



# Sri Lanka Veterinary Association 69<sup>th</sup> Annual Scientific Sessions

26<sup>th</sup> May 2017

Programme and Abstracts of Scientific Sessions

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# Sri Lanka Veterinary Association

## Annual Scientific Sessions

26<sup>th</sup> May 2017



## Programme and Abstracts of Scientific Sessions

Plant Genetic Resource Centre (PGRC)  
Gannoruwa, Peradeniya

# Programme

08.30 – 09.00	Registration
09.00 – 10.00	Opening Ceremony
09.00 – 09.10	National Anthem and Lighting of Oil Lamp
09.10 – 09.15	Welcome address: Prof. Basil Alexander, President, Sri Lanka Veterinary Association
09.15 – 09.50	Key Note Address: Prof. Ryan Brook, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5E2, Canada
09.50 – 10.00	Vote of Thanks - Dr. M.N.M. Fouzi, Assistant Secretary, Sri Lanka Veterinary Association
10.00 – 10.15	Tea – Lobby
	<i><b>Technical Sessions I (Common session)</b></i>
10.15 – 12.15	Clinical and Case Reports - <b>Main Auditorium</b>
12.15 – 13.00	Lunch – Lobby
	<i><b>Technical Sessions II (Parallel Sessions)</b></i>
13.00 – 15.30	Animal Health - <b>Main Auditorium</b>
13.00 – 14.45	Animal Production - <b>Small Auditorium</b>
15.30 – 15.45	Tea
15.45 – 16.30	<i><b>Technical Session III (Common Session)</b></i> Poster Session – <b>Lobby</b> End of the program

**Technical Session I (Common Session)**  
**Clinical and Case Reports - Main Auditorium**  
 Chairperson: Dr. D.S. Kodikara

10.15	Ultrasonographic changes of pancreas and peripancreatic structures of dogs attributed to ingestion of Durian <i>A.M.U. Atapattu, W.R.B. Kumara, D.R.A. Dissanayake</i>	3
10.30	Successful correction of congenital leg deformities of two puppies with Ponseti casting technique <i>M.L.W.P. De Silva, D.D.N. De Silva, W.R.B. Kumara</i>	4
10.45	Prognostic relevance of cytological grades and sub types of lymphomas (using the updated Kiel's classification) in forty cases of canine lymphomas <i>H.M.H.S. Ariyaratna, T.M.S.K. Piyadasa, A.M.U. Atapattu, D.G.S.S. Bulumulla, M.L.W.P. de Silva, A.S. Lenagala, P.N. Uyanwatta, E.R.K.V. Edirimanne, M.G.C.M. Jayasinghe, W.R.B. Kumara, R.A.D.E. Indrajith, A.M.R.B. Adikari, I.D. Silva, D.D.N. De Silva</i>	5
11.00	Diagnosis of canine cranial cruciate ligament disease and treatment using the Fabellotibial suture technique <i>M. Seneviratne, D. Siriwardene, J. Collure, N. Obeysekere</i>	6
11.15	Ultrasounographic characteristics of Sciatic nerve in Canine distemper myoclonus <i>W.H.M.T.C. Chinthana Wijekoon, K.A.N. Wijayawardhane, W.R.B. Kumara</i>	7
11.30	An outbreak of Haemorrhagic septicaemia in the Navithanveli veterinary range in Ampara district, Sri Lanka <i>M.W.D.C. Weerathunga, J.K.H. Ubeyratne, M.A. Nadheer</i>	8
11.45	<i>Anaplasma marginale</i> infection confirmed by Major Surface Protein (MSP) 5 gene sequencing <i>S.S. Iddamaldeniya, K.H.D.T. Kasagala, K.G.J.S. Disnaka, N.D. Senasighe, N.A.D.E.M. Gunasekera, A.M.H. Atapattu, N.C. Gamagedera, G.M. Ranasinghe</i>	9
12.00	Isolation and identification of <i>Brucella melitensis</i> from a swine herd in Sri Lanka <i>P.S. Fernando, K.M.S.G. Weerasooriya, N. Liyanagunawardena, W.M.P.B. Bandara, M.N.D. Munasingha, M.I. Wijemuni</i>	10

## Technical Session II (Parallel Sessions)

### Animal Health - Main Auditorium

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- 13.15 Prevalence and Economic impact of Contagious Pustular Dermatitis disease of Goats in Sri Lanka 12  
*S. Piratheepan, E.R.K. Perera, S. Puvanendiran*
- 13.30 Re- emerging of Haemorrhagic Septicaemia in Sri Lanka 13  
*P.S. Fernando, K.M.S.G. Weerasooriya, N. Liyanagunawardena, M.D.N. Jayaweera, H.P.V.D.S. Bandara, S.A. Thalagoda, S.K. Gunathilake, P.M.K. Abayaratne, D.M.S.N.B. Dissanayake*
- 13.45 Occurrence of Brucellosis in cattle and buffalo in Trincomalee district during 2011-2016 14  
*T. Mayurathi, T. Nirogini, K.G.S.K. Ranathunga, K.M.S.G. Weerasooriya, S. Puvanendiran*
- 14.00 Prevalence of *Salmonella*, *Campylobacter*, *Escherichia coli*, *Staphylococcus* spp and detection of antimicrobial resistance (AMR) associated with *E. coli* isolated from commercial poultry layer farms in Kurunegala district 15  
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- 14.15 Effect of water temperature on the occurrence of iridovirus in Asian sea bass (*Lates calcarifer*) 16  
*M.N.M. Fouzi, V. Sakajamary*
- 14.30 Monitoring of *Theileria orientalis* major piroplasm surface protein (mpsp) genotypes in cattle in Polonnaruwa and Nuwara Eliya districts of Sri Lanka 17  
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- 14.45 Reporting the preliminary results of synthesizing a thermosensitive nanoparticle to target solid tumors 18  
*S.M. Wijayarathna, B.R. Fernando*
- 15.00 Evaluating immune memory resilience using sheep lymphatic cannulation model 19  
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- 15.15 Sero-prevalance of *Neospora caninum* antibodies in a dairy farm 20  
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## Technical Session II (Parallel Sessions)

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Chairperson: Dr. S.P. Gunarathne

- |       |  |    |
|-------|--|----|
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| 13.30 | Study on Beta casein A1 and A2 polymorphism in Ayrshire and Holstein Friesian dairy cattle populations in two upcountry dairy farms<br><i>Ruwini K. Rupasinghe, Saumya Wickramasinghe</i>  | 23 |
| 13.45 | Antimicrobial resistance of contaminating bacteria of curd and yoghurt produced by small-scale processors in Kandy district<br><i>P.P. Jayasekara, J.C. Hapugoda, S.A.I.C. Subhasinghe, R.S. Kalupahana</i>  | 24 |
| 14.00 | Lessons learnt from the Heifer Calves Rearing (HCR) scheme in Vaddukkodai, Jaffna<br><i>Nirojini Arulrasa, Komathy Maheswaran, Muraliths Mahalingam</i>  | 25 |
| 14.15 | Evaluation of farmer attitudes on dairy cattle welfare in Central province of Sri Lanka<br><i>H.A.D.K. Senanayake, T.S. Samarakone, E. Rajapaksha, W.P.C.G. Weerasinghe, M.P.B. Wijayagunawardane</i>  | 26 |
| 14.30 | Assessment of suitability of indirect milk quality tests in determining the microbial load of raw milk<br><i>M.K.U.T. Amarasiri, B.R. Fernando, P.S. Fernando, R.S. Kalupahana</i>   | 27 |

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- |    |   |    |
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| 2  | Successful correction of a colorectal obstruction in a dog- A case Report<br><i>S. Pemachandra, W.S. Gothami, P.G. Eshwara, C. Kodithuwakku, L. Ranasinghe, L. Nanayakkara</i>  | 29 |
| 3  | Tetanus in a dog after whelping: A case-report<br><i>W.H.M.T.C. Wijekoon, N.C. Gunarathne, G.M.G.C.K. Premachandra, H.M.D.N. Shyamali, M.D.H.S. Mallikarachchi, A.M.R.B. Adhikari, M.G.C.M. Jayasinghe, K.A.N. Wijayawardhane</i>   | 30 |
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| 6  | Histopathological confirmation of pulmonary aspergillosis in a captive Flamingo in Sri Lanka<br><i>G.I.S. Perera, S.M.T.S. Manchanayake, P.S. Fernando, D.K. Sonnadara, D.M. Nambuge, S.K.S. Rathnasiri</i>   | 33 |
| 7  | Wild-type <i>Edwardsiella tarda</i> , possible cause of unnatural mass death of Asian Openbill ( <i>Anastomus oscitans</i> ) at Kotuathawala bird sanctuary in Nikawaratiya<br><i>J.K.H. Ubeyratne, G.M.C.R. Karunarathne, M.D.N. Jayaweera, K.H.D.T. Kasagala, W.M.P. Bandara, H.P.V.D.S. Bandara, G.A.T. Prasad, P.A.U. Kariyawasam, S.K. Gunathilake</i> | 34 |
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| 9  | Existing knowledge on Foot and Mouth Disease among livestock farmers in the Ampara district of Sri Lanka<br><i>P.M. Akalanka, R. Hettiarachchi, L.G.S. Lokugalappatti, Ryan K. Brook</i>  | 36 |
| 10 | Possibility of using <i>Cyclea peltata</i> plant extract as a substitute for Gelatin in set yoghurt<br><i>R.A.D.D. Ranathunga, M.N.P. Perera, R.M.C. Deshapriya</i>   | 37 |
| 11 | Possibility of application of TiO <sub>2</sub> photocatalytic technique to reduce the bacterial count in bovine milk<br><i>M.T.I. Hansika, R.M.C. Deshapriya, G.R.A. Kumara</i>   | 38 |

## Keynote Speech

# A Canadian Perspective on Risk Assessment and Management for Bovine Tuberculosis

Ryan K. Brook

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Bovine tuberculosis (TB) is a global disease that creates important risks for livestock and human health (Kuiken et al. 2005, Brook and McLachlan 2009). Historically, it was one of the most important globally important livestock diseases and was an important zoonosis. Intensive efforts to control TB have had much success. However, despite over a century of intensive research and management efforts worldwide, TB continues to be an important socio-economic risk. TB is a chronic disease of primarily hoofed mammals caused by the bacteria *Mycobacterium bovis*. It is listed in the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code, and must be reported to the OIE.

A key risk area for the intra-specific transmission of TB is at the wildlife-livestock interface. Wildlife are attracted to farms with livestock, often seeking high quality agricultural forage crops and/or shelter from predators. TB transmission at the wildlife-livestock interface occurs either indirectly through shared contaminated feeds or grazing lands infected with saliva, urine, or feces from TB infected animals shedding *M. bovis* to the environment (Briscoe 1912; Phillips et al. 2003; Hutchings and Harris 1997; Brook et al. 2012); or via direct contact through nose-to-nose contact and coughing, sneezing, or licking (Garnett et al. 2002). TB occurs at a community-scale, often in multiple reservoir and spill over species is a significant challenge for understanding and managing the bovine TB. TB often infects multiple hosts, each with a unique ecology, thus requiring a diverse set of approaches to understanding and managing disease (Olea-Popelka 2005; Nugent 2011).

Managing TB is particularly challenging due to its persistence in a wide range of wildlife species, including cervids (Brook and McLachlan 2009), badgers (Woodroffe et al 2009), possums (Ramsey and Efford 2010). These species are likely vectors for transmission to cattle (*Bos taurus*) with important economic impacts. Ongoing failures throughout the world to eradicate TB or even effectively manage it have been in part due to the challenging nature of the disease, which is persistent and has a long latent period (Maddock 1933, Phillips et al. 2003).

Bovine tuberculosis was mostly likely brought to Canada in infected cattle from Europe in the 1800's as agriculture expanded across the country. TB quickly became endemic to cattle. In 1897, Canada became one of the first countries in the world to offer livestock owners free testing and in 1907 a national meat inspection program was initiated (Koller-Jones et al. ). A formalized whole herd livestock test and cull program began in 1923 and from then up to 1961, the prevalence of TB in cattle declined from >4% to ~0.1%. This program continued to 1978 with repeated whole herd testing and removal of TB positive individual animals but the national prevalence remained at 0.1%. In 1980 the program was revised such that all susceptible livestock exposed to *M. bovis* on infected farms and any traced back to other farms were destroyed and by 2005 TB was considered eradicated from all cattle herds except in one small area around Riding Mountain National Park in central Canada.

Riding Mountain National Park in Manitoba Canada is 3,000 km<sup>2</sup> in size and is a largely forested island of protected habitat completely surrounded by intensive agriculture that includes annual cropland, perennial forage cropland, and approximately 50,000 beef cattle. Elk (*Cervus*



*canadensis*) and white-tailed deer (*Odocoileus virginianus*) are the two wildlife species having bovine TB in the same area and maintenance of TB may have been based on a combination of wildlife and beef cattle.

Efforts to eradicate TB from the Greater Riding Mountain ecosystem have included a wide range of activities. Barrier fencing of hay bales to keep elk and deer out has been highly successful, with over 100 farms in highest risk areas receiving fences for free as part of federal and provincial cost-shared program and these have been effective and keeping out most deer and elk (Brook 2010, Brook 2015). On-farm risk assessments have been developed that work with individual farmers to document environmental factors on and around farms with different farm management practices, including number of livestock, feeding strategies, and actions that influence elk and deer use of farms (Brook 2017). While the specific routes of TB transmission between elk/white-tailed deer and cattle are not known, efforts have been aimed at mitigating the most likely routes at hay bales (Gooding and Brook 2014, Letain and Brook 2017) and at cattle winter feeding areas (Brook et al. 2012).

Research around RMNP has also been focused on documenting farmers concerns regarding bovine TB within the context of other risks and impacts (Brook and McLachlan 2006). Efforts to document local farmer knowledge also resulted in considerable local engagement that greatly improved farmers' involvement in helping developing solutions to their own issues (Brook and McLachlan 2006, McLachlan 2009, Brook 2015). On-going efforts to estimate prevalence of TB in elk and deer have been instrumental in providing science-based evidence on the efficacy of management actions. Testing is done on all hunter harvested deer and elk heads and lungs turned into the government lab and through a test and cull program where elk and deer were captured by a net-gun fired from a helicopter to capture and restrain individual animals for blood testing and a VHF collar is attached so animals can be relocated and euthanized if they test positive from the blood (Shury and Bergeson 2011).

Overall, there have been considerable successes toward potential eradication of bovine tuberculosis in the one hotspot left in Canada where TB is found in wildlife adjacent to extensive livestock production and there has not been a positive cattle herd in the last six years, while prevalence in elk has declined as well and is largely only been found in older animals. However, bovine TB has been shown to be notoriously difficult to completely eradicate, especially when a wildlife reservoir is present.

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## **Ultrasonographic changes of pancreas and peripancreatic structures of dogs attributed to ingestion of Durian**

A.M.U. Atapattu, W.R.B. Kumara\* and D.R.A. Dissanayake

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Acute pancreatitis in dogs could be due to a number of etiological factors including high-fat diet, various medications or toxins. Durian (*Durio zibethinus*), which is an exotic fruit, is high in lipids and sulfur containing substances and it has been observed that the consumption of the fruit has induced symptoms of acute pancreatitis in dogs. Six dogs presented to the Veterinary Teaching Hospital, Peradeniya with history of consumption of durian flesh and seeds with signs of acute pancreatitis were subjected to transabdominal ultrasound scanning. All had reduced appetite, abdominal pain, vomiting, diarrhea, and were dehydrated. The hematological profiles of four patients were normal, whereas two had mild lymphocytosis. In all patients, serum amylase levels were significantly elevated with the mean of 2171 CU / 100 ml (Range: 1079 to 3462 CU / 100ml; normal: 972 CU / 100 ml). The ultrasonography indicated heterogeneous right lobe of the pancreas with heterogenous pancreatic parenchyma along with hyperechoic peripancreatic regions. The thickness of the right lobe of the pancreas had been increased significantly ( $p = 0.008$ ) and were ranged between 16.5 and 30.2 mm (mean:  $23.2 \pm 5$  mm). Moreover, the ratio between the right lobe of the pancreas and the mean diameter of the ascending duodenum were calculated. The elevated ratios which ranged between 1.29 and 2.17 (mean:  $1.67 \pm 0.3$ ) confirmed that the right lobe of the pancreas of all six patients was enlarged considerably. In addition, intestinal flatulence, reduced peristalsis and moderate to severely distended gall-bladder were observed. The pancreatic changes observed in these patients are highly suggestive of pancreatitis. The exact compound of durian which could cause this pancreatic pathology in dog is not known yet. The high fat content or sulphur compounds in the flesh of the fruit might have induced pancreatitis. All six dogs recovered fully after the symptomatic treatment with antibiotic, antiemetic, vitamin B and intravenous fluid therapy with Lactated Ringers. Findings of the present study indicated that the dogs may develop acute pancreatitis following consumption of durian and transabdominal ultrasonography of pancreas and peripancreatic structures is a quick method to diagnose this life-threatening condition.

## **Successful correction of congenital leg deformities of two puppies with Ponseti casting technique**

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Ponseti casting is an external coaptation technique used to correct orthopedic deformities using Plaster of Paris (POP) cast. This method was used on two cases of pups presented to the Veterinary Teaching Hospital with congenital limb deformities, with a remarkable outcome. The first pup was a 1-month old German shepherd male presented with severe right hind limb deformity from birth with impaired weight bearing. General clinical examination revealed medial rotation of the tibia from the level of stifle joint and tarsal laxity leading to hyperextension of stifle and tarsal joints resulting in restricted movement of the joints. Radiographs revealed dysplastic stifle joint. The second pup was a 2-month old mongrel female with medial deviation of carpal joints leading to bilateral forelimb adduction and limited palmar contact on the ground. The radiographs revealed dysplastic carpal joints. The muscle mass of affected limbs showed severe disuse atrophy. Results of neurological examination were unremarkable. The treatment protocol consisted of corrective manipulation, Ponseti casting and physiotherapy. Affected joints were manipulated under sedation to achieve near normal range of movement and positioning of the joints. Hind limb deformity was manipulated with dorsi-flexion and plantar-flexion of tarsal joint, abduction and adduction of stifle joint while the forelimb deformity was manipulated with dorsi-flexion and plantar-flexion of carpal joint. Encasing of the affected limbs with soft-roll cotton followed by application of POP cast ensured correct alignment of the displaced bones at each joint. This procedure was repeated 5 times at 2 weeks intervals, gradually increasing the degree of flexion at each joint. After removal of the cast each time, a gradual improvement was observed and finally a satisfactory range of movement of the joints was achieved. With the growth of pups, further enhancement of bone remodelling and proper alignment were confirmed radiographically. After 5 consecutive cast applications, the two pups showed marked improvement in positioning the legs and were able to walk satisfactorily. Thus, it can be concluded that the congenital angular limb deformities involving the stifle, tarsal and carpal joints could be corrected satisfactorily by this non-invasive method with less burden to pups and owners.

## **Prognostic relevance of cytological grades and sub types of lymphomas (using the updated Kiel's classification) in forty cases of canine lymphomas**

H.M.H.S. Ariyaratna\*, T.M.S.K. Piyadasa, A.M.U. Atapattu, D.G.S.S. Bulumulla, M.L.W.P. de Silva, A.S. Lenagala, P.N. Uyanwatta, E.R.K.V. Edirimanne, M.G.C.M. Jayasinghe, W.R.B. Kumara, R.A.D.E. Indrajith, A.M.R.B. Adikari, I.D. Silva and D.D.N. De Silva

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Lymphoma is a common neoplasm in dogs and accounts for approximately 7-24% of all canine neoplasms. They can develop at any nodal and/or extranodal sites. Cytology is a quick and cost effective technique for diagnosis and characterization of lymphomas which can be effectively used for prognostication. Among the lymphoma classification systems the updated Kiel classification 1988 (UKC), is the most appropriate for cytological classification. We describe the cytological classification of 40 canine lymphomas presented to the Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine using the UKC. The prognostic efficiency of the classification was tested against the overall survival (OS). Dogs with generalized lymphadenomegaly presented from 2015 January to 2016 January were selected for the study. In addition dogs which had a confirmed diagnosis of extra-nodal lymphomas were also included. Clinical staging of lymphomas were performed following the guidelines of World Health Organization. Cytological classification (grading and sub-typing) was performed according to the UKC using Diff-Quik stained smears prepared from fine needle aspiration biopsies. Chemotherapy (combinations of vincristine, cyclophosphamide/chlorambucil, doxorubicin and prednisolone) and/or surgical excision were used as treatments. Majority (30/40) of the dogs were >5 years old and there German shepherds predominated (18/40). Most frequent anatomical location was the peripheral lymph nodes (34/40) and there were uncommon locations such as sub-lingual (1/40) and renal (1/40). Lymphomas of all stages except stage II were identified. Stage III was the most common (28/40). Seven sub-types were identified among high, intermediate and low grade lymphomas. The most frequent sub-type was high grade (HG) pleomorphic centroblastic lymphomas (20/40). The least commons were HG lymphoblastic lymphoma (1/40) and peripheral mixed T-cell lymphoma (1/40). OS was comparatively low in high grade group (n = 26, 2-90+ days) compared to the intermediate grade (n = 8, 180 – 570+ days) and low grade (n = 6, 244 – 765+ days) groups. However there were considerable intergroup variations in the OS especially in the HG group (very short OS in untreated patients vs comparatively longer OS in the patients underwent treatments). Despite the intergroup variations these findings conclude the utility of cytological characterization of canine lymphomas for effective prognostication.

*Acknowledgement: This study was funded by university research grant RG/2014/48/V*

## **Diagnosis of canine cranial cruciate ligament disease and treatment using the Fabelotibial suture technique**

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Cranial cruciate ligament (CCL) disease is the most common cause of hind limb lameness in the dog. Progressive degeneration leading to rupture of the ligament is the commonest form of CCL disease. Diagnosis is based on historical findings, physical examination and radiographic evaluation. The preferred treatment option is surgical stabilisation of the stifle joint. Many surgical options including extra-capsular and intra-capsular repair techniques have been described but there is an increased trend towards using tibial osteotomy procedures. However, with the limited availability of equipment in Sri Lanka and the cost constraints faced, an extra-capsular technique, the fabelotibial suture, is used at our clinic. This case series describes the diagnosis and treatment of ten cases of CCL rupture presenting to our clinic over a period of 3 years. Breeds seen include the Labrador retriever (n=5), Rottweiler (n=2), crossbreed (n=2) and Tibetan terrier (n=1). All cases presented with hind limb lameness varying from moderate (5/10) to non-weight bearing (10/10) and had pain attributed to stifle joint movement, evidence of stifle joint effusion and medial peri-articular fibrosis on physical exam. One case had severe atrophy of the quadriceps muscles and two cases showed crepitation of the stifle joint with a prominent click. All dogs underwent radiographic evaluation of the stifle joint under sedation and changes consistent with stifle joint effusion and osteoarthritis were seen. Examination under sedation revealed a positive cranial draw and tibial thrust in all cases. Five cases underwent surgery for stabilisation of the stifle joint between 2-4 weeks following diagnosis. These cases underwent stifle arthrotomy followed by placement of fabelotibial suture for joint stabilisation. Meniscal injury was seen in one of the cases and mensicotomy was performed. One case had concurrent patella luxation and also underwent a wedge sulculoplasty. Five cases were managed conservatively due to owner preference and management included a combination of painkillers, physical therapy and weight loss. Three out of the five surgically managed cases had none to minimal lameness and normal quality of life as noted by the owners at a follow up of 3 years, 7 months and 3 months respectively following surgery. One dog has no consistent lameness 4 months after surgery but had a moderate degree of external rotation of the stifle. One dog is 5/10 lame at 14 days following surgery and is still in the recovery period. Of the five dogs managed conservatively, 2 dogs showed no improvement 3 months following diagnosis. One dog showed no improvement 1 month after diagnosis and passed away due to other causes. One dog showed improvement but remains 2/10 lame 6 months following diagnosis. One case was lost to follow up. This case series shows that the lateral fabelotibial suture can be used effectively as a stabilisation technique for the cranial cruciate ligament deficient stifle in situations where tibial osteotomy procedures are not practically possible. This case series also highlights the prevalence of and diagnostic techniques for Canine CCL disease.

## **Ultrasonographic characteristics of Sciatic nerve in Canine distemper myoclonus**

W.H.M.T.C. Chinthana Wijekoon\*, K.A.N. Wijayawardhane and W.R.B. Kumara

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Canine distemper is a multi-systemic disease caused by canine distemper virus, particularly affecting the nervous, respiratory and gastrointestinal systems. It has been reported that the disease can cause diffuse multi-focal lesions in many neuroanatomical sites including cerebrum, cerebellum, brainstem and spinal cord. Due to the grey matter involvement of the virus, myoclonus; a rhythmic jerking of single muscle or muscle group/s is/are manifested in canine distemper. The aim of the present study was to investigate whether the sciatic nerve of the dogs affected with distemper myoclonus have ultrasonographically detectable alternations in the sciatic nerve. One to four years old cross-bred dogs (n=3) presented to Veterinary Teaching Hospital, University of Peradeniya with spontaneous muscle twitches in hind limbs were used for the study. These dogs had not been vaccinated against canine distemper and showed clinical signs namely, hyperthermia, rhonchi, hyperkeratosis of foot pads and rhinarium, hyperplasia of gums and ocular discharges. The clinical presentations of three dogs were highly suggestive of canine distemper with myoclonus stage. The ultrasound scanner (MyLab30vet, Esaote, Italy) with a linear-array transducer Esaote LA 522 with frequency of 5 - 7.5 MHz was used for the study. The left and right sciatic nerves were scanned by positioning the ultrasound transducer on medial thigh area of the dog which was positioned in lateral recumbency. The color Doppler ultrasound was used to separate the sciatic nerve from femoral artery and femoral vein at the femoral triangle. Moreover, the ultrasound characteristics of sciatic nerve of three healthy dogs were evaluated as control. All dogs suspected to have distemper myoclonus showed heterogeneous echogenicity with intermittent hyperechoic areas in the sciatic nerve compared to healthy dogs who have homogenous echogenicity. Further, epineurium of the sciatic nerve was more hyperechoic in the effected dogs in comparison that of healthy dogs. These findings indicated that ultrasonographically detectable changes in sciatic nerve would occur during distemper myoclonus in dogs. Further studies need to be done to find whether similar lesions could be detectable in other peripheral nerves and to find out reasons for these changes.

## **An outbreak of Haemorrhagic septicaemia in the Navithanveli veterinary range in Ampara district, Sri Lanka**

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Haemorrhagic septicaemia (HS) is fatal septicaemic disease occurs in bovine in the tropical countries of Asia and Africa caused by specific serotypes of *Pasturella multocida* B: 2 and E: 2 respectively. Sri Lanka was endemic for HS since mid-1950s where cases reported in two third of the island with the onset of North east monsoon. Routine vaccination was introduced in 1984 while no HS cases were reported since 2005 in Sri Lanka. Hence preventive vaccination of HS has not been carried out in Navithanveli range from 2005. The index case was reported with the sudden death of 15 and clinically affected 5 local buffaloes (11 males and 9 females) in the age group of 1-3 years in October 2016. The outbreak was continued and reported deaths of 25 buffaloes and 03 cattle respectively. Severe respiratory distress, brisket oedema, hyperaemic mucous membranes, hyperthermia and hyper salivation were observed in clinically affected buffaloes but the severity of the clinical signs were less significant in cattle. Necropsy findings of three buffaloes and one cattle revealed congested lungs, petechial haemorrhages on cardiac parenchyma, abdominal organs and blood tinged fluid in the body cavities. Furthermore, laboratory identification carried out by collecting samples of heart blood swabs, lung, spleen and direct culture on 5% blood agar, MacConkey agar and direct mouse inoculation of heart blood swab. Culture was positive for *P. multocida* where confirmation was done with serology using rapid slide agglutination test and conventional bacteriological test. Moreover, multiplex PCR used for rapid, sensitive and specific identification. Confirmatory findings revealed that the isolate was *P. multocida* serotype B.2. Antimicrobial sensitivity test indicate the isolate was sensitive for tetracycline, penicillin, augmentin, cephalixin and cloxacillin. The index cases were diagnosed as HS by the clinical signs and necropsy findings and initiate treatment with oxytetracycline (20%) and dexamethasone. At the same time all the animals in the herds were vaccinated with alum precipitated vaccine as a control measure. However, 4 animals died despite treatment; therefore early intervention with antibiotic was success for recovery of 41 animals. In conclusion, This is the first re-emerging outbreak occurred in Navithanveli range, however previous outbreaks were not documented in history. Early detection methods, restriction movements of animals, routine preventive vaccination programmes and awareness programmes are suggested to prevent further occurrence of the disease.

## ***Anaplasma marginale* infection confirmed by Major Surface Protein (MSP) 5 gene sequencing**

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*Anaplasma marginale* is an endemic parasitic infection in Sri Lanka. Even though studies are conducted on *A.marginale* since 1961, there are only few reported detections. Traditionally, the species identification is being done by thin blood smear examination and sometimes by serological tests such as indirect fluorescent antibody test and card agglutination test. There is one report of molecular detection by PCR. Also the lack of positive control has prevented confirmation of *A.marginale* infections. This case report describes the confirmation of *A.marginale* found from Southern Province, Sri Lanka using Major Surface Protein 5 (MSP) gene sequencing. *Anaplasma* suspected deaths in *Bos taurus* cattle were reported from a government farm in Southern Province. Blood samples were taken from 12 animals with clinical signs. Thin blood smears were prepared and Packed Cell Volume (PCV) detection was performed. DNA was extracted from the blood using QIAGEN DNeasy Blood and tissue kit following manufacturer's protocol and stored at -20°C. Concentration of the extracted DNA was measured with a spectrophotometer prior to PCR. DNA extracted from an unexposed calf was used as negative control. No working positive control could be found; hence it was decided to sequence the PCR product. PCR was performed to identify the presence of two *Anaplasma spp*; *Anaplasma marginale* and *Anaplasma centrale*. For *Anaplasma marginale* 690bp fragment of Major Surface Protein 5 (MSP5) gene was targeted (msp5F: 5'-ATGAGAATTTTCAAGATTGTGTCTAACCTT-3' and msp5R: 5'-AGGAAAGCCCCCAAAGCC CCATACTT-3') and for *Anaplasma centrale* 394bp fragment of MSP4 gene was targeted (msp4F 5'- CATGGG GCATGAAGTG 3' and msp4R- 5'AATTGGTTCAGTGAGCGCA 3'). PCR product was subjected to 1% Agarose gel electrophoresis. Bands were visualized under UV light using Gene flash gel documentation system. One out of ten (1/10) positive PCR products was sequenced at Genetech Molecular Diagnostics, Colombo 07, for MSP5 gene and resulting sequence data were subjected to Basic Local Alignment Search Tool (BLAST) for species verification and confirmation. Ten out of 12 thin smears were positive for *Anaplasma spp*. and it was tentatively diagnosed as *A.marginale* according to the morphology. Average PCV levels were 12%. PCR revealed all smear positive samples were positive for PCR at 690bp (10/10). Gene sequence confirmed the *Anaplasma marginale* species. In contrast to other genes of *A.marginale* such as MSP1a and MSP4, MSP5 sequences are conserved between isolates of *A. marginale* and are not phylogenetically informative. Therefore, MSP5 has been used as a marker for the molecular diagnosis of *A. marginale* infection. In the absence of a positive control, we have used this gene for confirmation of species. BLAST results showed *A.marginale* strains Dawn, Liangdang, St. Maries and Florida are homologous to the Sri Lankan isolate by 96%. Out of these, whole genome sequences are available in the GenBank sequence database for strains Dawn, St. Maries and Florida. Also another isolate, Gaoan53, MSP5 gene complete coding sequences are also homologous to our isolate by 96%. Therefore, it concludes that the Sri Lankan isolate reported in this case is *A.marginale*.



## Isolation and identification of *Brucella melitensis* from a swine herd in Sri Lanka

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Brucellosis is a zoonotic disease caused by the bacterium, *Brucella*. Some of the species of *Brucella* are host specific and some are non host specific. Clinical manifestations may vary with the type of species and the host affected. *Brucella abortus* is the commonly identified species in Sri Lanka and there are no any records available on laboratory identification of other species. However, few reports of caprine and porcine Brucellosis in the country in past could be found based on the serological diagnosis. In 2016, post-mortem samples from a swine herd were submitted to VRI with the complaint of an acute infection and several deaths. From which an organism suspected as the *Brucella* was isolated from the uterine swabs of three sows which were with previous history of infertility and abortion. With the objective to identify and confirm the isolated organism to the species level and also to find out whether any relationship between the isolated organism and the reported history, phenotypical, biochemical and genotypical characterization were carried out. All tests were done comparing to the *B. abortus* S -19 reference strain. Genetic characterization was done after confirmed by the phenotypical and biochemical tests. Polymerase chain reaction was performed using genus- specific and species specific primers (*B. abortus*; 5'GACGAACGGAATTTTCCAATCCC3', *B. suis*; 5'GCGCGGTTTTCTGAAGGTTTCAGG3' and *B. melitensis*; 5'AAATCGCGTCCTTGCTGGTCTGA3' with common reverse primer; 5'TGCCCGATCACTTAAGGGCCTTCAT3') and the organism was confirmed as *B. melitensis*. It was further verified as the *B. melitensis* by 16s rRNA sequence analysis. As per the literature, this is the first incidence of isolating *B. melitensis* from swine in Sri Lanka. In addition, all the pigs above 6 months of age in the affected farm were tested by Rose Bengal Plate Test and 36 out of 165 serum samples (23.6%) were found as serologically positive. However, relationship between the incidence of mortality of sows in that particular herd and *B. melitensis* couldn't be identified.

## **Identification of *Mycoplasma gallisepticum* from Chronic Respiratory Disease (CRD) suspected poultry flocks**

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Avian Mycoplasmosis caused by *Mycoplasma gallisepticum* (MG) is one of the most economically significant Mycoplasma pathogen of poultry, and has a world-wide distribution. MG is the etiological agent of Chronic Respiratory Disease (CRD) that can cause vertically or horizontally transmitted infections. The incidence rate of CRD in Sri Lanka had been reported as 8% in 1979 and thereafter published records of the disease are not available. However, evidences of CRD were reported in local poultry farms during last few years and poultry producers affirmed of high economic loss. Therefore, this study was aimed at identifying MG in CRD suspected poultry flocks. Sampling comprised eighty choanal swab samples; out of which fifty from two breeder farms with two suspected flocks in each and thirty from three broiler flocks. Aseptically collected samples were then transported to the laboratory in modified PPLO mycoplasma broth medium, where it was incubated at 37<sup>o</sup>C for 4-14 days. Afterward, the incubated samples with positive color reaction were inoculated into the PPLO agar to identify MG colonies under Phase Contrast microscope. The results revealed 12 (45%) breeder birds and 10 (33.3%) broiler birds to be culture positive. Subsequently, MG-type specific PCR was performed using extracted DNA to reveal 60% and 83% of breeder birds and broiler birds respectively to be positive. Moreover, PCR amplicons of breeder and broiler groups were subjected to 16srRNA sequence and genetic determination thus confirmed as MG. Hence, type-specific PCR assays demonstrated in the study could be useful as a sensitive means to determine and confirm MG diagnosis in poultry flocks where possible, in comparison to conventional culture techniques. It is noteworthy, the genotypic similarity of MG sequences from breeder and broiler flocks, providing insights in to same origin of infection. In addition, MG sequences obtained could be clustered to form a sister clade to available NCBI MG sequences.

## **Prevalence and Economic impact of Contagious Pustular Dermatitis disease of Goats in Sri Lanka**

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Contagious Pustular Dermatitis (CPD) is a zoonotic disease reported from all parts of Sri Lanka. In the North Central, Northern and Eastern provinces where 60% of national goat population is found, its incidence is higher. Accurate information on prevalence and economic impact of CPD in Sri Lanka is scarce. This study was conducted to investigate the prevalence and economic impact of CPD in goat farms. A questionnaire survey was conducted among 239 farms and 59 Veterinary Investigation Officers (VIOs) and Veterinary Surgeons (VSs) in Ampara, Kurunegala, Anuradhapura, Vavuniya, Trincomalee, Mannar, Batticaloa, Puttlam and Kandy districts. All 239 goat farms were visited and information on breeds, management practices, numbers and ages of animals infected, immunization, therapeutic procedures, production and price of mutton and farmers' knowledge on CPD were collected through questionnaire survey. Information from the VSs and VIOs were collected through questionnaire survey and telephone interview. Data were subjected to descriptive analysis. Results revealed that the majority of goats (91%) was of indigenous type while the rest (9%) was Jamunapari crosses. Most farmers (85.7%) preferred sole livestock farming to integrated farming, and adopted extensive (66.7%) or semi intensive (24.6%) management practices. Over 50% of the surveyed farms was affected by CPD. Apparent prevalence of CPD was higher in Trincomalee (64.7%) and in extensively managed farms (64.2%). Although infected animals showed characteristic symptoms, they were not separately reared. Most farmers (79%) were unaware of the zoonotic nature of CPD. No farms were vaccinated during outbreaks but were treated with antibiotics and anti-inflammatory drugs. In 35.5% and 20% farms, CPD has lowered growth rate and milk yield, while in 27% and 81.5% farms CPD has increased mortality rate and cost of production. Estimated farm gate price of cured goat meat (Rs.315.22±47.31/kg) was lower than that of healthy goat meat (Rs.625.5±62.52/kg).

## Re- emerging of Haemorrhagic Septicaemia in Sri Lanka

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Haemorrhagic Septicaemia (HS) is a highly fatal disease in domestic large ruminants of tropical regions. It is a primary pasteurellosis caused by two specific serotypes of *Pasteurella multocida*, i.e. Asian 6: B (also known as B:2) and African 6:E (also known as E:2). In Sri Lanka, HS remained as the principal disease causing deaths of cattle and buffaloes for many decades. Nevertheless, the last reported outbreak was in 2004 and Sri Lanka was able to declare itself free of HS in 2012. Subsequently, from end of the year 2015, several isolated cases of similar clinical signs were reported specially in North-Central and Eastern provinces, and organism isolated of which was identified as *Pasteurella multocida* type B:2. In the light of the re-appearance of this killer disease after a decade, particularly in the absence of latent carriers as discovered by island wide survey done in several years back, a comprehensive analysis of the present pathogen is a prudent necessity. Hence, this study was aimed at establishing a confirmatory PCR assay and thereby to compare the re-emerged pathogen with previous isolates. For this purpose, ten isolates each obtained before 2004 and after 2015 and which were confirmed through biochemical tests, Indirect Haemagglutination test and Agar Gel Precipitation Test were used. Three pairs of primers (*P.multocida* specific primers: KMT1T7 : 5'-ATCCGCTATTTACCCAGTGG-3' and KMT1SP6: 5'-GCTGTAAACGAACTCGCCAC-3', HS causing type B specific primers: KTT72: 5'-AGGCTCGTTTGGATTATGAAG-3' and KTSP61: 5'-ATCCGCTAACACACT CTC-3', *P.multocida* type A specific primers: RGPMA5: 5'-AATGTTTGGCGATAGTCCGT TAGA-3' and RGPMA6: 5'-ATTTGGCGCCATATCACAGTC-3') were used as described in OIE terrestrial Manual 2002. The specificity of primer pairs was confirmed using known reference cultures, after which PCR assays were done to determine the entire outbreak isolates. Consequently, all the isolates were able to produce amplification products for *P. multocida* species-specific as well as type B-specific in particular, providing the proven of re-emergence of HS. Further study being continued for sequencing and phylogenetic determination of PCR amplicons and whole genome of bacterial isolates obtained before 2004 and after 2015.

## Occurrence of Brucellosis in cattle and buffalo in Trincomalee district during 2011-2016

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Bovine brucellosis is an important disease, which is manifested by late term abortions due to placentitis, weak calves, still births, infertility epididymitis and orchitis. Brucellosis causes a significant economic loss and is one of the most devastating transboundary animal diseases and also a major trade barrier. Brucellosis is considered as a major cause of abortions among cattle and buffaloes in Sri Lanka too and the first confirmed case was reported in 1956. The prevalence was high in North Central, North Western, Eastern and Southern provinces and lower in Western and Central provinces. However, no studies were carried out recently especially in Trincomalee district. Hence, a study was conducted by Veterinary Investigation Center, Trincomalee from 2011 to 2016 to determine the seroprevalence of *Brucella abortus* in cattle and buffaloes in Trincomalee district. Serum samples from 3111 of unvaccinated, healthy and aborted cattle and buffalo between 03 months to 12 years were collected within the eleven veterinary ranges. Eighty percent of samples were collected from cattle where as 20% were from buffaloes. *Brucella* antibodies were screened by Rose Bengal plate test (RBPT) and the positive samples were confirmed by Complement Fixation Test (CFT). One hundred samples were serologically positive for *Brucella abortus* antibodies and this is 3.2% of the tested population and within that positive samples, 82% from cattle and 18% from buffalo, which is 3.3% of the tested cattle population and 2.9% of the buffalo population and there is no brucellosis-free range within the district. Interestingly, 25% of the positive samples were from Kanthalai veterinary range and majority of positive animals (43.29%) were found between the ages of 4 - 6 years. When farmers were interviewed individually, it was found that introducing new animals without screening, lack of awareness and milk testing in collecting centres were found as risk factors for transmission of disease among herds. Therefore, vaccination and creating awareness among people are an effective ways to control brucellosis in cattle and buffaloes. S19 vaccination was started in 2011 in Trincomalee district and from year 2011 to 2016, 6654 female cattle and buffalo were vaccinated by S19 vaccine and efficiency of brucellosis vaccination will be studied in future.

**Prevalence of *Salmonella*, *Campylobacter*, *Escherichia coli*, *Staphylococcus spp* and detection of antimicrobial resistance (AMR) associated with *E. coli* isolated from commercial poultry layer farms in Kurunegala district**

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Foodborne pathogens arising from poultry and poultry products have become a great public health concern throughout the world including Sri Lanka. Poultry is the leading, well developed livestock industry in the country producing table eggs and broiler chicken mainly to local market and also some considerable amount to export market. Therefore aims of this study were to detect the prevalence of main foodborne pathogens including *Salmonella*, *Campylobacter*, *Staphylococcus spp.* and *E. coli* and AMR patterns of selected *E.coli* isolates from 50 commercial poultry layer farms located in Kurunegala district where the highest poultry population of Sri Lanka is reported. Fecal samples, drag swabs from poultry litter, cloacal swabs and skin swabs from birds were collected to isolate and identify *Escherichia coli*, *Salmonella*, *Campylobacter* and *Staphylococcus spp.* respectively. Isolation and identification of the above mentioned four pathogens were performed according to accepted standards with certain modifications. Profiling of antimicrobial resistance in *E. coli* isolates was done following the method described in Clinical and Laboratory Standards Institute for 12 antimicrobials using disk diffusion assay. Out of the 50 farms tested, all farms were positive for *E.coli*. The prevalence of Staphylococci was 98% whereas, the prevalence of *Campylobacter* and *Salmonella* were 20% and 4% respectively. Antimicrobial susceptibility profile of 25 *E.coli* isolates from 25 different farms indicated different levels of resistance against tested antimicrobials. The highest resistance was observed for tetracycline (64%) followed by nalidixic acid (48%), trimethoprim-sufamethoxazole (44%) and ampicillin (44%). Lower levels of resistance; 12%, 12%, 8%, 4%, 4% were seen for streptomycin, ciprofloxacin, chloramphenicol, ceftazidime and amikacin respectively. Except one isolate with intermediate resistance all other isolates were susceptible for ceftriaxone. Further all the isolates were susceptible for imipenem and gentamicin. Results of this study indicate the presence of foodborne pathogens associated with commercial poultry layers in considerable amount. It also shows the higher levels of antimicrobial resistance for commonly use antimicrobial groups in poultry sector such as tetracyclines, quinolones and macrolides. Considering above results, it is advisable to adopt measures to prevent pathogens associated with poultry layer houses by implementing proper biosecurity, good management practices and prudent use of antimicrobials.

## **Effect of water temperature on the occurrence of iridovirus in Asian sea bass (*Lates calcarifer*)**

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Asian sea bass (*Lates calcarifer*) usually called Barramundi is one of the tasty and nutritious food fish cultured both in marine and freshwater. High mortalities due to irido virus infection were frequently observed in the sea cages of barramundi cultured in the Eastern sea of Sri Lanka, especially during the post stocking at the fingerling stage. The present study was conducted to evaluate the effect of the heat treatment on the presence of virus in the Asian sea bass. Liver, spleen, kidney and brain samples from sea bass collected during a previous outbreak of Sri Lankan sea bass irido virus disease (SLSBIVD) that had been confirmed by PCR were used for the artificial infection of sea bass through oral route. The experiment was designed with five treatments each with three replicates. Four experimental treatments (35, 40, 45 and 50 °C) and a control treatment. Each experimental tank of 10 l capacity was stocked with 50 fish weighing around 20g. The fingerlings of barramundi in the all experimental tanks (15 tanks) of five treatments were maintained for two weeks with minimal feeding and confirmed for negative for SLSBIVD. Fish paste prepared soon after thawing from -80 °C was given to the fish in four treatment tanks. Fish in the control tanks were not given the fish paste. After 5 days of oral infection, fish fed with fish paste were showing the signs of irido viral infection such as off food, scales off etc. The presence of SLSIV was confirmed by PCR in all four treatment except the control. Water was heated by a boiler and given in respective temperature for one hour. Mortality of fish in the treatment tanks of 40 °C was stopped while fish in all other treatment tanks were started to die. Observed mortality were all fish, 28 and 42 in the tanks of 35, 45 and 50 °C respectively. PCR results revealed that there were no virus in treatment tanks of 40, 45 and 50 °C. The experiment concludes that the best heat treatment is 40 °C for one hour, while 35 °C is not effective and more than 40 °C would be harmful to the fish, as the fish mortality in treatment tanks of 45 °C and 50 °C was due to the high temperature.

## Monitoring of *Theileria orientalis* major piroplasm surface protein (*mmsp*) genotypes in cattle in Polonnaruwa and Nuwara Eliya districts of Sri Lanka

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*Theileria orientalis* occasionally induces clinical disease in cattle. In Sri Lanka, recent cattle surveys reported four *T. orientalis* genotypes, including types 1, 3, 5, and 7, based on major piroplasm surface protein (*mmsp*) gene. Proper knowledge on the diversity and temporal changes in the genotypes are essential before any immune control strategies can be designed, as the immunity against *T. orientalis* is usually genotype-specific. In the present study, therefore, we monitored the *T. orientalis mmsp* genotypes in cattle in two districts, namely Polonnaruwa (dry zone) and Nuwara Eliya (wet zone). DNA samples extracted from blood collected in Polonnaruwa (n=75; *Bos indicus*) and Nuwara Eliya (n=161, *Bos taurus*) in June, September, and December 2014 and March 2015 were screened by a *T. orientalis*-specific MPSP-PCR assay. The findings indicated that 47, 42, 44, and 44 samples in Polonnaruwa and 86, 111, 117, and 126 in Nuwara Eliya were positive on 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> sampling, respectively. In addition, 58 and 147 animals in Polonnaruwa and Nuwara Eliya, respectively, were parasite-positive at least on one sampling occasion. The *T. orientalis*-positive samples were then analyzed by PCR assays specific to genotypes 1, 3, 5, and 7. The findings demonstrated that the rate of infections with multiple genotypes in Polonnaruwa (63.3 – 77.8%) and Nuwara Eliya (67.1 – 72.5%) were significantly higher ( $p < 0.05$ ) than that of single genotypes on all four sampling occasions. *T. orientalis*-parasite negative animals on 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> sampling became infected with multiple or single genotypes on 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> sampling, respectively, and the rates of multiple and single infections among such animals were comparable to each other ( $p > 0.05$ ). These findings suggest that the mixed infections might be due to simultaneous transmission of multiple genotypes by co-infected ticks and/or re-infections with different genotypes. However, surveys to detect *mmsp* genotypes in ticks are essential to confirm our assumption. Additionally, the genotypic profiles of *mmsp* in individual animals markedly differed between consecutive samples in Polonnaruwa (63.3 – 64.3%) and Nuwara Eliya (73.5 – 82.6%), suggesting that the parasitemia of *mmsp* genotypes fluctuate temporally to escape from the host immunity. On the other hand, 17, 14, 8, and 6 samples from Polonnaruwa, and 10, 13, 8, and 11 samples from Nuwara Eliya were negative for all four genotypes tested, suggesting the presence of additional genotypes or that the concentration of DNA template of individual genotypes may not be sufficient to yield visible PCR product. The future investigations, therefore, should analyze the *mmsp* sequences derived from the samples that were negative by type-specific PCRs. In conclusion, the findings of the present study suggest that the genotypic diversity of *T. orientalis* undergo drastic changes temporally within cattle populations as well as within individual animals.



## **Reporting the preliminary results of synthesizing a thermosensitive nanoparticle to target solid tumors**

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A significant proportion of systemically injected chemotherapeutic agents end up in healthy organs due to lack of specificity. Consequently, patients subjected to chemotherapy undergo life threatening toxicities with only a modest survivability. Low Temperature Sensitive Liposomes (LTSLs) are modified stealth liposomes which rapidly release their contents in response to mild hyperthermia (< 42 °C), and are being widely explored for energy deployed targeted drug delivery. The objective of this study was to synthesize and characterize doxorubicin loaded LTSL (Dox-LTSL) to evaluate the feasibility of achieving targeted therapy. This research work has been initiated to establish a strong nanomedicine program in the country to achieve the government's goal of making Sri Lanka a leader in nanotechnology based research. A combination of three phospholipids, 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine (S-lysoPC), 1,2-Dipalmitoyl-sn-glycero-3-phospho choline (DPPC), and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3 phosphoethanolamine (MPEG -2000-DSPE) were dissolved in a minimum volume of chloroform at a molar ratio of 85.3:9.7:5.0. Then, the organic solvent was evaporated to dryness in a rotary evaporator and the resulting thin film of phospholipids was hydrated using 300mM citrate buffer at pH 4.0. Next, the hydrated lipids were extruded 5 times through a polycarbonate filter of 200nm pore size to yield bland liposomes (LTSL). Encapsulation of doxorubicin into LTSLs was carried out actively using a pH-gradient method. Then, LTSLs were characterized for size, and encapsulation efficiency. The average size of the nanoparticles were 138nm with a 7.3% coefficient of variation and a polydispersity index of 23.5%. At the optimum loading efficacy (2%, i.e. 2mg of doxorubicin loaded to liposomes prepared with 100mg of lipids), the encapsulation efficiency was over 88%. While quiescent at normal body temperature (37°C), Dox-LTSL could trigger release doxorubicin in response to treatment with a mild hyperthermia of 41-42°C. The above nanoparticle formulation was stable up to a week at 4°C without a significant deviation of the size. The results of this study revealed that doxorubicin can be entrapped effectively within thermosensitive liposomes, with a potential to trigger release the therapeutic agent inside a tumor, in response to application of heat, and thereby minimizing the exposure of healthy tissues to the chemotherapeutic agent. The next step of the study would be to validate this preliminary results using cell culture based experimental models.

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## **Evaluating immune memory resilience using sheep lymphatic cannulation model**

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Vaccines provide protection through inducing rapid and magnified immune recall following pathogen encounter. With the evidence that pathogens manipulate host immunity, the quality of the vaccine-induced immune memory becomes important when vaccines are against such pathogens. In this context, the quality of vaccine-induced immune memory can be seen as the ability of the immune response to resist manipulation by immuno-modulatory pathogens; we have termed this characteristic “immune memory resilience”. In order to measure immune memory resilience we used sheep pre-femoral efferent lymphatic duct cannulation model. Taking advantage of the fact that immune responses could be studied in real time as they occur at the level of the local lymph node, we compared two adjuvants (alum and Quil A) in the same animal. Switching adjuvants for the booster allowed us to assess the resilience of the induced immune memory response. While both sides of the animal received the same total treatment, the sequence of adjuvants used was reversed. If the immune memory induced is resilient the sequence would matter and each side would be different following this treatment. However, without immune memory resilience, the outcome would be essentially the same. Multiple T tests were used to compare IgG1 and IgG2 titre on daily collected lymph samples. Following a single vaccination with the model antigen, ovalbumin, and one adjuvant (OVA+ alum or OVA+ Quil A) the induced memory response was not resilient and was easily manipulated by the opposite adjuvant (4 sheep) as measured by antibody isotype concentration (IgG2 ELISA for Th1 and IgG1 ELISA for Th2). Importantly, two vaccinations with the same adjuvant dramatically increased the level of resilience (i.e. quality) of the immune memory (5 sheep), *in vivo*, upon opposite adjuvant challenge. Thus vaccines against immuno-modulatory pathogens would possibly be benefited from multiple injections.

## Sero-prevalance of *Neospora caninum* antibodies in a dairy farm

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The protozoan parasite *Neospora caninum* is one of the major causes for bovine abortions worldwide. As the infection can pass from cow to its calf and produce persistently infected offspring, Neosporosis can be a devastating disease to the livestock industry. An outbreak of abortions was encountered in a dairy farm from December 2015 to May 2016 and a study was carried out at the height of abortions to determine the sero-prevalance of *Neospora caninum* antibodies in the affected herd. Blood samples were collected randomly from 72 cows of reproductive age, serum separated and stored at -200C until testing. *Neospora caninum* antibodies were detected by an indirect ELISA assay (Svanova, Sweden). Samples were run in duplicates and optical density (OD) was measured at 450nm. Samples were considered positive when Percentage Positivity (PP = OD sample or Negative Control / OD Positive Control X100) was 20 or above and negative if PP was <20. History of abortions in sampled animals and presence of dogs in the farm were recorded. Chi Square test was done to see the association between sero-positivity and abortion status at p< 0.05. Sero-prevalance of *Neospora caninum* antibodies in the farm was 63.9% (n=46/72). Of the sampled cows, 38.9% (n=28/72) experienced abortions during the outbreak while 61.1% (n=44/72) of the animals did not abort. The majority of the aborted animals (71.4%) were sero-positive for *Neospora caninum* antibodies. Abortions were encountered in 11.1% (n=8) sero-negative animals. All the abortions were between 5 to 8 months gestation. Out of the 44 cows that did not abort 26 (59.0%) were sero-positive. Chi square analysis revealed that there was no significant association between sero-positivity and abortion status. At the time of sampling 8 dogs were found roaming in the farm. According to the authors' knowledge this is the first report of finding *Neospora caninum* sero-positive cattle in Sri Lanka. Since the sero-prevalance is high in the farm abortions may be encountered in future pregnancies of sero-positive animals. Dogs in the farm may be a contributing factor to the high sero-prevalance of *Neospora caninum* antibodies. *Neospora caninum* could be an emerging threat to the livestock industry in Sri Lanka.

## **Development of an online feed formulation platform by transforming grouped square method into a computer automated software**

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Calculation of nutrient components in various feed materials is one of the key steps involved in the process of formulating feeds for both companion and farm animals. Thus, numerous methods such as Trial and Error, Pearson Square and Linear Programming (Least Cost Method) are being used for that purpose. Satisfying of only two feed ingredients is the major disadvantage of the Pearson Square method. Furthermore, Linear Programming is not simple to be used by an ordinary farmer as it requires advanced knowledge to operate. Further, both Trial and Error method as well as Pearson's Square method involves several manual calculations which make them difficult to be used. Group square method is a modification of Pearson's square method where user can use more than two feed ingredients in the process of feed formulation. However, there is no computer automated platform to make this method a user-friendly. Therefore the objective of this project is to develop a user friendly easily accessible online platform to make a simple ration using Group Square Method, which fulfils the nutritional requirement of the animals. Therefore online feed formulation platform has been developed using web based programming languages know as HTML 5 and PHP 5.4 and the associated database is developed using MySQL 4.0. In the process of evaluation proximate analysis of the sample created according to formula generated from the software gives accuracy of 90.55%. Currently platform can be accessed directly from <http://vetfeed.esy.es>. Final formula can be obtained based on the selected ingredients and it's availability. The developed platform can be effectively used to formulate feed rations to be used in poultry, aquaculture and swine industry but the limitation is the availability of internet facility. This program will estimate the proportions of each ingredient in the final formula, metabolizable energy (ME) and the estimated value of cost per kg of feed. This can be used in the field to formulate a ration by balancing required crude protein level but the limitation is currently the platform is generating only one formula form selected ingredients.

## **Antimicrobial use and Emerging Multi-Drug Resistant ESBL producing *Escherichia coli* in northern poultry production in Sri Lanka**

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Antibiotic use in farm animals, are critically important in public health which implicated in the emergence of new forms of multi-resistant bacteria that may infect people. In food animals, antibiotics are usually being administered for therapeutic, metaprohylactic, prophylactic purposes but also as growth promoter and for better feed efficiency bearing the risk of selecting resistances against antibiotics in zoonotic and commensal bacteria of animals and humans. The production of extended-spectrum Beta Lactamases (ESBL) in *Escherichia coli* represents a considerable emerging problem in human and veterinary medicine. This preliminary cross-sectional study was focused on antimicrobial use (AMU) and antimicrobial resistance (AMR) by identifying the presence of multi drug resistant ESBL producing *Escherichia coli* (ESBL-EC) in commercial broiler chicken farms in Jaffna district. Potential risk factors of antimicrobial usage practices were obtained by means of a questionnaire. Odds ratio (OR) and 95 % confident levels (CL) with the percentages were calculated from univariate analysis for quantifying significant associations by using Epi Info™ 7 statistical software. 25 g pooled fecal samples were collected from 83 broiler farms and the isolation of ESBL-EC were carried out by the method of MacConkey supplemented with 1mg/L Cefotaxime agar plate and the ESBL confirmation by double disk diffusion methods by using ESBL antibiotics discs. The ESBL-EC was detected in 40.96 % (34/83) of the broiler farms. Broiler farmers use antibiotics either for prophylactic or therapeutics purposes. The antimicrobials commonly used (AMU) were Enrofloxacin (100%), Tetracycline (55.4%), Trimethoprim/Sulfamethoxazole (49.4 %), Doxycycline (44.6 %), Amoxicillin (41 %), Chloramphenicol (12 %), Cloxacillin (8.4 %). In which the fecal ESBL-EC isolates showed resistance to Ciprofloxacin (91.2%), Nalidixic Acid (91.2%), Enrofloxacin (85.3%), Trimethoprim/Sulfamethoxazole (85.3%), Tetracycline (85.3%), Doxycycline (52.9%), Amoxicillin/Clavulanic acid (17.6%), Chloramphenicol (14.7%). At this point, the resistance to therapeutic importance antibiotics becomes a major concern, since these antibiotics usages select ESBL producing *E.coli* against these antibiotics through co-selection. The critical factors such as 'More than 4 groups of antimicrobial used 65 % (54/83) throughout the fattening period (OR=16.65, CI=4.96 - 55.80), antibiotic treatment for other rearing animals 57 % (47/83) (OR=4.4, CI=1.73 - 11.17), rearing layer birds at same farm 66 % (55/83) (OR=2.82, CI=1.15 - 6.89) were found to be the risk factors for the occurrence of ESBL-EC in the broiler farms studied. Our study result showed, nearly 70 % (58/83) of broiler farmers adhere to buy-back system (OR=4.3, 95 % CI 1.71 - 10.78) through middle man which showed four times more likely to be ESBL-EC farm positive than those not practicing the buyback system with middle man. These alarming first findings should result in properly planned measures to ensure prudent use of antibiotics linked with improved management practices to prevent further colonization and transmission of ESBL-EC to eventually the human and animal population.

## **Study on Beta casein A1 and A2 polymorphism in Ayrshire and Holstein Friesian dairy cattle populations in two upcountry dairy farms**

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Beta casein (CSN2) is an important protein in bovine milk and histidine to proline substitution in the encoded protein causes variants CSN2-A1 and CSN2-A2. Recent studies have shown an association of CSN-A1 milk with certain health issues such as increased cardiac diseases in humans. It was revealed that histidine in A1 variants causes the release betacasomorphin-7 in gastrointestinal proteolytic digestion which leads to health issues while A2 hinders the cleavage at this position due to the presence of proline. There is an increased demand for CSN2-A2 type milk and in some countries there are selective breeding programs to remove the A1 allele from milking herds. Therefore a preliminary study was performed to analyze the A1/A2 allele frequencies in two major dairy breeds in Sri Lanka. Blood samples were collected from 123 Ayrshire and 101 Holstein Friesian cows in two upcountry dairy farms and DNA was extracted. CSN2 polymorphism (A1/A2) was analyzed by PCR-RFLP method using the restriction enzyme DdeI. The results of this study revealed A1A2 genotype to be predominant in both Holstein Friesian and Ayrshire populations. CSN2 genotypes and their frequencies in Ayrshire cows were A1A1 (0.18), A1A2 (0.68), A2A2 (0.14). Genotype frequencies in Holstein Friesian cows were A1A1 (0.07), A1A2 (0.63), A2A2 (0.30). Frequencies of A1 and A2 alleles were 0.52 and 0.48 in Ayrshire cows and 0.39 and 0.61 in Holstein Friesian cows. The Chi square test showed that the genotype distribution of the both tested Ayrshire and Holstein Friesian populations to have a significant departure from the Hardy-Weinberg equilibrium ( $p < 0.05$ ). Both populations had relatively high heterozygocities and high negative FIS values (-0.3681 and -0.3366). This indicates less inbreeding that can be explained by the selective breeding practiced in the both population. This study concluded that the A1 allele of CSN2 gene is present in considerable frequencies in the two farms. Further studies will be required to genotype more dairy cattle breeds (*Bos indicus*, *Bos taurus* and indigenous) in Sri Lanka.

## **Antimicrobial resistance of contaminating bacteria of curd and yoghurt produced by small-scale processors in Kandy district**

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Bacterial contaminants in ready-to-eat food and associated antimicrobial resistance (AMR) pose serious threat to human health. Currently, AMR of common food contaminants such as *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* is a global public health issue. Curd and yoghurt are popular ready-to-eat milk products in Sri Lanka but often reach retail markets without quality certification. Poor quality of these products from small scale processors was revealed by studies in North Central, Sabaragamuwa and Southern Provinces. High bacterial counts in raw milk under local field conditions result in poor quality of dairy products. Thus there is a greater possibility of curd and yoghurt getting contaminated with AMR bacteria. Therefore, a study was designed to assess microbiological quality of curd and yoghurt from small scale processors in Kandy district while investigating the resistance profiles of contaminating bacteria. Isolation and identification of bacterial contaminants and testing of their resistance profiles were performed according to guidelines of Sri Lanka Standards (SLS) and Clinical and Laboratory Standards Institute respectively. Samples were obtained from 6 curd and 9 yoghurt processors. A total of 30 curd pots from six batches and 98 yoghurt cups from 27 batches (3 batches from each processor) were tested. Results indicated that 66% of curd and 38% of yoghurt batches were microbiologically unacceptable according to SLS. Finding is in agreement with published literature and warrants the need of educating small-scale processor on hygienic processing. Identified contaminating bacteria were *E. coli* (57), *K. Pneumoniae* (7) and *S. aureus* (1). Considerably lower level of resistance to tested antimicrobials was seen in *E. coli* isolates. The highest resistance was for ceftazidime (3.5%). More than 98% of the isolates were sensitive for nalidixic acid, ceftriaxone, ampicillin, gentamycin, and imipenem. All *Klebsiella* isolates were resistant against ampicillin, while 14.3% were resistant to cefotaxime and ceftriaxone. *S. aureus* isolate was resistant to erythromycin. Unhygienic food handling is a major reason for having contaminated organisms bearing AMR in dairy products. Although only small percentage of bacteria were resistant to antimicrobials, continuous surveillance is required to ensure that public health risk from AMR bacteria in fermented dairy products is minimum.

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## **Lessons learnt from the Heifer Calves Rearing (HCR) scheme in Vaddukkodai, Jaffna**

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The dairy industry has enormous potential to contribute to Sri Lanka's economic development. The Department of Animal production and Health (DAPH) implemented the "Heifer Calf Rearing" (HCR) scheme to further strengthen livestock production and livestock-based farmer livelihoods. This preliminary cross sectional study was conducted in the Vaddukkodai Veterinary Surgeon's (VS) Range in the Jaffna district, to find out the impact of the HCR programme by investigating heifer calves born by artificial insemination (AI) and to assess the risk factors that hinder the success of this program. Details of 223 registered AI-born female calves during 2013 to 2015 were collected from the available records. Additional information was obtained through a questionnaire. Out of 369 registered calves, 223 were female (male:female = 1:1.43). Forty three percent of the farmers that owned a female calf (98/223) had received the first payment. The percentages of farmers that had received for second, third and fourth payments were 8%, 3% and 1%, respectively. Calf mortality rate was 11%. Calf death or selling the cow with calf within one month of calf-birth without proper ownership transfer (OR= 1.16, 95% CI 1.01- 3.04) and failure to report birth of female calves (OR= 2.32, 95% CI 0.92- 5.87) were the main reasons for non-issuance of the 1st payment. Allowing the calf to graze with the cow (OR= 2, 95% CI 1.12- 4.86) and allowing the heifer to breed naturally (OR= 1.91% CI 1.01- 4.58) were the major factors for non-issuance of the 2nd payment. Repeat breeding (OR= 3.50% CI 1.81- 15.15), inappropriate document maintenance (OR= 1.52% CI 0.98- 6.62) and failure to report parturition (OR= 2.28% CI 0.86- 22.79) were reasons for non-issuance of 3rd and 4th payments and subsequently dropping out from the HCR scheme. Furthermore, it was observed that all heifer calves which obtained 4th payment had shown first behavioural oestrus before 15 months of age. Fulfilling the main objective of the HCR project, i.e. to increase local milk production, the annual milk production of Vaddukkodai veterinary range has increased by 20% (1,208,065 to 1,455,860 liters) from 2013 to 2015. Availability of breedable heifer calves has also increased. These findings can be useful to the DAPH and district veterinary surgeons in implementation of the HCR scheme or similar programmes linked with improved management practices with awareness and follow-up monitoring, in the future.



## **Evaluation of farmer attitudes on dairy cattle welfare in Central province of Sri Lanka**

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Welfare of dairy cattle is important for higher milk yield in dairy industry. Dairy farmer is responsible for management decisions, housing and cattle handling and, farming involves high level of human-animal interactions which affect welfare of cows. This study investigated the attitudes of farmers on cattle welfare and its relationships with on farm management aspects. Study consisted of farmer interviews, observations of cattle-human interactions and a questionnaire survey. Study included 129 farms in the central province of Sri Lanka. Farmers were interviewed and their answers reflect their own attitude towards welfare cattle. Farmers were categorized as negative or positive based on the number of positive and negative answers given. Several questions in the questionnaire evaluated the attitude while others related to farmer observations on welfare of cows. Management practices were recorded with the aim to find actual way of treating animals, as it mirrors the real attitude of farmer. Presence or absence of hoof trimming, clinical mastitis, waterer and feeder, controlling ectoparasites, disease prevention methods were recorded during the questionnaire. Behavioural observations on both farmer and cattle were recorded during milking time in 42 farms to cross check the farmer attitudes with animal handling. Flinch-step responses and flinch step kick responses were measured as cow behavioural traits and number of pats or resting hand on flank or back, number of moderate slaps, number of forceful slaps, number soft vocalizations, number of loud vocalizations per cow per milking were observed for behavioural traits of farmers. Observers kept a reasonable distance to avoid cows from getting excited. According to the questionnaire survey 52.8% of farmers had positive attitudes and 48.2% had negative attitudes towards dairy cattle welfare. Statistical analysis revealed that number of forceful slaps per cow per milking is significantly higher ( $P < 0.05$ ) when the farmer has a negative attitude. This study could not establish any other significant relationships for farmer attitudes with welfare aspects tested in the study. Most important was that irrespective of farmer attitude many farms had problems in housing, management and sanitation conditions that adversely affected cow welfare.

## **Assessment of suitability of indirect milk quality tests in determining the microbial load of raw milk**

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Cow milk is a highly nutritive and perishable food that needs careful handling to avoid bacterial contamination at each level of production. High bacterial counts in raw milk may cause food poisoning and gives rise to poor quality dairy products. Therefore, assessing bacterial counts in raw milk at the level of collection is important. The alcohol test, methylene blue dye reduction test (MBRT) and resazurin dye reduction test (RDRT) are commonly used in Sri Lanka to determine the hygienic quality of milk. Therefore, the objective of this study was to compare results generated by the above mentioned indirect tests and the true bacterial counts present in the raw milk. Ninety-three milk samples were collected from the milk collectors in Udunuwara (38) and Doluwa (55) areas in Kandy district. The standard plate count method was utilized to determine the true bacterial numbers in the milk samples. The above mentioned indirect tests were carried out on the same samples according to FAO guidelines. However, the alcohol test was conducted using two different concentrations of ethanol (68% and 80%). Out of the 93 samples, 91 samples had a standard plate count above the milk acceptance level of the EU standard (> 100000 cfu/ml) indicating that 97.8% of the samples had an unacceptable hygienic quality. The false negative rates of the MBRT, RDRT, 68% alcohol test and 80% alcohol tests were found to be 62.3%, 48.3%, 18.2% and 12.9%, respectively when compared with the EU standard. Therefore, there is a need for developing a quick and reliable test method for testing the hygienic quality of raw milk under Sri Lankan field conditions.

## **Correction of Premolar and Molar tooth malocclusions in a Rabbit: A case study**

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Rabbits are considered to be the third most popular companion animal species in the world. They are presented with dental conditions more often than dogs and cats. Because undetected dental issues can lead to potentially life threatening conditions, dental evaluation is critically important in all sick rabbits. “Rabbit”, a 3 year old, 1.9kg, New Zealand White crossbred, accurately vaccinated and de-wormed male rabbit was presented to Pet Vet Clinic, Colombo in 2016 with reduced appetite and weight loss over a few months. It has been force-fed with finely chopped matter and juices of a variety of green leaves. Oral examination with the otoscope revealed malocclusion of premolar/molar teeth due to buccal and lingual spikes. Except for emaciation, all other clinical parameters were normal. It was decided to correct the condition under general anaesthesia. No fasting was required as rabbits cannot vomit. Meloxicam (1mg S/C) was given as an analgesics while metoclopramide (1mg S/C) and ranitidine (8mg S/C) were given prophylactically against gut-stasis and gastritis, respectively. Anesthesia was induced with 20mg ketamine / 0.3mg medetomidine / 1mg butorphanol combination, and maintained with 1%-2% isoflurane with oxygen, using a mask. The rabbit was placed on a hot water bag covered with a towel to prevent hypothermia. Oral examination under anaesthesia with a rabbit mouth gag and a cheek dilator confirmed dental spikes causing malocclusion. Further, lingual spikes of the left mandibular cheek teeth had led to severe erosion at the tongue base. Spikes were trimmed using an electric burr to get a functional grinding occlusion. Overheating of teeth was prevented by spraying distilled water at regular intervals. Isoflurane was then discontinued, medetomidine was reversed with 1mg atipamezole, and the rabbit was transferred to an oxygen cage for recovery. The recovery was smooth and took five minutes. Dextrose (50%, 1.5 ml) was given orally to prevent hypoglycemia, and “Rabbit” was discharged within 45 minutes. Metronidazole oral gel was prescribed to be applied on the tongue wounds. The patient started to eat normally by the following morning and a 24% weight increase was evident within a few weeks. Rabbits are hypsodonts; a balance between tooth eruption and attrition is crucial for normal alignment of teeth and a healthy life. A high fiber diet and a normal anatomy of the skull are important to maintain that balance. Therefore, pet rabbit owners should be educated on proper husbandry practices to prevent/minimize dental malocclusions.

## **Successful correction of a colorectal obstruction in a dog - A case Report**

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Most of the reported tumors in the large intestine are either benign tumors or polyps which can be later developed in to neoplasms. Adenocarcinoma, alimentary lymphoma and leiomyosarcoma also have been described in digestive system of the dogs. This case presents successful surgical correction of a colorectal obstruction possibly due to an adenomatous polyp. A seven years old male German shepherd dog was presented to the City Pet Animal Hospital with Tenesmus, hematochesia and vomiting lasting over a period of year. The dog had been treated with broad spectrum antibiotics previously suspecting colitis and had not responded to the treatment. On admission, a moderate anemia (PCV 19%, HB8.45g/dl, RBC 3.0, 106/ $\mu$ l) was noted. Clinical examination suggested an abdominal involvement and subsequent abdominal radiograph showed a distended area in the large intestine with multiple radiopaque structures suggesting a calcified mass. In addition, intussusception also was noted. (Upon examination of lateral and DV radiograph of the abdomen telescoping of intestinal loop was evident). After induction of general anesthesia on this dog, an exploratory laparotomy was performed. A distended hard area was palpated along the intestines and the lumen was narrowed at this point due to the intussusception. After assessing the bowel viability, the affected part was resected and “End to end anastomosis” was performed. Upon dissection of the removed portion of the intestine a calcified solitary intraluminal mass was detected which could be a polyp or a benign tumor. Histopathology could not be performed on this tissue and therefore, diagnosis could not be confirmed. Postoperative management with broad spectrum antibiotics and appropriate supportive therapy continued. The response to treatment was highly satisfactory and the patient was discharged seven days post surgically.

## **Tetanus in a dog after whelping: A case-report**

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Tetanus is a rare disease in dogs caused by vegetative forms of the *Clostridium tetani*, which is an obligate anaerobic, spore forming Gram-positive bacillus. Tetanospasmin, a lethal exo-toxin excreted by this bacterium, results in characteristic muscle spasms and tonic contractions. Tetanus usually follows injuries such as deep dog bite wounds (teething), surgical sites, puncture wounds and sites of tick bites. This case report highlights the importance of anti serum therapy in the successful clinical management of an unusual tetanic case developed after whelping. A female Rhodesian Ridgeback, two and a half years old with all vaccinations and deworming properly done was presented to the Veterinary Teaching Hospital with one day history of lethargy, recumbence and dyspnoea. She had delivered (normal) six live pups two weeks earlier with no complications at whelping or thereafter. She was hyperthermic, recumbent with opisthotonus, hyper extended limbs, neck and tail due to muscle contraction. The animal showed erected ears and contracted facial muscles with retracted lips. Mouth was difficult to open and drooling of saliva was present. Animal also showed evidence of shallow rapid thoraco abdominal breathing, sluggish reflexes, hyper excitability to external stimuli and was hospitalized. Blood samples were collected on Day 1 for complete blood count and serum biochemistry of which the results showed mild leukocytosis with normal serum calcium concentrations. Presence of nitrite, protein and blood in catheterized urine samples were suggestive of urinary tract infection. On Day 3 she started a clear mucoid vaginal discharge and ultra sound scanning revealed postpartum endometritis. A presumptive diagnosis of tetanus with postpartum endometritis and cystitis was therefore made. Treatment was commenced with antimicrobials ((Penicillin G (Na<sup>+</sup>), Metronidazole), muscle relaxants with sedative properties (chlopramazine, phenobarbital), analgesics (meloxicam) and vaginal pessuries containing povidone iodine. Animal was assigned with a darkened kennel with minimum disturbance. On Day 8, tracheostomy followed by endotracheal intubation and artificial ventilation was done. Freshly extracted serum (45ml) from a dog treated 45 days ago with tetanus toxoid was transfused. In addition, treatment for decubitus ulcers and physiotherapy was done while urination and defecation were monitored. On Day 12, her tongue movements started; hence hand-feeding was commenced. Her limbs got relaxed, joint movements gradually improved and ate on her own. Most clinical signs disappeared and she was almost normal by Day 38 and therefore, was discharged. We would like to highlight the anti serum therapy in managing this case.

## **Successful management of canine diabetes using human insulin injection**

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Diabetes mellitus is caused by lack of the hormone insulin due to dysfunction of pancreatic beta cells or inadequate response to insulin or impaired expression of insulin receptors. It is a treatable condition which requires a committed effort by veterinarian and client; Diagnosed based on history, polyuria, polydipsia, dehydration, weight loss, sweet odourous breath and laboratory findings of hyperglycemia (>120mg/dl) and glycosuria. In the absence of canine insulin, four diabetic dogs were given intermediate acting 70/30 human insulin isophane injections twice daily morning and evening. Initial blood glucose values of case 1, 2, 3 and 4 were 402, 584, 418 and 498 mg/dl respectively. Case 2 was on once daily dose of human insulin for last three years and Case 3 was on canine insulin for last three years at the time of presenting. Initial insulin dose was 0.25 IU/Kg and gradually increase to maintain blood glucose level between 80 – 250 mg/dl based on blood glucose curve. Owners were advised to give only two equal sized meals per day at time of insulin injections and daily check of blood glucose levels at home prior to giving insulin to minimize hypoglycemia. Case 1 was followed up daily while other cases were followed up periodically to evaluate clinical progression with monitoring of blood glucose, urine, and kidney and liver functions. Improvement of both clinical condition with maintaining of blood glucose level between 80 to 300 mg/dl, and survival time were observed after initiating the therapy. Case 1 was survived for four years and three months, while case 2 survived for five years after initiating insulin therapy. Case 3 and 4 are still being managed with human insulin for last three and four months respectively with controlled diabetes. In the absence of twice daily administration of insulin in case 1 and 2, patients died due to diabetic ketoacidosis with signs of lethargy, anorexia and dyspnea, and ketonuria. Case 2 had elevated liver enzymes and serum creatinine levels. Urinary infections, injection site reactions and hypoglycemia were occasionally encountered. In the absence of recommended canine insulin, human insulin is a good alternative to treat canine diabetic patients.

## **Ovarian Adenocarcinoma in backyard poultry in Homagama veterinary range, Sri Lanka**

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The most frequent tumor of the reproductive system is adenocarcinoma of the albumin-secreting glands of the oviduct of laying hens. Neoplastic cells are shed from tumors in the oviduct into the abdominal cavity. These cells implant on the ovary, pancreas, and other viscera and produce multiple, hard, yellow nodules. They may block lymph drainage and result in ascites. The incidence increases with age, and this tumor may be a cause of death after 2 years of age. A few back yard poultry hen carcasses were presented to Veterinary Investigation Center (VIC) Homagama. The carcasses weighed (3 - 4Kg) with protruded abdomen. The post mortem examinations were carried out and the results revealed that the abdomen was fully filled with grapes like multilobular structures which were attached to the ovary, oviduct, gizzard, intestine mesentery and other adjacent serosal surfaces. Histopathological findings with H & E staining of affected tissue revealed that the tissue mass was consisted of pleomorphic epithelial cells with an eosinophilic cytoplasm and relatively large vesicular nucleus. Some cells consisted of eosinophilic granules in the cytoplasm. Cells were loosely arranged in bundles or nests and separated with some fibrous connective tissues. Ovary and mesentery consisted of same type of cells with vascular and lymphatic invasion. Clinical etiology, gross pathological and histopathological findings revealed that hens had Ovarian Adenocarcinoma involved in ovary, oviduct and also to the adjacent serosal surfaces.

## **Histopathological confirmation of pulmonary aspergillosis in a captive Flamingo in Sri Lanka**

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Aspergillosis is an air-borne fungal infection primarily of the respiratory system caused by *Aspergillus* species. The disease is common in chicken, turkey and other captive birds. Decreased immunity, stress etc. act as predisposing factors of the infection. The gold standard in diagnosing aspergillosis is culture positivity with histopathological evidence. Histopathology alone is a valid diagnostic tool when the fungal morphology could be specifically identified. In 2016, Pathology laboratory of Veterinary Research Institute (VRI) received tissue samples of a captive greater flamingo (*Phoenicopterus roseus*) from National Zoological Gardens. It had died following debilitation and the post mortem examination of lungs revealed numerous small white foci and patches. Histopathological observations of the lung disclosed evidence of congestion, haemorrhages, oedema and presence of abundant septate, branching, filamentous fungal hyphae within bronchioles and interstitium, surrounded by numerous lymphocytes, eosinophils and some macrophages. Most interestingly, several short, smooth fungal conidiophores were observed in secondary bronchioles with conical terminal vesicles. The conidial heads were uniseriate, and phialides were present only on the upper two thirds of the vesicles indicating infection with *Aspergillus fumigatus*. Diagnosis was further clarified with visualization of fungal hyphae using special stains, PAS and Grocott's. The condition was morphologically diagnosed as severe, diffuse, sub acute fungal pneumonia/ aspergillosis. To our knowledge, this is the first detailed histopathological description of pulmonary aspergillosis with specific identification of aetiology by means of histopathology alone, in a captive bird in Sri Lanka. Although brooder pneumonia (aspergillosis) was commonly detected in young chicks in the country, only a few sporadic cases of disease had been detected in adult poultry (mainly via gross pathology, sometimes associated with histopathology/fungal culture). In many countries it is observed that the incidence of aspergillosis in captive birds other than domestic poultry is also high. In Sri Lanka the situation may be similar, as three more cases received by VRI during 2016 for histopathological assessment, were suggestive of pulmonary aspergillosis, and all three were exotic pet birds. Since the disease also has a zoonotic potential, prevention of aspergillosis in captive pet and wild birds is equally important as it is in domestic poultry.



## Wild-type *Edwardsiella tarda*, possible cause of unnatural mass death of Asian Openbill (*Anastomus oscitans*) at Kotuathawala bird sanctuary in Nikawaratiya

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*Edwardsiella tarda*, a member of the family *Enterobacteriaceae*, is both human and animal pathogen. It is a Gram-negative, short rod shaped facultative anaerobic bacterium. The organism is phenotypically tight and displays little biochemical variability and divided in to two groups, wild-type and biogroup 1. More than 80% of strains of *E. tarda* are known to hemolyse red blood cells in a wider range of host species including marine and freshwater fishes, invertebrates, amphibians, reptiles, mammals, humans, cattle, swine, dogs, and bird species. Virulent *E. tarda* strains have been isolated from aquatic and semiaquatic host species in different geographical locations of the world. In January 2017, unusual deaths of resident birds of *Anastomus oscitans* (Asian openbill) were reported at Kotuathawala bird sanctuary, Nikawaratiya in Kurunegala district. More than 500 bird deaths among the fledgling birds at the age of 2-3 months were reported within four days of onset of this epidemic. Central Veterinary Investigation Center of the Veterinary Research Institute was requested to elucidate the cause of death of these birds. Rapid diagnostic test for Avian Influenza was conducted using the cloacal swabs obtained from bird carcasses and sick live birds (total of 6 birds) at the field and the samples were negative for Avian Influenza virus. Postmortem findings were haemorrhagic enteritis, haemorrhagic, enlarged liver and whitish diarrhea. Aseptically collected liver, lung, cloacal swab samples from three birds were streaked on to 3% and 5% sheep blood agar and MacConkey agar respectively for bacteriology. The plates were incubated at 37°C for 24 hrs. Isolated colonies from liver tissues on blood agar were characterized by primary identification tests (Gram staining, motility, catalase, oxidase), biochemical tests and macroscopic sign of hemolysis. Cloacal swab samples (2 samples) and droppings (5 samples) were tested by egg inoculation and negative for Ranikhet and Avian Influenza viruses. The bacterial strain that was isolated from *Anastomus oscitans* was identified as wild-type (L-arabinose, D-mannitol, and sucrose negative) *Edwardsiella tarda* which is the possible cause of death in this epidemic. According to published literature isolation of *E. tarda* from liver tissue is more frequent than other organs, which corroborates with the present isolation from Asian openbill liver samples. *E. tarda* is sensitive to a wide variety of antimicrobials and therefore, infections can be treated effectively using common antimicrobials. We found out that the isolates were sensitive to tetracycline, doxycycline, flumequine, enrofloxacin, augmentin, gentamicin, trimethoprim-sulfamethoxazole and resistant to neomycin and bacitracin as determined by the disk-diffusion method performed using Muller-Hinton agar. As per the literature, this is the first incidence of isolating wild type *E. tarda*, an important zoonotic pathogen from wild aquatic birds in Sri Lanka.

## Survey of bacterial flora in the nasal cavity of horses in an upcountry stable

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The nose of the normal horse harbor a diverse flora out of which a majority are harmless. Occasionally few opportunistic pathogens from nasal flora can cause serious infections including pulmonary conditions. *Rhodococcus equi* is a major such pathogen causing pneumonia in foals. The objective was to characterize the nasal flora of a group of healthy horses in an upcountry stable in Sri Lanka. Nasal swabs from 17 horses were cultured on blood agar and incubated at 37<sup>o</sup>C for 24 hours. The colonies from each sample were sub cultured on blood agar and Gram's staining was performed for each culture. Gram positive cocci were sub cultured on Mannitol Salt agar and further identified using tests including Catalase, Oxidase, Coagulase and CAMP test. Gram negative rods were further characterized using tests including Catalase, Oxidase, Urease, Citrate, Indole and Triple Sugar Indole test to identify the bacterial species. Gram positive coccobacilli were further characterized by culturing on nutrient agar and using tests including Catalase, Oxidase, Urease and CAMP to test if they were *Rhodococcus equi*. Out of the total 100 isolates, 31% were coagulase negative Staphylococcus (CNS), 6% were *Klebsiella* spp, 5% were *Staphylococcus intermedius* group (SIG), 4% of each *Yersinia*, *Aeromonas* and 1% of each of *E.coli*, *Proteus*, *Salmonella*, *Pseudomonas* and *Rhodococcus equi* were identified. The rest were unidentified Gram positive coccobacilli (21%), non-spore forming Gram positive rods (17%) and spore forming Gram positive rods (7%). *Staphylococcus* was isolated from all 17 horses. Out of the 17 horses, 94% had CNS while the SIG was isolated from 23% of horses. Gram negative rods were isolated from 9 horses. *Klebsiella* was isolated from 23%, *Aeromonas* from 17%, *Yersinia* from 12%, *Proteus*, *E.coli*, *Salmonella* and *Pseudomonas* were isolated only from 5% of the horses. Majority of the horses had at least one identical bacterial isolate in both nostrils. Therefore this study demonstrates the presence of a diverse nasal flora in horses including CNS, SIG, *Klebsiella*, *Yersinia*, *Aeromonas*, *E.coli*, *Proteus*, *Salmonella*, *Pseudomonas* and Gram positive rods. Furthermore it demonstrates the presence of *Rhodococcus equi* in nasal mucosae of foals which is sensitive for ampicillin, cefuroxime, erythromycin, gentamicin and tetracycline.

## **Existing knowledge on Foot and Mouth Disease among livestock farmers in the Ampara district of Sri Lanka**

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Foot and Mouth Disease (FMD), is a highly contagious viral disease affecting cloven hoofed livestock and wild animals. The economic impact of the disease is huge throughout the world due to direct production losses and indirect costs associated with control measures. Based on recent epidemiological data Ampara district is considered as a hotspot for FMD out breaks in Sri Lanka. As such thorough awareness on the FMD by the livestock farmers rearing susceptible animals would be extremely important to devise and implement an effective integrated control strategy. Hence, farmer education and awareness programs, with appropriate knowledge translation tools would be an initial step needed to minimize the economic impact of FMD outbreaks in Sri Lanka. This survey based study investigated the existing level of knowledge on FMD among livestock farmers (n = 144) in five selected veterinary service ranges in the Ampara district. The study was conducted during July to October 2016 using a pretested structured questionnaire as a part of a study involving assessing the effectiveness of different knowledge translation tools. The results indicated that 59% of the farmers had heard about the disease and its occurrence as outbreaks in Ampara. Their knowledge about the; causative agent and mortality rate were 3% and 13% respectively even though 87% of the farmers had direct contact with the area veterinary surgeon. 54% of the farmers had a basic idea about mode of transmission of the disease while the majority (85%) said that it was from direct contact of infected animals. Even though (83%) farmers knew about susceptible animal species, 80% said that it was only cattle. Forty four percent of the respondents mentioned that vesicles in limbs was the major symptom of the disease. Farmers (34%) have comparatively poor understanding about main consequences of the disease except reluctance to eat. The awareness of morbidity rate and prevention and control methods was 30% and 46% respectively. The study also revealed the daily presence of potential wild hosts/ reservoirs such as elephants, wild boar, deer, sambar, and wild buffalo in the vicinity of livestock. Presence of potential wild hosts/ reservoirs poses a serious threat for controlling and eradicating FMD especially for the farmers who manage their livestock under extensive and semi-intensive management system. These risk factors are magnified by the overall lack of understanding of FMD by farmers in the Ampara region. We envisioned our results would facilitate selection of appropriate knowledge translation interventions to close the knowledge-to-practice gaps in FMD control and eradication programs in Sri Lanka.

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## **Possibility of using *Cyclea peltata* plant extract as a substitute for Gelatin in set yoghurt**

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Gelatin is an animal origin stabilizer commonly used in dairy products industry in Sri Lanka. Some lacto-vegetarians are reluctant to consume gelatin incorporated product due to several cultural and religious taboos. Therefore, this study was carried out to investigate the feasibility of using *Cyclea peltata* plant extract as a substitute for gelatin in vegan set yoghurt. Initially, several trials were conducted to find the the maximum level (10%) of plant extract that can achieve the maximum gelation effect. A series of set yoghurts were made using three variables (1.5%, 3% and 4.5%) of plant extract from the initially made extraction (10%). In these three set of yoghurts fat and SNF were balanced as 3.5%, 8.5%; 3.15%, 8.57% and 4.2%, 11.43%, respectively. A sensory evaluation was carried out with ten untrained panellists. The sensory evaluation revealed that 1.5% plant extract incorporated yoghurt was found to be the most acceptable yoghurt. Whey syneresis percentage and pH of yoghurt were significantly higher ( $P < 0.05$ ) than the control, while titratable acidity of both were not significantly different ( $P > 0.05$ ) throughout the storage at refrigerated temperature. As proximate analysis showed that herbal set yoghurt had Moisture ( $76.26 \pm 0.4\%$ ), Dry matter ( $23.57 \pm 2.53$ ), Fat ( $4.24 \pm 0.026\%$ ), Protein ( $6.83 \pm 0.39\%$ ) and Ash ( $4.11 \pm 0.19\%$ ) similar to that gelatin incorporated yoghurts. There was no coliform, Yeast & Mould growth during storage at refrigerated temperature for two weeks. Further, physical and the organoleptic properties can be improved by incorporating Carrageenan, Kelco-gel or Pectin from natural fruits. In conclusion, this study highlighted that *Cyclea peltata* plant extract can be used to produce the gelatinous effect of set yoghurt.

## **Possibility of application of TiO<sub>2</sub> photocatalytic technique to reduce the bacterial count in bovine milk**

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The poor microbiological quality of raw milk is a common issue in Sri Lankan dairy sector. Therefore, there is a high demand for the improved milk quality free from spoilage microorganisms. The main purpose of this study was to investigate the possibility of applying TiO<sub>2</sub> photocatalytic technique to reduce the bacterial count in raw cow milk. In the method 4×4cm<sup>2</sup> soda lime glass plates were coated using 10mL and 20mL of 52824 ppm TiO<sub>2</sub> colloidal solution and three light sources namely Sunlight, UV light and 30W CFL bulb's light were used. 30 milk samples of 20mL with and without these glass plates were exposed to three light sources for one hour and samples were taken in 15 minutes time intervals (0, 15, 30, 45 and 60minutes) for microbiological analysis. Milk sample without glass plate was used as the control. All microbiological analysis was done according to the standard methods using nutrient agar. The total plate count revealed a significant decrease (P<0.05) in colony forming unit per ml when both 10mL TiO<sub>2</sub> (10%) and 20mL TiO<sub>2</sub> (16%) sprayed plates were used in milk samples exposed to sunlight. The survival bacterial count was reduced significantly (P<0.05) by 15% and 26% in milk samples with same TiO<sub>2</sub> plates when exposed to UV light. However, there was no significant effect when it was exposed to 30 W CFL bulb's light. These results support the conclusion that there is a possibility to apply TiO<sub>2</sub> photocatalytic technique to reduce bacterial count in raw milk. Further, it could be suggested that the efficiency of the method could be increased by increasing the mass of TiO<sub>2</sub>.

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