially high, gradually reduced. Both elephants became obedient, were released from musth stable earlier than usual and were used in their regular activities. Jana raja who was in apparent post-musth with temporal gland secretions, did not have elevated serum testosterone and hence was released from must stable. Thereafter, his must discharge stopped and became obedient. It is suggested that elevated testosterone may not be essential for post musth behavior and temporal gland secretions to continue in elephants. Actions of elevated testosterone could be suppressed by the presence of other dominant males around. Three GnRH vaccine doses two weeks apart could rapidly reduce post-musth signs and therefore could be used in male elephant management. Role of cortisol during post must is important and must be studied further because must could be stressful event. The human laboratory provided comparable serum levels of both testosterone and cortisol in elephants.

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#### Non-Communicable Health Conditions in Workers in Pinnawela Open Zoological Garden

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Those who work closely with animals are at higher risks of attracting several infective agents. Similarly, the non-communicable health status of zoo workers could also depend on the type of animals and work involved. For example, elephant management is known to be physically demanding. Elevated non communicable health risks of keepers of privately owned elephants have been previously studied. Such risks, if studied and remedied in Pinnawela open zoo, would help management while encouraging workers for better performance. A medical clinic on non-communicable health status was held with permission within Pinnawala zoological garden on 26<sup>th</sup> October 2018 using 3 doctors, 3 nurses 2 attendants and 2 health workers. After obtaining consent from participant zoo workers, a questionnaire with personnel details was also collected. The definitions by Ministry of Health were used to decide on the health status of participants using all measurements involved. Altogether, there were 82 workers at all levels within Pinnawela open zoo, 82 of them participated in the clinic out of which 22 were involved with elephant management. From among those who work with elephants (n=22), according to the data on Body Mass Index (BMI), 5 were overweight while 1 each was underweight and obese. Only 4/22 had elevated systolic blood pressure, 6 had evidence of heart ailments, 1 was with defective vision and all had satisfactory random blood glucose levels. A total of 5 smoked while 5 regularly consumed alcohol. From among those who do not work with elephants (n=60), 2 were overweight while 30 and 3 were underweight and obese respectively. Fourteen (14) had elevated systolic blood pressure, 2 had elevated random blood glucose levels, 7 had vision problems and 14 were having heart ailments. Four of them smoked and 9 regularly consumed alcohol. None of the comparisons between those who work with elephants and those who do not, did not show statistical significance. However, those who do not work with elephants had unsatisfactory values for BMI and systolic blood pressure. These findings suggest further studies, regular exercise and dietary changes for those who do not work with elephants within Pinnawela zoological garden.

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#### **Collection and Cryopreservation of Goat Semen**

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Collection of semen from a buck with a good breeding soundness is a key step in goat semen processing. The aim of this study was to investigate whether buck semen collection and freezing can be carried out in the field condition successfully. The study was conducted at the Veterinary Teaching Farm in Udaperadeniya using a four year six month old Saanen buck. Semen collection was performed in one-week intervals within a period of two months by using electro-ejaculator after buck evaluation. The samples were macroscopically evaluated according to semen volume, color and motility. During microscopic evaluation sperm concentration, live: dead ratio and morphological defects were assessed. The Mean sperm concentration was  $14.1 \pm 2.4$  million/ml. The Mean motility of raw samples was detected as  $57.1 \pm 5.1\%$ . The percentage of primary abnormalities in eosin; nigrosin stained sperms was within the normal range (30-40%) and the percentage of secondary abnormalities was higher than the standard values. Collected semen was processed in order to produce deep frozen semen by adding Tris and egg yolk-based extender through one step method. After adding extender, the semen was loaded into mini straws (0.25ml) and was sealed with cryo sealer under room temperature. The straws were cooled to 4°C for four hours and frozen at -196°C. Post thawing evaluation was performed after three days of freezing. According to the obtained results the Mean value of live sperms in post thawing samples was  $14.8 \pm 1.7\%$  and less than normal range (30-40%). In conclusion, results were suggestive of multiple factors that affected for the low motility percentage observed in this study and necessity of further studies to improve the technique.

#### A Microsatallite-based Genetic Diversity Analysis of Four Cattle Populations of Sri Lanka

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Cattle represent a major proportion of livestock population in Sri Lanka. Cattle genetic resources in Sri Lanka are comprised of indigenous zebu (Bos indicus) breeds, exotic zebu breeds, exotic European breeds (*Bos taurus*) and various crosses of these three types. Among locally adapted indigenous zebu cattle in Sri Lanka, at least three distinct breeds/populations (Lanka cattle/ Batu Haraka, White cattle/ Thamankaduwa breed and Thawalam cattle) can be recognized based on their phenotype and management system. At present only limited information is available on demography, population structure and genetic diversity of above indigenous zebu cattle types. In this study, 3402 genotypes of 126 cattle generated using 27 FAO/ ISAG recommended short random repeat (STR) loci from three indigenous zebu breeds (Batu Haraka, White cattle and Thawalam cattle) and an exotic taurine (Holstein Friesian) population in Sri Lanka were compared to assess genetic diversity and relationship among them. Among the studied cattle breeds, Batu Haraka displayed high genetic diversity (mean number of allele  $(n_a=8.26)$  and heterozygosity (H<sub>e</sub>=0.74918)) while Thawalam cattle displayed relatively lower diversity measures ( $n_a=7$  and  $H_e=0.709263$ ). The overall mean estimated inbreeding coefficient (Fis) was 0.04648 and it varied from 0.033679(Holstein Friesian) to 0.065434(White cattle). Bayesian clustering of individuals without prior population information revealed the most appropriate K with two genetic clusters representing two main cattle types (zebu and taurine). All three indigenous zebu populations did not indicate significant population substructure while only Batu Haraka showed evidence for taurine introgression and genetic admixture. Analysis of molecular variance clearly indicates genetic differentiation between taurine and zebu cattle (10.49%) and little (1.78%) genetic differentiation among the three indigenous populations. Most of the observed genetic variation was due to within populations among individual variation rather than among populations. In conclusion, the present study showed that three indigenous zebu breeds/populations are not very different but are differ from Holstein Friesian. Yet some admixture is taking place in Batu Haraka population perhaps due to artificial insemination services that has been done for long.

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#### Evidence on Possible Immunosuppression in Male Elephants during Musth

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Musth in adult male elephants corresponds with elevated circulating testosterone and is characterized by annually occurring behavioral, physical and physiological changes in them. Since off late, elevation in circulating glucocorticoid concentrations during musth also has been reported. Since steroids are well known immunosuppressants, field evidence in this regard could be valuable in captive musth elephant management. This information is important in providing better health care for approximately 100 captive elephants currently available in the country. It has been documented that some, not all captive male elephant during musth possibly are more prone for certain rare helminth infections. A questionnaire was prepared in this regard, to be administered during personnel interviews with respective captive elephant keepers of males during Esala Perahera in Kandy in 2019. The questionnaire included questions on details of particular elephants especially with regard to origin, age, must period and its regularity and number of keepers in the past. In addition, the body condition as noticed by keepers, was also questioned during musth and immediately beforehand. A total of 19 keepers of privately-owned male elephants responded to the study that had brought their elephants to Kandy Esala Perahera 2019, from various districts in Sri Lanka. All of these elephants have had more than 3 keepers during their lives. All elephants were sexually matured and come into regularly musth with the durations of 3 -7 months. Only one respondent keeper reported a possible reduction in body condition during musth, in the elephant under his care. There was no strong evidence to a possible immunosuppression during musth in the elephants as measured by a reduction in body condition as noticed by the respective keepers. It is documented that the keepers of privately owned elephants in Sri Lanka, are likely to distort information for various reasons. It is impossible to rule out the musth associated immune suppression and detailed studies in this regard are warranted to provide conclusive remarks.

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