8th Proceedings of the Seminar on Veterinary Sciences

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PREFACE

With the grace and blessings of Allah we managed to publish the 8th Proceedings of the Seminar on Veterinary Sciences. As in previous years the proceedings consist of short and extended abstracts of final year project reports. This year 76 veterinary students conducted their final year projects. Areas of research interest of the students now seems to become more varied, ranging from animals as small as crickets to laboratory and aquatic animals and as large as elephants. Almost 24% of all research conducted by the students were on food animals. This is a healthy development because the food animal industry in the country is on the down-slide. We hope that with growing interest shown by the new veterinarians, the animal industry in Malaysia will prosper and contribute to the expansion of the national economy. This is essential to ensure food security for the country. Presently most of food animal products for human consumption in Malaysia are imported. We cannot keep on relying on other nations to provide food for our citizens. Eventually the food animal industry needs to develop and progress until we attain self-sufficiency. This can only be achieved through a paradigm shift by the veterinary profession to address the animal industry holistically from veterinary medicine and surgery to animal production.

The Editorial Board is most grateful to the students and their supervisors even amongst their busy schedule had taken great pains to prepare the abstracts and again making this annual procedings a reality. We wish to also express our gratitude to the Faculty of Veterinary Medicine, Universiti Putra Malaysia and all government and commercial organisations for providing the facility and time for the final veterinary students to successfully conduct their researches. Last but not least, we thank the internal and external supervisors and co-supervisors for their commitment to the student projects.

May Allah bless us all.

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TOTAL METABOLISABLE ENERGY AND FAT COMPOSITION OF HOUSE CRICKET (*BRACHYTRUPES PORTENTOSUS*) MEAL IN POULTRY

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ABSTRACT

A study was conducted to determine total metabolisable energy of house cricket (*Brachytrupes portentosus*) meal (HCM) fed to broiler at 6, 7 and 8 weeks of age. Proximate analysis and fat composition were conducted on HCM to determine the nutritional quality and their potential value as an alternative animal energy ingredient. The TME values were determining using precision-fed broiler assay in which three groups of broiler aged 6, 7 and 8 weeks were used in the experiment. Each age group consisted of 18 birds and divided into three subgroups with 6 birds per group. Each subgroup within age group was crop-intubated with 15 g of HCM, corn and palm kernel cake (PKC) and excreta collected for 48 hours. The results of proximate analysis for HCM showed that crude protein (CP), crude fiber (CF), ether extract (EE) and ash content were 64.4, 8.3, 22.7 and 5.4 %, respectively. The lipid composition of HCM consist of 60.25 % unsaturated fatty acids and 39.75 % saturated fatty acids. The average gross energy for HCM was 5923 cal/g. The TME values for HCM at 6, 7 and 8 weeks of broiler age were 19.7, 18.0 and 19.2 MJ/kg, respectively. These values were significantly higher ($P < 0.001$) than those recorded in broilers fed either corn or PKC in broiler of all ages. The experiment results showed that HCM has higher caloric content than either corn or PKC and it could be used as a good energy feed ingredient in poultry diets.

Keyword: house cricket meal, fatty acids, total metabolisable energy, broiler

INTRODUCTION

The chemical composition and nutritional value of some insects have been extensively investigated in various parts of the world. In fact, insects are potential sources of proteins, lipids, carbohydrates and certain vitamins. In Malaysia, crickets are not reared in large volume because it is customer demand-driven. The present study is designed to determine the total metabolisable energy content, proximate chemical and fatty acid composition in HCM for growing broiler.

MATERIAL AND METHOD

Preparation of House cricket meal

The house crickets (HC) was about eight-weeks-old when bought from an insect breeder farm in Kuala Selango, Malaysia. The crickets were frozen at -20ºC for 24 hours. Before experimentation the crickets were washed with tap water and then dried in the oven at 60ºC for 48 hours. House cricket meal (HCM) was prepared by freeze-drying in liquid nitrogen before grinding into 60 mesh size. The HCM was stored in a freezer until required for analysis (Ismasyahir *et al*., 2012).

Chemical analysis of House cricket meal

The procedures of the Association of Official Analytical Chemist (1990) were used to determine the dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash, minerals (Ca and P) and gross energy. Crude protein was determined by the Kjeldahl method using a MicroKjeltech apparatus.

Total Metabolisable Energy (TME) assay

Total metabolisable energy determination was conducted according to the method of Parsons *et al*. (1982). Fifty-four broilers were divided into different age groups namely 6, 7 and 8 weeks old. The age groups with 18 broilers each were each subdivided into 3 groups of 6 birds per subgroup and fed HCM, corn or PKC. All birds were fasted for 48 hours before force-fed with 15 g of the assigned feed by crop-intubation technique. Excreta were collected during the fasting period. At 48 hours post-feeding, all endogenous excreta produced were collected. Feathers and debris were removed and the excreta dried in the oven for 48 h at 60 ºC. Gross energy for HCM, endogenous excreta and excreta were determined using the bomb calorimeter. Calculation of TME was done using the following formula:

$$ME = GE\ intake - (GE\ excreta + GE\ endogenous\ droppings)$$

Fatty acid analysis

Fatty acid (FA) compositions in HCM were determined using gas-liquid chromatography (Sukhija and Palmquist, 1988). The fatty acid methyl ester (FAME) was separated using a 100m × 0.25mm ID (0.20 µm film thickness) Supelco SP-2560 capillary column (Supelco, Inc., Bellefonte, PA, USA). All GC conditions were the same except the oven temperature which followed the column temperature programme-initiated runs at 120 °C held for 5 min, increased by 2 °C/min up to 170 °C, held at 170 °C for 15 min, increased again by 5 °C/min up to 200 °C, and held at 200°C for 5 min and then increased again by 2 °C/min to a final temperature of 235 °C and finally held for 10 min. The fatty acid concentrations are expressed as % total identified fatty acids. A reference standard (mix C4-C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, MO, USA) was used to determine recoveries and correction factors for the determination of individual FA composition.

Identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to standards (Sigma, St. Louis, MO, USA). Both

gravimetric calculations and normalised percentage (%) of total FA were used to determine the differences in FA composition. Peak areas were determined and calibrated using a personal computer integrator (Hewlett-Packard, Avondale, PA). Automatic expression of peak areas as absolute and percentage amount of a detected fatty acid was obtained with a programmed PC using Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

The amount of fatty acid is determined quantitatively by concentrations (gravimetric calculation) of specific fatty acids and qualitatively by their relative proportions (normalised percentages to total fatty acids) (Huerta-Leidenz *et al*., 1991). The normalised percentages describe the interactive and comparable relationship among fatty acids regarding lipid quality, while the gravimetric concentration shows the actual amount of fatty acids in tissues, which relates to nutritional intake.

Statistical analysis of data

Data from TME assays were subjected to one-way analysis of variance for completely randomized design. Statistical significance of differences among treatments was assessed using the Turkey's test (SPSS version 20).

RESULT

Table 1 shows fatty acid composition of HCM. The HCM contained 60.25 % unsaturated fatty acid and 39.75 % saturated fatty acid. **Table 2** shows the chemical composition of HCM, PKC and corn. The results of proximate analysis for HCM showed that crude protein (CP), crude fiber (CF), ether extract (EE) and ash content were 64.4, 8.3, 22.7 and 5.4 %, respectively. The CP percentage of HCM was 64.4 % which was higher than corn (9.8 %) and PKC (22.7 %). The CF percentage of HCM (8.3 %) was lower than PKC (16.7 %) but higher than corn (2.4 %). The results also indicated that the fat content in HCM (22.7 %) was higher than either corn (4.2 %) or PKC (1.0 %) suggesting that it has high caloric value. The ash percentage in HCM (5.4 %) was higher than corn (1.3 %) and PKC (4.4 %) indicating higher mineral content. However, the percentage of Ca in HCM (1.4 %) was the higher than either corn (0.3 %) or PKC (0.3 %). Gross energy in HCM, corn and PKC were 5.92, 4.2 and 4.9 MJ/kg, respectively.

Total metabolisable energy (TME) of HCM, corn and PKC in broilers at 6, 7 and 8 weeks of age

Table 3 shows mean total metabolisable energy (TME) values in broilers at 6, 7 and 8 weeks old and fed HCM, corn and PKC. The mean TME values for HCM in broilers at 6, 7 and 8 weeks old were 19.7, 18.02 and 19.16 MJ/kg. This value were significantly higher than TME of corn for 6, 7 and 8 weeks old broilers at 13.76, 14.28, 15.82 MJ/kg respectively. Broilers fed with PKC showed significantly lower TME values of 6.68, 6.75, 7.83 MJ/kg for 6, 7 and 8 weeks old broiler respectively, compared to those fed HCM or corn.

Table 1. Fatty acid composition of house cricket meal

		Fatty Acid Composition
Name Fatty Acid	[a]Structure	Total fatty acid (%)
Capric Acid	C10:0	0.2475
Myristric Acid	C14:0	2.465
Myristoleic Acid	C14:1	0.8025
Palmitic Acid	C16:0	29.1225
Palmitoleic Acid	C16:1	0.7075
Stearic Acid	C18:0	7.935
Oleic Acid	C18:1	26.8925
Linoleic Acid	C18:2 n-6	30.88
α-Linolenic acid	C18:3 n-3	0.5725
γ-Linolenic Acid	C18:3 n-6	0.3175
Arachidonic Acid	C20:4 n-6	0.0525
Total saturated		39.75
Total unsaturated		60.2525
Total monoenes		27.47
Total PUFA n-3		4.5725
Total PUFA n-6		31.255

[a]Structure carbon number with 'zero' double bonds are saturated fatty acids, with 'one' double bonds are monounsaturated and with 'two' and 'three' double bonds are polyunsaturated fatty acid (PUFA)

Table 3. Mean total metabolisable energy of house cricket meal, corn and palm kernel cake in broiler chicken.

Broiler age (weeks)	Feed	[1]Mean TME Value (MJ/kg)
6	HCM	19.70[a]
6	PKC	6.68[b]
6	Corn	13.76[c]
7	HCM	18.02[a]
7	PKC	6.75[b]
7	Corn	14.28[c]
8	HCM	19.16[a]
8	PKC	7.83[b]
8	Corn	15.82[c]

[1]Mean of 3 groups at 6, 7 and 8 week age of broilers
[a,b,c]Means within a column differ significantly ($p<0.05$).

Table 4.4 Differences mean TME value (MJ/kg) at 6, 7 and 8 week age of broiler and at 20 weeks age of layer cockerel.

Feed	Mean TME values (MJ/kg)	
	Broiler Cobb (6-8 weeks)	Layer Cockerel (20weeks)
HCM	18.96*	13.03[a]
Corn	14.62*	13.4[b]
PKC	7.09*	5.74[c]

*Data from the experiment conducted. [a]Ismasyahir (2012). [b]Macdonald (1995). [c]Noraini (2000).

Total metabolisable energy (TME) of HCM, corn and PKC at 20 weeks age of layer cockerel

According to Ismasyahir *et al.*, (2012) the TME of HCM in 20-week old layer cockerel of 13.03 MJ/kg was similar to layer cockerel fed with corn, which was 13.4 MJ/kg (Macdonald *et al*., 1995). Another study showed that the TME value of PKC was 5.74 MJ/kg for layers (Noraini, 2000). This value is much lower than that obtained in this trial.

DISCUSSION

Based on the fatty acid composition, HCM contributed 60.25 % of unsaturated fatty acids and 39.75 % of unsaturated fatty acids. According to Zollitsch (1997), unsaturated fatty acids produce lower faecal energy losses and consequently higher TME and this is expected to cause higher fat deposition. The additional energy is stored as triglycerides, which are important for growing broilers. The omega-3 contributed about 4 % and omega-6 contributed about 31 % to the total fatty acid. According to Seyed Reza Hashemi (2012), the omega-3 fatty acid which is an unsaturated fatty acids that may play a role in the improvement of immune response and resistance to to infectious bursal disease, while omega-6 fatty acid influence growth and reproductive performance of male and female birds.

In this study, the average TME value for HCM of growing broiler was 18.96 MJ/kg. This value is higher than the mean values recorded for corn (14.62 MJ/kg) and PKC (7.09 MJ/kg). The higher TME value for HCM is due to its higher lipid content than in corn or PKC. The TME value for soybean meal was 13.14 MJ/kg (Ozugul *et al.*, 2006) and this is also lower than in HCM or corn. That study also reported that TME value for anchovy fishmeal was 17.2 MJ/kg while that for herring fishmeal was 16.8 MJ/kg. However, due to the high price of fish in Malaysia, fishmeal cannot be used as a source of feed ingredient.

The mean TME values of HCM in broilers at 6, 7 and 8 weeks old were higher compared that of corn and PKC. This suggests that HCM can be used as a potential new source of energy and could replace traditional feedstuff and grains. There is a difference between the TME value in broilers and matured layer cockerel. Broilers fed HCM had higher TME values compared to layer cockerel fed the same meal. This is an indication that broilers utilise lipid more efficiently for growth than old layers that use energy for maintenance only. Similarly broilers fed corn and PKC diets have higher TME values than layer cockerel fed the same diets.

CONCLUSION

House crickets which have high an energy value and have good potential as an energy source feed ingredient for broilers and it would be extremely beneficial as an alternative feed ingredient in poultry diet complement to the poultry industry.

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GROWTH PATTERN FOR BODY WEIGHT AND MORPHOMETRIC MEASUREMENTS FROM HATCHING TO FLEDGING OF CAPTIVE AFRICAN PENGUINS (*SPHENISCUS DEMERSUS)* AT UNDERWATER WORLD LANGKAWI, KEDAH, MALAYSIA

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ABSTRACT

African penguins (*Spheniscusdemersus*) originated from the southern part of Africa and are currently being displayed at Underwater World, Langkawi (UWL), Kedah Malaysia. These penguins are also known as black-footed penguins and Jackass penguins. Two other species can be seen in Malaysia: Rockhopper penguins at UWL and Humboldt penguins at the National Zoo in Kuala Lumpur. Since the performance of these captive birds in Malaysia has not been documented it is pertinent to examine the performance of the penguins when raised in a tropical environment so that the management of these birds can be further improved. Therefore a study was conducted to analyse the growth pattern of the African penguins for body weight and morphometric measurements (flipper length, foot length, bill length and width) from hatching to fledging. The records included data collected from 2006 to January 2013 and the samples were made up of 18 parent birds and 56 chicks that were born in a captive tank at UWL. The adults were imported from South Africa about 7 years ago when they were between 7- 24 years.

The growth patterns for body weight and 2 morphometric measurements (flipper and foot length) from Day 1 to Day 82 of the 56 chicks are shown in Figure 1. African penguin chicks showed a smooth increment in body weight from hatching until fledging stage. The heaviest body weight gained was about 3.1kg at around 64 days of age. There was an obvious reduction of about 1 kg of body weight of the chicks after about 64 days of age. The mean weight of the day-old chicks was 70 g and the range of flipper length was 18.5 to 21.12 cm, foot length was 10.95-11.58 cm and the total bill length was 2 to 6 cm (Figure 2). Bodyweight was found to have reduced from around Day 82 to Day 100 which may be due to the chicks were in the period of fledging. It is characteristic of African penguins for body weight to decrease slightly before fledging after which the weight increases very slowly to eventually reach the normal adult weight at around 70 days of age. The fledging period for single chicks averages around 73.4 days, twin chicks

average 80.5 days and synchronous hatching broods average about 96 days. For the morphometric parameters, most of the chicks in UWL were within the range of the values documented for *Spheniscus* penguins in EAZA (1993). For the juvenile chicks, moulting age is around 13 – 18 months, with a mean of 15.2 months.

In conclusion, the growth performance of the captive African penguin chicks in UWL is similar to that reported by Williams (1984). This indicates that these chicks have been well-managed in the captive environment. The results of the current study provide targeted performance indicators for body weight and morphometric measurements which could assist the management of these penguins at UWL and other captive enclosures in the tropical region that mimic the original environment of the African penguins.

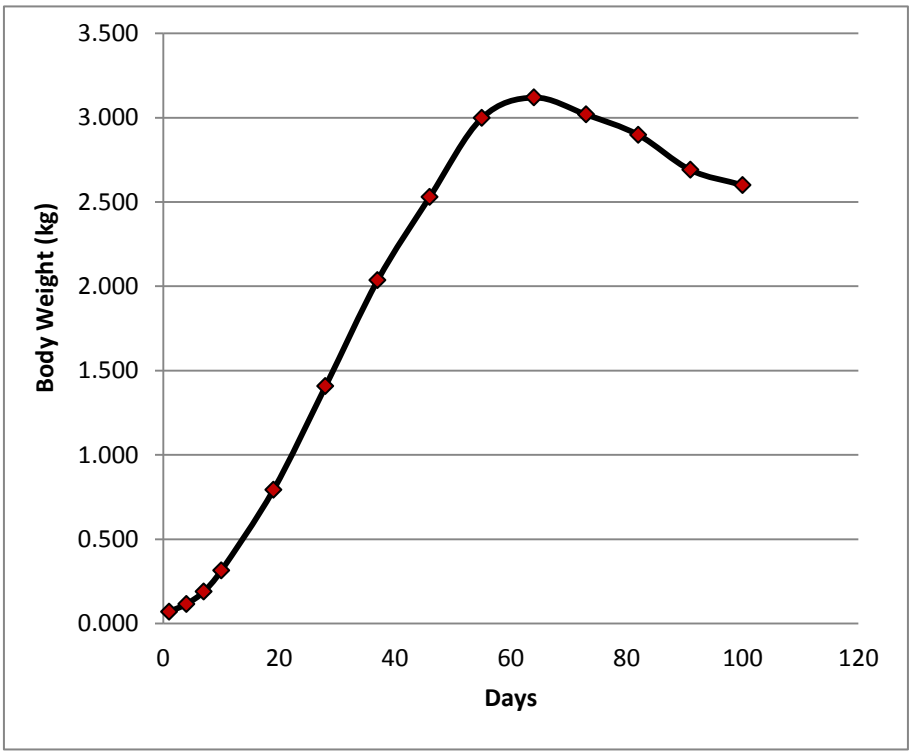

Figure 1: Growth pattern of African penguins at Underwater World, Langkawi, Malaysia.

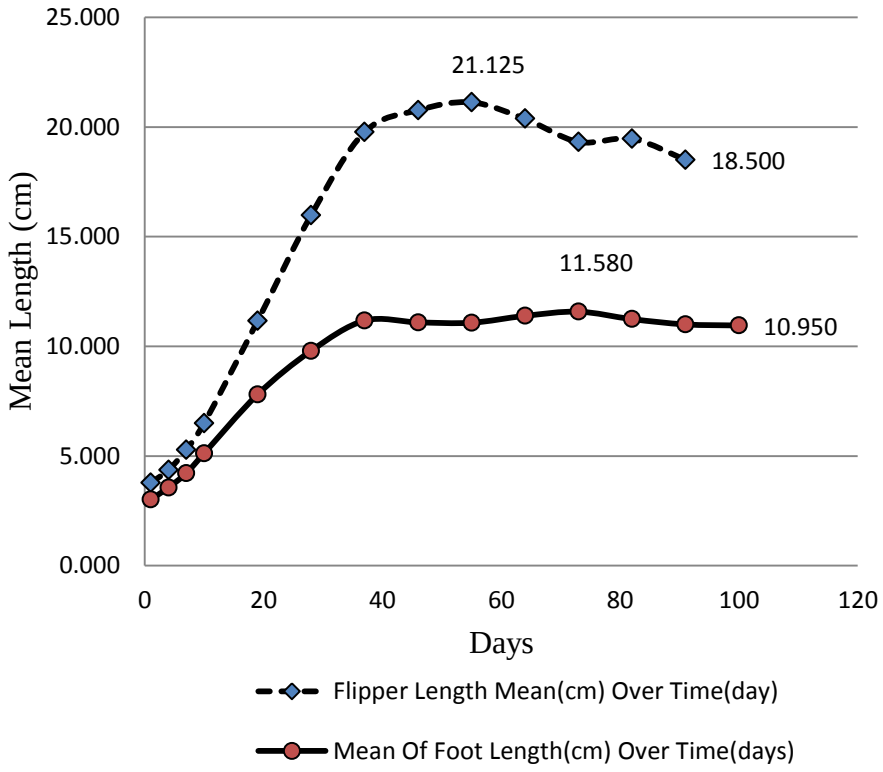

Figure 2: Flipper and foot length of African penguins at Underwater World, Langkawi, Malaysia.

Keywords: African penguins, growth pattern, morphometric measurement, Underwater World Langkawi

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COMPARATIVE EFFICACY BETWEEN LEMONGRASS (*CYMBOPOGON CITRATUS*) EXTRACT AND IVERMECTIN AGAINST TICKS IN HAIR SHEEP

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ABSTRACT

Rhipicephalus (Boophilus) microplus is considered as the most important tick affecting livestock worldwide especially ruminant species. Ivermectin is the major class of acaricide commonly used worldwide to control ticks (Davey *et al*., 2010). Its repeated use has posed resistance threat in many farms (Lane and Crosskey, 1996). Lemongrass (*Cymbopogon citratus*) has been reported to have repellant, larvicidal, and acaricidal effects against arthropods (Hanifah *et al.*, 2011) however, its use in tick control has not been extensively promoted. Therefore the present study was conducted to compare the efficacy of *Cymbopogon citratus* extract and ivermectin in controlling the tick population in hair sheep.

Fifteen (15) male Barbados Blackbelly x Santa Ines crossbred hair sheep, aged between 2 to 3 years old with grazing history and naturally infested with ticks, were randomly and equally divided into 3 treatment groups: untreated control group, ivermectin treated group [Ivermectin 1 % (Biomectin) 0.2 mL/kg of bodyweight via subcutaneous injection] and lemongrass treated group (10 % lemongrass aqueous extract, 5ml/kg of bodyweight sprayed to whole body. Tick counts were obtained on both left and right inner ear pinnae during pre-treatment (day 0), days 7, 14, and 21 of post-treatment for each group. The sheep were kept in raised slatted floor houses and fed with cut signal and napier grasses in the morning and concentrates in the evening.

The ivermectin and lemon grass treated groups showed significantly lower ($P < 0.05$) mean tick count on days 14 and 21 post-treatment compared with the control group (Table 1). Lemongrass treated group showed significantly higher mean tick count ($P < 0.05$) compared with ivermectin treated group on days 7 and 14 but not on day 21. The ivermectin treated group showed sudden reduction in mean tick count within a week of treatment and later maintained constant mean tick count for the rest of the treatment weeks. On the other hand lemongrass treated group showed gradual reduction of mean tick count from week 1 to week 3. The efficacy of ivermectin was constant between 82-83 % throughout the treatment period whereas the lemongrass extract showed gradual increase in efficacy starting from 43 % on day 7 of post-treatment to 51 % on day 14, and 61 % on day 21 post-treatment.

However, compared with 1 % ivermectin, the efficacy of lemongrass extract was lower but its efficacy reached an accepted level of 40 % after 1 week of application. All the sheep treated with ivermectin and lemongrass extract showed no external clinical conditions such as alopecia, pruritus and photosensitisation.

Empel and Konacki (1990) recorded that a single subcutaneous injection of ivermectin was sufficient for the complete cure against ectoparasites. It was concluded that ivermectin at 0.2ml/kg of bodyweight given subcutaneously was the drug of choice for treating tick infestation in cattle which have the same dosage as sheep. The constant efficacy of ivermectin can be attributed to its stable chemical components. The tick reducing effect of lemongrass can be attributed to the presence of alkanoids and flavonoids normally found in many plant materials having bactericidal, fungicidal, and pesticidal properties. This finding is in agreement with Heimerdinger *et al.* (2006) whereby lemongrass was shown to have acaricidal activity in different preparations. They reported that alcohol extraction containing 23.08 and 37.5 % of macerated lemongrass used in three immersions had high *in vitro* efficacy against hard and soft ticks.

Table 1. Tick count and efficacy rate of hair sheep treated with ivermectin and lemongrass extract.

	Tick count			
Treatment	**Day 0**	**Day 7**	**Day 14**	**Day 21**
Control	14.8±8.0	17.0^{a}±10.90	18.0^{a}±11.0	22.0^{a}±14.3
Ivermectin	21.5±15.9	3.75^{b}±0.96	$3.702.99^{b}$	3.50^{b}±2.52
Lemongrass	12.20±7.32	10.60^{a}±8.79	6.00±3.46^{c}	4.80^{b}±4.66
	Efficacy rate, %			
Ivermectin		82.6	82.6	84.0
Lemongrass		43.4	50.8	60.7

Values are either expressed as mean ± SD or just means.
^{a,b,c}Means of tick count in the same column with different superscripts are significantly different at $P < 0.05$.

The lemongrass extract has acaricidal properties that are able to reduce the number of ticks in hair sheep. The extract is safe to be used externally on sheep as no sign of external toxicosis was found following its application on sheep skin. Therefore, lemongrass extract can be considered as a potential anti-ectoparasitic agent in hair sheep.

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EFFECT OF DIET SUPPLEMENTED WITH DIFFERENT SOURCES OF OMEGA-3 FATTY ACIDS ON BLOOD LIPID PARAMETERS IN RATS

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ABSTRACT

Omega-3 fatty acids (n-3 PUFA) have been proven to have many health benefits and one of it is to alter blood lipid parameters especially lowering serum triglycerides. There are many readily available supplements of n-3 PUFA from both animal and plant sources. Animal sources especially marine sources are excellent sources of n-3 PUFA in the form of docosahexaenoic acid(DHA) and eicosapentanoic acid (EPA) while plant sources are high in ALA which is the precursor of DHA and EPA. Four groups of male Sprague–Dawley rats were fed with a high fat diet, and the three treatment groups were supplemented with 10 % (wt/wt) n-3 PUFA sources, namely fish oil, barramundi fish or grouper fish, while the control group remained on the high fat diet without any n-3 PUFA supplementation. The results revealed that fish oil and barramundi fish significantly reduced serum triglyceride levels up to 49 %, while grouper fish caused some non-significant reduction. Effects on total serum cholesterol, LDL and HDL were not significant and inconclusive. In conclusion, barramundi fish showed good potential in lowering serum triglycerides by up to 49 % which is just as good as commercial fish oil supplements.

Keywords: n-3 PUFA, fish oil, triglyceride, eicosapentanoic acid (EPA), docosahexaenoic acid (DHA).

INTRODUCTION

Hypertriglyceridaemia and high low-density lipoproteins (LDL) in blood have been associated with cardiovascular diseases such as coronary heart diseases (Maki *et al*., 2012). Arteriosclerosis and stroke can also result from the increase of these lipids in blood. Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been shown to reduce triglyceride levels in blood by up to 20 to 40 % in hypertriglyceridaemic patients (Kris-Etherton *et al*., 2003). Also, n-3 PUFA may increase the level of high-density lipoprotein (HDL) which is beneficial in reducing LDL plaque accumulation that

may lead to arteriosclerosis (Herold and Kinsella, 1986). However, according to Covington (2004) the effects of n-3 PUFA on LDL are variable.

Fish is a rich source of n-3 PUFA in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Covington, 2004). Plants are rich sources of n-3 fatty acids in the form of ALA (α-linolenic acid) which is the precursor of EPA and DHA. The bioconversion of ALA to EPA and DHA is restricted in humans (William and Burdge, 2006).

Most of the studies on the effect of n-3 PUFA used commercial fish oil, such as cod liver oil and cold water fish, thus similar to the barramundi fish (*Lates calcarifer*) and areolate grouper (*Epinephalus areolatus*) used in this study. Wan Rosli *et al*., (2012) who compared commonly consumed fish in the East Coast of Peninsular Malaysia reported that the barramundi had the highest EPA content (55.38 ± 7.38 µg/g) while the grouper had the highest DHA content (165.21 ± 6.17 µg/g).

MATERIALS AND METHODS

Feed Preparation

Fish was prepared by oven-drying and the fillet was ground to 1 mm size. The Glen Forest rodent pellet used in this study was similarly ground. The fish oil used was the commercial brand Scott Emulsion (cod liver oil) and Anchor Brand pure unsalted butter was used to induce hypertriglyceridaemia.

Daily Feed

Each rat was given 15 g of feed daily, where the percentage of supplement was given according to % (wt/wt). All groups were given basic high fat content food, which was prepared by adding 10 % of butter to the rodent pellet. The control group was fed with only the basic high fat diet, while other groups were supplemented with 10 % of different omega-3 supplements namely the fish oil, barramundi fish or grouper fish.

Experimental Design

Fifty male Sprague-Dawley rats aged 8 weeks were obtained from the Animal Resource Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia and placed in an air-conditioned laboratory. During the period of adaptation, the rats were placed randomly in cages, with 5 rats per cage and fed with 15 g of pellet/rat/day. After a 7-day adaptation period, 5 rats were anesthetised and blood was sampled intracardiacally for blood lipid analysis was labelled as 'normal blood lipid'. The remaining 45 rats were then fed with high fat diet (pellet and 10 % butter) for another period of 4 days to induce hypertriglyceridaemia and 5 rats were sacrificed to collect pre-treatment (day 0) blood sample. The remaining 40 rats were then divided into 4 equal (5 rats) groups namely the Control, Fish oil, Barramundi and Grouper group. The treatment group was then fed with n-3 PUFA supplemented

feed (15g/rat/day) and water was provided *ad libitium*. Blood collection was performed on days 6 and 11 and after 5 days of the feeding trial.

Fatty Acid Profile Determination
Total fatty acids from the plasma and feed were extracted using chloroform: methanol 2:1 (v/v) by the method by Folch *et al*. (1957) and modified by Rajion *et al*. (1985). Transmethylation of extracted fatty acids to their fatty acid methyl esters (FAME) were carried out using 14 % methanolic boron trifluoride (BF_3) according the previously described method (AOAC, 2007). The FAME was separated using a 30m x 0.25mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). The gas chromatography conditions were as described by Ebrahimi (2013). The identification of fatty acids was carried out by comparing relative FAME peak retention times of samples to commercial standards (Sigma, St. Louis, MO, USA). The fatty acid concentrations are expressed as % of total identified fatty acids.

Serum Lipid Parameters Determination
The serum triglyceride, total cholesterol, LDL and HDL was analysed using a chemistry analyser (Hitachi 902, Japan).

Statistical Analysis
All statistical tests were conducted using the Statistical Package for the Social Sciences (SPSS) V. 19 at 95 % confidence level using repeated measures ANOVA.

RESULTS AND DISCUSSION

The grouper fish significantly ($p < 0.05$) had the highest percentage of total n-3 PUFA (31.92 %) compared to fish oil (23.92 %) and barramundi fish (21.08 %). The grouper fish also contained a higher percentage of DHA (24.13 %) while the Barramundi had higher EPA (7.61 %). The ratio of DHA: EPA in the fish oil was almost 1:1.

The n-3 PUFA in plasma was determined at pre-treatment (day 0) and post-treatment (day 6 and day 11) periods to whether supplementation had an effect on the n-3 PUFA level in plasma or not. There was a significant ($p < 0.05$) increase from day 0 to day 11 for the Fish oil and Barramundi groups and a significant increase in the Grouper group from day 0 to day 6. In the Control group, there was no increase in n-3 PUFA throughout the experiment because n-3 PUFA supplementation was not provided. However, the differences between groups were not significant ($p < 0.05$).

The effect of n-3 PUFA supplementation on serum triglyceride was determined by comparing the pre- and post-treatment blood triglyceride concentrations. Only Fish oil and Barramundi groups showed significant ($p<0.05$) differences at pre- (day 0) and post-treatment (days 6 and 11) periods, and both showing about 49 % reduction in serum triglyceride concentrations. Other groups also showed some

insignificant reduction serum triglycerides. Despite of having a higher percentage of n-3 PUFA than the barramundi fish, the grouper fish only showed slightly lower serum triglyceride. There was no significant ($p<0.05$) differences between the group means observed

The effect of n-3 PUFA supplementation on serum total cholesterol, LDL and HDL was not significant in this study, which consistent with most previous reports.

CONCLUSION

In conclusion, supplementation of fish oil and barramundi fish to the rat diet significantly reduced the serum triglyceride concentration by up to 49 % in these rats. This suggests that barramundi fish is a good commercial supplement to be used to decrease serum triglyceride. However the fish oil, barramundi and grouper fish had no significant effect on the blood total cholesterol, LDL, and HDL cholesterol in rats.

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A STUDY OF CLASSICAL SWINE FEVER AND PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME IMMUNOLOGICAL STATUS IN TWO FARMS IN SELANGOR, MALAYSIA USING IDEXX® ELISA

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ABSTRACT

Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS) are porcine diseases that are highly contagious and of great economic importance in Malaysia. In the evolving pig industry, reliable and useful diagnostic methods are needed to reduce the impact of these diseases. This preliminary study evaluates the CSF and PRRS immunological status in two farms in Selangor using commercial IDEXX® ELISA test kits. By sampling 30 percent of a breeding herd, a more representative farm immunological status was aimed to be achieved. In this study, 115 sows from a 400 sow population were sampled from Farm A, and 60 sows from a 200 sow population were sampled from Farm B. In addition, 50 porkers were sampled from both farms to determine their general immunological status. Both farms are practicing intensive, farrow-to-finish, open house system and located in a densely populated pig farming area. The samples were tested for CSF and PRRS antibodies using IDEXX CSFV Antibody Test Kit and IDEXX PRRS X3 Antibody Test Kit respectively. For the CSF test kit, the results revealed an average of 64 % blocking percentage among the sows in Farm A, and 56 % in Farm B. For the PRRS test kit, the results revealed an average S/P ratio of 2.8 among the sows in Farm A and 2.3 in Farm B. The results suggested that Farm A had a better immunity status compared to Farm B. In conclusion, commercial ELISA allows mass screening of a herd and can be a useful diagnostic and monitoring tool when combined with proper interpretations and field diagnosis.

Keywords: Classical Swine Fever (CSF), Porcine Reproductive and Respiratory Syndrome (PRRS), IDEXX CSFV Antibody Test Kit, IDEXX PRRS X3 Antibody Test Kit.

INTRODUCTION

Classical Swine Fever (CSF) is a highly contagious viral disease characterised by high morbidity and high mortality in susceptible pigs. In the acute form of CSF,

outbreaks with high mortality and severe illness can occur. Subacute and chronic forms of CSF show less severe symptoms, but can also cause losses due to poor performance and complications from other pathogens (Too &Seneque, 2002). The CSF is endemic in Malaysia and vaccination with live attenuated vaccines is widely performed to control the disease. However, lapses in vaccination programs or rapid changes in the herd demographics can result in unsatisfactory vaccination which leads to disease outbreaks (Too and Seneque, 2002).

Porcine Reproductive and Respiratory Syndrome (PRRS) is another major threat to the pig industry and is characterised by reproductive failure in sows and gilts, and respiratory problem in young growing pigs (Benfield *et al*., 1999). This disease which is due to reproductive failure, production failure, mortality and increased culling rates will cause significant economic losses to swine producers. According to the Malaysian Veterinary Protocol on PRRS, the overall status of PRRS in Malaysia is yet to be determined as there is lack of surveillance programs to date. Based on two individual surveillance studies done in 2008 and 2012, the results showed a high seroprevalence of PRRS in Malaysia and suggested that Malaysia is endemic for PRRS.

The Enzyme-Linked Immunosorbent Assay (ELISA) is one of the serological tests that enable detection of antibodies in pig serum. In this study, two commercial ELISA test kits namely the IDEXX CSFV Antibody Test Kit and IDEXX PRRS X3 Antibody Test Kit will be used to determine the CSF and PRRS immunological status in two pig farms by sampling 30 percent of the sow herd. The data obtained will provide useful information for future serology profiling and vaccination program evaluation in pigs.

MATERIALS AND METHODS

Animals

This study was conducted in two commercial pig farms located in TanjungSepat, a densely populated pig farming area in Selangor. Farm A had a total sow population of 400 where 115 sows and 50 porkers from different age groups were sampled. Farm B had a total sow population of 200 where 60 sows and 50 porkers from different age groups were sampled. For the CSF vaccination history in both Farm A and B, the porkers were vaccinated at 4 weeks and revaccinated at 7 weeks, while the sows were vaccinated when its piglets were weaned. For the PRRS vaccination history, Farm A and Farm B practised a 3-4 month and 5-6 month routine vaccination, respectively. Blood samples were obtained via jugular venipuncture and serum from each blood sample was extracted, labelled and stored at -20 °C until further use.

Serological Tests

The serum samples were tested for CSF and PRRS using IDEXX CSFV Antibody Test Kit and the IDEXX PRRS X3 Antibody Test Kit, respectively according to the manufacturer's protocols. For IDEXX CSFV Antibody Test Kit, positive result indicating the presence of CSFV specific antibodies is when the blocking

percentage of the test sample was ≥ 40 %. The test sample was negative if the blocking percentage was ≤ 30 %. Samples having a blocking percentage between 30 % and 40 % required re-verification; however, they were grouped as negative results in this study. For the IDEXX PRRS X3 Antibody Test Kit, the presence or absence of PRRS antibody in the serum sample was determined by calculating the sample to positive control mean (S/P) ratios. Samples with S/P ratios < 0.4 were classified as negative for PRRS antibodies, while samples having S/P ratios ≥ 0.4 were classified as positive.

RESULTS AND DISCUSSION

Based on the results of the immunological status, the sow herd was divided into 4 categories because of their different immunological status. Different age group were included in this study in order to observe the time of seroconversion of the pigs studied (Rice *et al*., 2008). Ideally, sampling should be done in replicates (Blome *et al*., 2006), but due to time and cost, only a single sample was collected in this study. The IDEXX CSFV Antibody Test results for Farm A and B are presented in Table 1 and 2, respectively. Farm A had a better CSF immunological status compared to Farm B as the average blocking percentage was higher. Farm A also had higher percentage of sows with desirable antibody levels which could provide better maternal antibodies to the production herd.

Table 1: Farm A sow herd IDEXX CSFV Antibody Test results

	Negative: <40 % Blocking (Suspicious: 31-39 % Blocking)	Positive: ≥40 % Blocking (Desirable: ≥55 % Blocking)	Average Blocking %	Total Sows
Gilt	5 (1)	20 (18)	64	25
P1-P2 (Young)	2 (1)	17 (13)	62	19
P3-P5 (Mid)	11 (5)	45 (38)	64	56
≥P6 (Old)	1 (0)	12 (12)	67	13

The IDEXX PRRS X3 Antibody Test results for Farm A and B are presented in

Table 2: Farm B sow herd IDEXX CSFV Antibody Test results

	Negative: <40 % Blocking (Suspicious: 31-39 % Blocking)	Positive: ≥40 % Blocking (Desirable: ≥55 % Blocking)	Average Blocking %	Total Sows
Gilt	7 (1)	10 (9)	43	17
P1-P2 (Young)	4 (0)	8 (5)	48	12
P3-P5 (Mid)	2 (1)	11 (11)	71	13
≥P6 (Old)	2 (1)	7 (4)	60	9

The IDEXX PRRS X3 Antibody Test results for Farm A and B are presented in Figures 1 and 2, respectively. For PRRS, both Farm A and B should enhance the control measures as the results indicated high antibody titres for PRRS. Few precaution control measures such as preventing introduction of new strains of PRRS virus, good vaccination practice and proper monitoring may reduce active infection of PRRS in the farm.

This project was a preliminary study to obtain a base line result on the immunological status of CSF and PRRS in swine farms using IDEXX ELISA test kits. Further studies should also be extended in more farms in different regions in Malaysia to assess the immunological status of these two important diseases in different farm scales, farming systems and management systems, as the base line result for each farm may differ significantly. The sample size should be a minimum of 15 pigs per group regardless of farm size. The increase in sample size can produce more reliable results to obtain the true scenario of a farm herd, to help in improving the farm conditions

The Commercial ELISA test kit allows mass screening of a herd within a shorter time, at a lower cost and uses automated procedures. It can be a useful additional diagnostic and monitoring tool to check the immunological status of a swine herd when combined with field diagnosis and virus isolation.

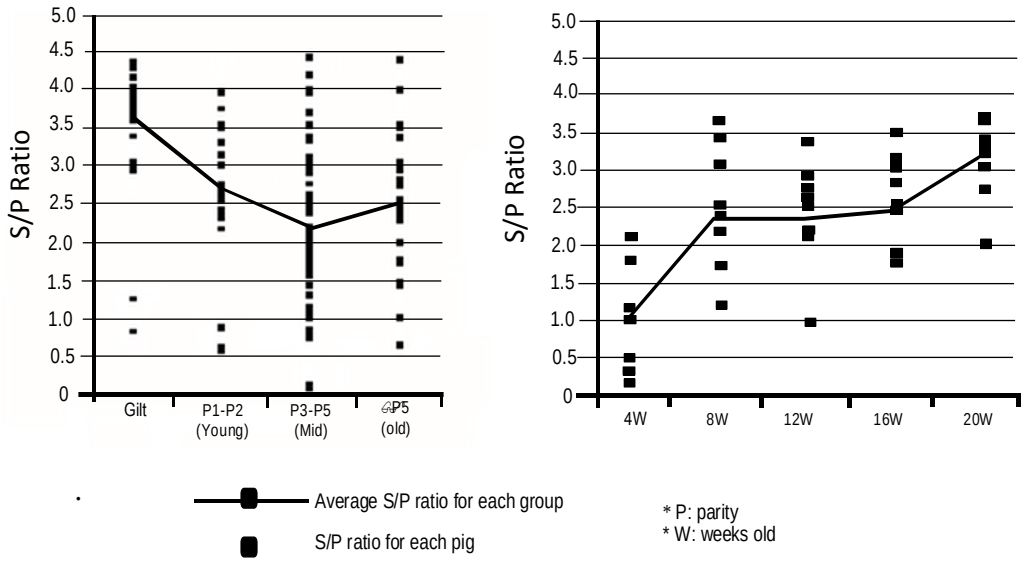

Figure 1: Average PRRS antibody levels (S/P ratio) of sow herd and porkers in Farm A. The average S/P ratio for the sows was 2.8, while the porkers had an increasing average S/P ratio as they aged.

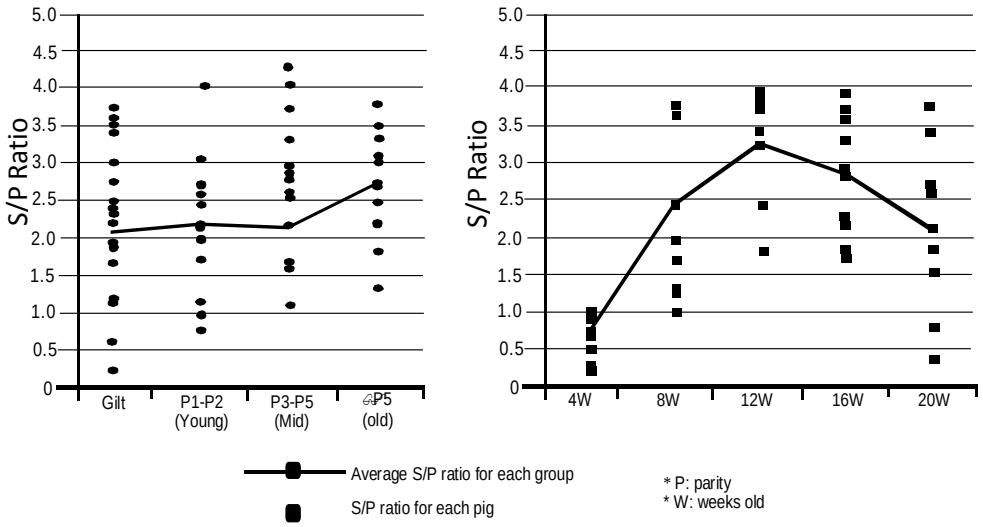

Figure 2: Average PRRS antibody levels (S/P ratio) of sow herd and porkers in Farm B. The average S/P ratio for the sows was 2.3. The porkers had an increasing average S/P ratio from 4 to 12 weeks, and decreased S/P ratio at 16 and 20 weeks.

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RETROSPECTIVE EVALUATION OF COMPOUND FRACTURE TREATMENT AND HEALING IN CATS AND DOGS

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ABSTRACT

A retrospective study of compound fracture treatment and healing in cats and dogs at University Veterinary Hospital (UVH), University Putra Malaysia (UPM) conducted for the period of January 2003 to December 2012 recorded 30 cases of compound fractures in cats and only 6 cases in dogs. The factors on the final outcomes and bone healing quality were evaluated based on the clinical records and radiograph examination. From 12 cats that have been treated and healed, 4 cats (33.3 %) age ranging from 6 months to 1 year had satisfactory outcomes with good alignment and reduction ($p<0.05$) using IM pin with C-wires. Six cats (50 %) age ranging from 1 to 3 years had unsatisfactory outcome where they had fair to poor reduction. Two (16.7 %) of the cats had unknown history due to incomplete records. Bone healing quality was evaluated in the dogs that have been treated. This study showed that 2 (66.7 %) of the 3 dogs treated had satisfactory bone healing. These 2 dogs age ranging from 6 months to 1 year had satisfactory outcome, moderate to well-formed callus, good and fair reduction, fair alignment, correct type fixation (IM pin and Cerclage wire).

Keywords: retrospective study, compound fractures, treatment, healing

INTRODUCTION

Open fracture contributes about 4 % to all fractures incidents (Robert and Darryl, 2006). Although this percentage is small, open fractures do not usually respond to treatment and develop complications that makes amputation the only measure to save the animals. The common complications associated with compound fractures were osteomyelitis and non-union. Compound fractures are classified and the prognosis of treatment differs according to their severity (Nunamaker, 1979). Fractures were considered clinically healed if there were radiographic evidences of

bone union and no pain upon palpation of the fracture site and during exercise (Stampley and Delamin, 1991).

MATERIALS AND METHODS

Thirty-six cases (30 cats and 6 dogs) of compound fractures at Universiti Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM) were retrieved. Radiographs were retrieved for these cases. For proper analysis, three radiographs were taken at three different time intervals. The first set of radiographs was taken pre-operatively to assess the type of fractures involved. The second set of radiographs was taken just after treatment or surgical procedure to assess the surgical reduction, alignment and the fracture gap. The third set of radiograph was taken to observe and monitor bone healing. Medical records which did not have enough or complete data were supplemented with information obtained from the owners through direct contact. The later process is important to obtain adequate information regarding the cases involved as well as to determine the condition and progress of the animals after treatment. The data were arranged in Microsoft Excel and analyzed by using SPSS version 20. Since the data were not normally distributed, the data were further analyzed by non-parametric tests using the Kruskal-Wallis and Mann-Whitney U test.

RESULTS

For cats, there was a significant difference ($p<0.05$) in fracture severity and the type of treatment. The same was not true for dogs. The influence of the time interval to the types of treatment was significant ($p>0.05$) for cats but not for dogs. The influence of the bone reduction to the bone healing quality was significantly ($p< 0.05$) different in cats but not dogs. The influence of gender, body weight and fixation factor to bone healing quality was insignificantly difference ($p>0.05$) for both cats and dogs.

DISCUSSION

In this study, gender is not an important factor in bone healing quality and this finding supports that observed in a previous study (Metha *et al*., 2011). That study suggested that gender is not a risk factor in bone healing in males and females, when determined by callus properties (Mehta *et al*., 2011). Bodyweight is also not a factor in the healing quality outcomes (Augat *et al*., 1998). For cats, 4 (30.8 %) had satisfactory outcome, 6 (46.2 %) had non-satisfactory outcome, while 3 (23 %) had unknown outcome. From 4 cats that had satisfactory outcomes, three were treated with IM pin and C-wires while one treated with external skeletal fixation (ESF). Although IM pin and C-wires are generally not recommended in compound fracture

treatment because of risk in introducing infection, in this case the cat had satisfactory outcome. This is because the age, reduction, time interval and severity of the fracture all involved young cats. These cats had good reduction, time admission of less than 8 hours and were classified as class 1 and 2 severities. For 6 cats that had unsatisfactory outcome, 4 (66.7 %) cats had time interval of less than 8 hours. Three of these cats were treated with bandages and one treated with IM pin and C-wires. These treatments had resulted in fair and poor reduction that finally led to the unsatisfactory outcomes. In case of dogs, two (66.7 %) had satisfactory outcome while one (33.3 %) showed unsatisfactory outcome. Although these two dogs were treated with IM pin and C-wires, these procedures are not recommended for compound fracture. The two dogs had all the factors associated with satisfactory treatment outcome of class 1 and 2 severity, good and fair reduction and young age with time interval of less than 8 hours. One dog showed unsatisfactory outcome, although for this dog the time interval was less than 3 hours and it is of class 1 severity. Conservative treatment using external coaptation could be associated with the poor fracture reduction, which consequently led to the unsatisfactory outcome in this dog.

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REASONS AND RISK FACTORS FOR LIMB AMPUTATION AND SURVEY ON QUALITY OF LIFE OF AMPUTATED CATS BASED ON OWNERS' PERCEPTION

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ABSTRACT

A retrospective study was carried out to identify the reasons and risk factors for limb amputation in cats treated at the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM) from January 2010 to July 2012. A survey was also conducted by a telephone interview to assess the quality of life (QOL) of the feline amputees based on the owners' perception. Clinical data of 43 cats including signalment, reasons for limb amputation and medication given post-surgery were retrieved from the UVH medical records. The most common reason for limb amputation in cats was osteomyelitis (58 %). The domestic shorthair cat was the only breed treated. Limb amputation was significantly higher in males, intact cats and hindlimbs compared to forelimbs. Findings from the QOL survey by telephone interview using a standardized questionnaire with 23 cat owners revealed that none of the cats showed sign of pain at home even though some cats did not receive any prescribed drugs for analgesia or inflammation. About 97 % of owners agreed that their cats returned to routine activity and lead a good quality of life and 87 % of them would recommend limb amputation if a similar condition occurs to other cats.

Keywords: cat, amputation, osteomyelitis, questionnaire, quality of life (QOL)

INTRODUCTION

Amputation, defined as the removal of a body or other appendage or outgrowth of the body (Blood *et al.*, 2007), is a surgical procedure commonly performed in cats to remove diseased or injured tail or limb(s). Limb amputation was recommended for malignant tumours (both of musculoskeletal and cutaneous origin), complex fractures with poor prognosis for a functional limb, infected fractures that failed to heal, severe trauma causing neurologic dysfunction, and when primary therapy is too costly.

Depending on the location of lesions and its severity, amputation can be performed on the front or hind limb, either partially or in full length. Additionally,

multiple limb amputation has also been reported but it is rare, probably due to restriction in activity the cat after first amputation. Medication such as analgesic, anti-inflammatory, antibiotic, and supportive drugs such as multivitamins or muscle stimulant may be provided to promote healing. Physiotherapy, hydrotherapy or acupuncture can also be performed to assist in the rehabilitation of animals after amputation.

The quality of life of amputated cats depends on the lifestyle of owners and management of the cat. Owners who adopt their cats as family members tend to take greater care of their pet. In the Malaysian context, some cat owners have difficulty to accept recommendation of amputation for their cats. This is mainly due to their own perception that cats fared poorly on 3 limbs, sympathy for the cat, abnormal appearance and fear that the cat will become a burden to them later on. Thus, owners prefer long term medical therapy where possible. Unfortunately, in some cases, cats are eventually put to sleep or die.

There is a dearth of information on limb amputation in the cat population of Malaysia. This study was conducted to identify reasons for limb amputation in cats treated at the UVH, UPM from during the period of January 2010 to July 2012, to determine the risk factors associated with limb amputation in cats, and to assess the quality of life of amputated cats based on perception of the owners. The findings form this study will assist practitioners in client education of future feline amputation cases.

MATERIALS AND METHODS

Source of Data

Fifty-one cat limp amputation cases at the Small Animal Hospital, UVH, UPM during the period of January 2010 until July 2012 were obtained and medical records, owner and patient information recorded. Forty-three cases were complete 8 cases were excluded from the study of which two died during surgery and 6 records were missing. The questionnaire used was adopted from a previous study conducted by Forster *et al*., (2010).

Questionnaire survey by telephone interview

All 43 owners with complete information were contacted by telephone. However, only 23 agreed to be interviewed. The information obtained included the condition of the cat after surgery, whether the cat returned to normal or not, length of recovery period, signs of pain, and adaptation after amputation.

Data management and analysis

Data were compiled and explored for normality and descriptive analysis was computed using SPSS version 16. Age distribution is presented as mean ± standard error (SE). Where necessary, statistical analysis was conducted using non-parametric approach for small data. A P value <0.05 was considered significant.

RESULTS AND DISCUSSION

Reasons for limb amputation in cats

The most common reasons for limb amputation in cats treated at the UVH were osteomyelitis (58 %), and none for neoplasia. Other reasons were bone fracture (16 %), nerve damage (14 %), non-healing wound (7 %) and necrosis (5 %). There were significant difference between reasons of limb amputation ($X^2(5)=4$, $p<0.001$).

Factors associated with limb amputation in cats

The average age of the amputated cats was 22 ± 3.6 months and age was a significant factor ($p<0.001$) in which junior cats cases (age from 6 months to 2 years old) was the highest (56 %) among age groups. This finding supports a previous study (Forster *et al.*, 2010), where it was shown that the age of majority of cats that were amputated (63.2 %) were less than 4 years old. Limb amputation cases were higher in male cats than in females however, this difference was not statistically significant. Intact cats recorded a significantly higher number of cases than neutered cats ($p<0.001$). Amputation was performed significantly more in hindlimbs than in the forelimbs ($p=0.014$). This result is consistent with the findings from a previous study (Forster *et al.*, 2010) where the incidence of amputation in cats was 2 times higher in hindlimbs compared to forelimbs. Rochlitz (2004) reported that during road accident, injuries to hindlimbs were 3 times more common than forelimbs.

Quality of life of the amputated cats based on the owner's assessment

All 23 respondents stated that their cats did not show sign of pain during rehabilitation process and returned to normal activity (96 %) regardless of medication given (Spearman's rho= -0.83, n=23, $p>0.05$). However since perception of pain sign in the cats is visual assessments by a lay person, it may be subjective and biased (Christiansen and Forkman, 2007).

The average time period for cats to return to normal was 20 days and there was no significant difference in this period between age groups ($X^2(2) = 2$, $p= 0.799$), although there is an increasing trend with increase in age. It is possible that in older cats recovery takes longer because tissue regeneration take longer in older animals (Gosain and DiPietro, 2004; Swift *et al*., 2001).

The majority of respondents stated that in their cats there was no change in time spent indoor or outdoor (35 %), activity level at home (52 %), playfulness (57 %), speed of movement (61 %), interest level (74%), self-grooming (91%), appetite (65 %), coat condition (70 %), and friendliness towards human (74 %) and other pets at home (83 %) and friendliness towards outside animals (83 %). However, some changes were observed in body posture (4 %) and the cat developing phobia (17 %) and in body condition. Almost equal number of respondents indicated that there is no change in mood or their cats were in better mood after amputation. As also shown by a previous study (Forster *et al.*, 2010), these findings indicate that limb amputation does not affect personality, behaviour and lifestyle of cats. This study also showed that the majority of the owners (87 %) would consider amputation if

their cats were challenged with situations requiring amputation. This is solely based on their positive experience and observation of their cat's ability to adapt and lead a normal life after amputation.

CONCLUSION

Osteomyelitis (58 % of 43 cases) was found to be the most common reason for limb amputation in cats treated at the UVH, UPM from January 2010 to July 2012. Significantly more cases of limb amputation done in junior cats (6 months to 2 years old). In intact cats more amputations were done on hindlimbs. Most owners agreed that their QOL of their cats improved after amputation and agreed that amputation was the best option for their sick cats. All owners reported that their cats did not show any sign of pain.

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IDENTIFICATION OF PARASITES IN FERAL FRESHWATER FISH FROM DIFFERENT HABITATS IN KEDAH, MALAYSIA

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ABSTRACT

A study was carried out to determine the prevalence, sites of attachment and species of parasites infecting five species of feral freshwater fishes from different habitats in Kedah. A total of 92 feral freshwater fishes were examined for ectoparasites as well as endoparasites; only 73 fishes were positive for parasite infestations. These fishes comprised of Nile Tilapia *(Oreochromis niloticus)*, Gouramy *(Trichogaster trichopterus)*, Climbing perch *(Anabas testudineus)*, Snakehead *(Channa striata)*, and Silver Carp *(Barbonymus gonionotus)*. The morphology of the parasites species was determined using light microscopy and the number of parasites were counted and tabulated according to host fish species, fish habitats and sites of infestation. In the study it was found that the prevalence of parasites infestation on each fish species was 100 % and seven main species of parasites were identified to belong to Monogenea *(Dactylogyrus sp.)*, Trematode (Digenean metacercariae), Ciliates *(Trichodina sp.)*, Phylum Acanthocephala, Nematode *(Camallanus sp.)*, Myxosporea *(Hennaguya sp.)*, Protozoan *(Epistylis sp., Heteropolaria sp.)* and two unidentified species. Amongst the five fish species, climbing perch had the highest parasitic infestation at 78 %, while Nile tilapia had the lowest degree of infestation (1 %). The commonest parasite infestation site found in this study was at the gills region and class Monogenea was the most prevalent as it was found in all five fish species. Irrigation canal located at Pangkalan Bujang, Merbok supported the highest prevalence of parasites infestation at 44.8 % than other habitats.

Keywords: freshwater fish, parasites, infestation site.

INTRODUCTION

Feral freshwater fish are defined as untamed fishes living in freshwater bodies with less than 0.05 % of salinity, such as rivers, lakes, irrigation canal or ponds. They are excellent sources of high quality protein, well-balanced in essential amino acids and highly digestible. Thus, the parasites infestation is one of the major causes of loss of fish productivity and the infestations may cause direct or indirect illness in fish. Parasites are typically divided into two groups, which are ectoparasites and

endoparasites. Currently, there is less information on prevalence of fish parasites in local feral fishes in their habitats than in cultured fishes (Rahman and Bakri, 2008). A study is necessary to determine methods to control parasites infestation from becoming epidemic in local indigenous fishes. This study was conducted to identify common parasites infesting feral freshwater fishes, to determine the relationship between the parasites abundance, host and its habitat and to assess the gross changes and clinical manifestation associated with the parasite infestation in these fishes.

MATERIALS AND METHODS

Sampling Sites and Collection of Fishes

A total of 92 apparently healthy feral freshwater fishes were collected from different habitats that were natural ponds, irrigation canals and streams. Five indigenous species of freshwater fish were caught, which are Nile tilapia *(Oreochromis niloticus)*, Gouramy *(Trichogaster trichopterus)*, climbing perch *(Anabas testudineus)*, snakehead *(Channa striata)*, and silver carp *(Barbonymus gonionotus)*. The fishes were caught with a casting net or fish scoop and were kept alive as long as possible an aquarium equipped with a battery-operated portable aeration system. The captured fishes were examined within 24 to 48 hours. The water quality parameters also were measured *in situ*, viz., for temperature, turbidity, pH, nitrite, and ammonia and nitrate level by using standard water quality equipment and commercial test kits.

Collection of Parasites and Infected Tissue or Organs

The skin (mucous and scales), fins and gills of each fish were examined for ectoparasites using direct wet smear technique. Large parasites were collected and stored in sterile bottle with 70 % alcohol.

Identification of Parasites

The parasites were identified, sketches were made from observation under light microscopy and photographic records were made with digital microscope (Dino-Lite). The identification of various phylum, class and genera was carried out based on pictorial guide from Kabata (1985) and Williams *et al*., (1994) and the modified guide by Woo (2006).

RESULTS

Prevalence and Degree of Parasites Infestation on Fish Species

From the current study a total of 92 feral freshwater fishes were caught and examined for ectoparasites as well as endoparasites; of these 73 fishes were positive to parasites infestation. The prevalence of parasites infestation was 79.3% for all fish species. Seven major species of parasites belonging to Monogenea

(Dactylogyrus sp.), trematode (Digenean metacercariae), ciliates *(Trichodina sp.)*,Phylum Acanthocephala, nematode *(Camallanus sp.)*, Myxosporea *(Henneguya sp.)*, protozoan *(Epistylis sp.*, *Heteropolaria sp.)* and two unidentified species were recorded. Among these fish species, climbing perch had the highest parasitic infestation at 78 %, followed by snakehead (11 %), gouramy (5 %) and silver carp (5 %) while Nile tilapia had the lowest degree of infestation (1 %). Monogenean was found in all the five fish species.

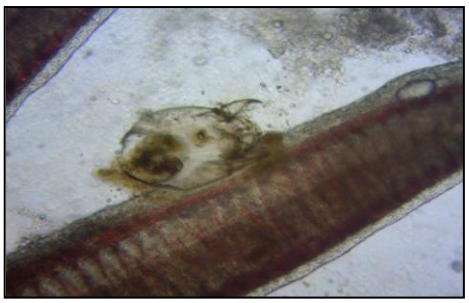

Figure 1: Unidentified arthropod-like species found on the gills of the Silver Carp (B. gonionotus).

Figure 2: *Pallasentis sp*. (*Phylum Acanthocephala*) in small intestine of climbing perch *(A. testudineus).*

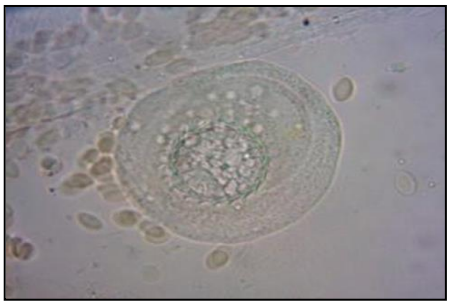

Figure 3: Trichodina sp. on the skin mucous of the Nile tilapia (*O. niloticus*)

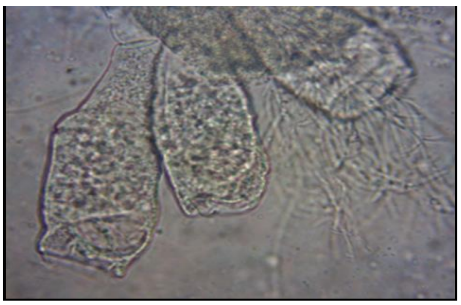

Figure 4: *Heteropolaria sp.* on the gills of the Silver carp *(B.gonionotus).*

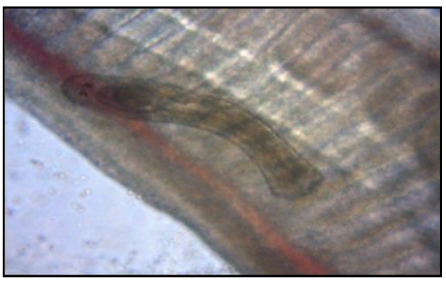

Figure 5.Dactylogyrus sp. on the gills of Snakehead (C. striata).

Gross Changes Associated with Parasites Infestation

The gross lesions seen at the gills lamellae include excessive mucous production on the gills. The deformity of the gills filament due to hyperplasia, hypertrophy and necrotic changes in the epithelium of the gills could be observed under light microscopy.

Water Quality Parameters of Different Habitats

The temperatures were within normal range; the lowest was 27 °C and the highest was 32 °C recorded at the irrigation canal, Pangkalan Bujang. However these values may not be of significance because the temperature was taken at almost hottest time of the day at 1.44 pm. All water habitats showed pH to be within the normal range, between 7 and 7.5 and considered as normal alkaline water. In secchi disk measurement, the lowest water depth clearance was 24.1 cm and this was in the irrigation canal, Bukit Kecil. The water was turbid indicating that there was a high amount of suspended solids. The results also showed that all the habitats were high in ammonia, especially in the irrigation canals of Bukit Kecil and Pangkalan Bujang at 8 mg/L, followed by the Sungai Petani stream at 2 mg/L and the natural pond in Sungai Lalang at 0.25 mg/L. The study also shows that the irrigation canal in Bukit Kecil had high nitrite content at 5mg/L. Other water samples were normal at less than 0.01 ppm nitrite. Meanwhile, the water nitrate was high in stream in Sungai Petani and irrigation canal in Pangkalan Bujang at 5 mg/L.

DISCUSSION

The results obtained from this study revealed that water quality of the irrigation canal Bukit Kecil was the poorest amongst the three habitats. As shown in this study that poor water quality can decrease immunity to parasites and induced immunosuppression in the fish which can lead to increase susceptibility to diseases (Poulin, 1992). The study also suggest that the low fish parasites burden in the irrigation canals may be associated with low number of fish population because of suboptimal water quality. Fishes in nature are often exposed to stress due to sub-lethal contaminants (heavy metals, organic and inorganic chemicals), water velocities, sediment loads, fluctuating temperatures, amount of dissolved oxygen, pH, salinity or food availability. In irrigation canal at Bukit Kecil because of poor quality water only two fish species, the climbing perch and snakehead were found. These fish species are air-breathers and thus can survive longer in water with high ammonia and low dissolved oxygen. Usually air-breather fishes will absorb oxygen from atmosphere and do not rely much on dissolved oxygen. This is particular beneficial to the fish when the canal becomes dry during draught.

The gills, skin (scale and mucous) and gastrointestinal tract (stomach and intestines) were the organs chosen to access parasites. The gills showed high preference for many species of the parasites and this is true for all fish species in the present study. Gills are a very susceptible to parasite infestation due their cell structure i.e. one-cell thick as to allow the oxygen and carbon dioxide to exchange efficiently between blood and water. The gills are supplied with main blood vessels

and in direct contact with aquatic environment and thus favoured by the parasites to attach and feed. Among the parasites found, the monogenean was the commonest ectoparasites parasitizing the gills. This finding is similar to that of Modu *et al.* (2011).

Many more different parasite species were found in the irrigation canal in Pangkalan Bujang than other sites. This may be due to the fact that the water in this irrigation canal was deeper and canal wider than other locations. Two endoparasites species were obtained from climbing perch and snakehead and they were the nematode, *Camallanus* sp. and thorny-headed worm phylum Acanthocephala. Intestinal nematode infections should be suspected if the fishes eat well but still appea thin. This is a manifestation of wasting disease, which is a major concern in aquaculture (Yanong, 2002). Climbing perch and snakehead are predatory fish and they are also act as paretenic host because they feed on small fishes infested with larval nematode.

The relationship between the parasites and habitats was determined conclusively in this study. However there was a direct relationship between habitats and presence of fish species, where habitats determine presence of particular parasites species.

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ISOLATION OF PATHOGENIC LEPTOSPIRA FROM SOW URINE IN SELECTED PIG FARMS IN PENINSULAR MALAYSIA

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ABSTRACT

Ninety urine samples from sows were collected in selected pig farms in Peninsular Malaysia for detection of Leptospira. Samples were then inoculated into Johnson-Seiter media for culture purpose. After 3 weeks of inoculation, all samples were then observed under dark field (DF) microscopy. Only two samples were positive under DF microscopy and remaining samples were reincubated until 12 weeks with weekly observation under DF microscopy. Subculturing of the positive samples was done in order to reduce contamination load. Subcultures of the positive samples were then used for extraction of DNA sequence by Polymerase Chain Reaction (PCR) procedure which is more sensitive than culture. Based on PCR, both samples were positive towards genus specific Leptospira and only one out of two samples were positive towards pathogenic Leptospira.

Keywords: sows, dark-field microscopy , polymerase chain reaction

INTRODUCTION

Leptospires belongs to Leptospiraceae family and from the order of Spirocahetales. Leptsopires is also known as spirochetes. Spirochetes has slender body, flexous, flexible cell wall and can utilize carbohydrates, amino acids, long chain fatty acids or long chain fatty alcohol as carbon and energy sources. Leptospira is a gram negative, obligate aerobic spirochete, motile with a length of 10-20 µm bacteria which can be either pathogenic or saprophytic. Pathogenic *Leptospira* causes disease in animals and humans, while saprophytic *Leptospira spp*. is considered free living and harmless to living organism. Pathogenic *Leptospira spp*. is maintained in nature in the renal tubules and genital tracts of certain animals that works as reservoir host before the organism disseminates in the urine excreted out from animal's body. Different pathogenic serovars have specific reservoir hosts. Pigs as a reservoir host for *Leptospira interrogans* serovar *Pomona* and serovar *Tarassovi***.** Leptospirosis is a disease caused by pathogenic strain of *Leptospira spp*. In Thailand, the most prevalent serovar causing reproductive problem in sows was *Leptospira interrogans* serovar *Grippotyphosa* which accounted for 55 % of all

positive cases with various titres and minor serovars were *Leptospira interrogans* serovar *Canicola*. Prevalence of Leptospirosis in Vietnam predominantly caused by *Leptospira interrogans* serovars Icterohaemorrhagiae, *Pomona* and *Bataviae* (Ho and Tran, 2000). In Japan the most prevalent serovars causing reproductive failure in sows were *Leptospira interrogans* serovar *Icterohaemorrhagiae* and *Leptospira interrogans* serovar *Canicola* (Ayako, *et al.*, 2002). *Leptospira interrogans* serovar *Pomona* is condemned mostly for reproductive failure in all parts of the world. It can be transmitted by carrier pigs and a continous pig raising system permitted its spread among pig herds in the village (Ho and Tran, 2000).

MATERIALS AND METHODS

Ninety samples of urine were collected from 18 different farms in three different states at Peninsular Malaysia: Penang, Melaka and Selangor. In each farm five random urine samples were collected from sows. The age group was chosen because it stayed in the farm for longer period of time and had higher chance of developing Leptospiros compared to other stages. A 50 ml hygiene container was used to collect spontaneous urine excretion from the sows. Samples were then directly transferred into sterile universal bottles. For each urine sample, 100 µL of concentrated urine was inoculated directly into Johnson-Seiter (JS) semi-solid medium containing 1.0 g of 5 flourouracil. Subsequently, 1 mL of concentrated urine was inoculated into 9 mL of basal medium in order to dilute the sample. Next, 1 mL of diluted urine inoculated into JS semi-solid medium. For both concentrated and diluted urine samples, replicates were produced. For further investigation and culture purposes, samples that were inoculated into JS semi-solid medium were used. These cultures were kept in CO_2 incubator with 30 °C temperature and monitored for four to seven days for inoculation.

After three weeks of inoculation, 1 µL was taken from each of the remaining urine samples and placed on a glass slide in order to be observed under dark field (DF) microscope. *Leptospira spp.* appears fluorescent in dark background. This organism can be observed as motile, moving forward quickly and spinning on its axis with one or both ends hooked. Presence of *Leptospira spp.* in the sample indicates positive samples and requires further diagnosis. Positive urine samples identified in DF microscope were subjected to Polymerase Chain Reaction (PCR) test in order to detect DNA of *Leptospira spp.* Extraction of DNA was done based on manufacturers recommendation by using commercial Promega® Wizard Genomic DNA Purification Kit.

RESULTS

Both Selangor and Melaka samples showed a band weight at 331bp which indicated positive for Leptopsira genus. To further differentiate either it was pathogenic or saprophytic *Leptospira sp.,* both samples underwent another pathogenic primer and

result showed that only Selangor sample was positive with the band size of 531 bp. Based on the result obtained, only two from 90 samples were positive for genus Leptospira and one of them was pathogenic strain of Leptospira.

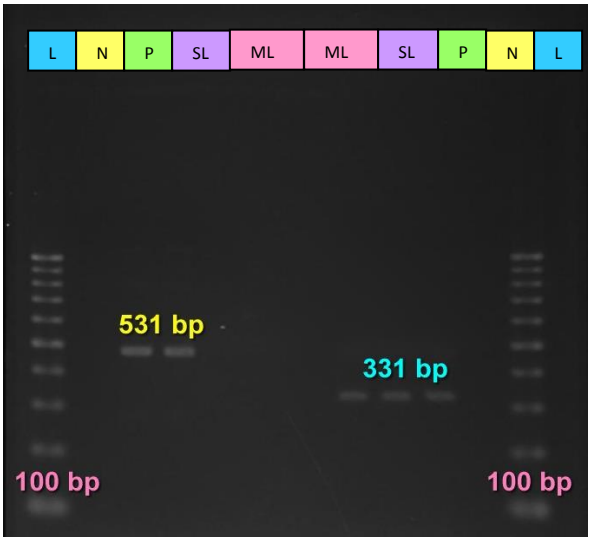

Figure 1. Lane M, 100bp DNA ladder; Lane N, negative control; Lane P, Canicola DNA taken as positive control; Lane SL, Selangor sample; Lane ML, Melaka sample

DISCUSSION

The detection and characterization of leptospires are currently done with culture isolation and serological techniques. Culturing of the organism is the most demonstrative approach. Slow growth and interference by other contaminants make isolation not a suitable option for rapid diagnosis (Rapiphan *et al*., 2011). Similar findings were observed from culture that has been inoculated in the field. Contaminated culture appears black in color with pungent odor. After 3 weeks of field inoculation, culture can be observed under dark field microscope for positive sample.

DNA-based techniques including Polymerase Chain Reaction method have been introduced into the field of Leptospirosis. PCR based molecular technique can potentially provide rapid and specific means of Leptopirosis (Cheema *et al.*, 2007). PCR has been used for the diagnosis of fastidious organisms such as Leptospira. PCR assays are sensitive, but quality control procedures and sample processing for PCR are critical and must be adjusted. A variety of primer sets to conduct PCR assays have been established. Some primers are only specific for the genus Leptospira and others designed to identify only pathogenic species.

Leptospirosis has been recognized as an important emerging global public health problem because of its epidemic and increasing incidence in both developing and developed countries. Direct transmission among animals can be transplacental,

haematogenous, by sexual contact or by suckling milk from infected dam. Direct transmission from animals to human beings is common amongst the occupational groups who handle animals and animal tissue such as butchers, veterinarians, cattle and pig farmers, rodent control workers.

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OCCURRENCE OF *AEROMONAS* SPP. AND *STREPTOCOCCUS* SPP. IN ORNAMENTAL FISH

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ABSTRACT

Ornamental fish keeping is a worldwide hobby and the aquaria can be found in various places, ranging from the house to the workplace. Ornamental fish aquarium water was reported to carry potential zoonotic pathogens, which could cause human health hazard, especially to fish hobbyist. *Aeromonas* and *Streptococcus* infections are the two emerging diseases associated with ornamental fishes. Emergence of multiple drug resistant bacteria is another rising concern in public health due to the increase use of antibiotics in aquaculture. This study was conducted to determine the occurrence of *Aeromonas* spp. and *Streptococcus* spp. in ornamental fish, as well as the antimicrobial profile of the isolates. Thirty aquarium water samples and 60 fish skin swabs were obtained and cultured onto blood agar (Oxoid) for *Aeromonas* spp. and *Streptococcus spp*. isolation. The antibiotic resistance of the isolates against 6 antibiotics was carried out using the disc diffusion method. There was high occurrence of *Aeromonas* spp. (30 % in aquarium water and 8.3 % on fish skin); but low occurrence of *Streptococcus* spp. (3.3 % in aquarium water and 1.7 % on fish skin) in the samples. Thirty-three percent of the isolates showed multiple drug resistance. Hence, it can be concluded that ornamental fish and its aquarium water do carry potential zoonotic *Aeromonas* spp. and *Streptococcus* spp. that are multiple drug resistant.

Keyword: ornamental fish, *Aeromonas* spp., *Streptococcus* spp., multiple drug resistance

INTRODUCTION

In 1974, Trust and Bartlett isolated several potential zoonotic bacteria from aquarium water of ornamental fishes. Chacko *et al*. (2006) reported the transmission of fish borne zoonotic diseases via ingestion of aquarium water and contamination of lacerated skin upon contact with aquarium water and fish, particularly among the fish hobbyists.

Despite being the major fish pathogens, both *Aeromonas* spp.and *Streptococcus* spp. are considered as emerging zoonotic pathogens. Chacko *et al.* (2006) found that gastroenteritis and localised wound infection were the two common clinical

signs of *Aeromonas* spp. infection seen in immunosuppressed humans. Zoonotic streptococcosis is frequently caused by *S. iniae* and *S. agalactiae*; associated with bacteraemia, cellulitis and meningitis.

Nowadays, the challenges faced in the ornamental fish industry is bacterial diseases and the option to use antibiotic for therapeutic and prophylaxis purposes. The issue that arises following the use of antibiotics in aquaculture is the emergence of multiple drug resistant bacteria in the fish and its aquarium water.

Hence, the objectives of this study were to determine the occurrence of *Aeromonas* spp. and *Streptococcus* spp. in tropical freshwater ornamental fishes and aquarium water as well as to determine the antimicrobial resistance profile of the isolates.

MATERIALS AND METHODS

Sampling

Fourteen home aquaria and 16 freshwater aquaria from 5 aquarium shops in Selangor were sampled in this study. Aquarium water was collected into a sterile 30 mL universal bottle. The number of fish selected for surface skin sampling was 10 % of the total fish population in each aquarium. Randomly selected fish was gently restrained with the aid of a fish net. Sterile glove was worn during handling. Fish skin surface was swabbed gently, bilaterally and aseptically with sterile swabs. Each fish skin swab was then kept in a sterile universal bottle. All fishes selected for sampling were apparently healthy, with no apparent skin condition. Water samples and skin surface swabs were kept in ice-packed cold box and transported to laboratory within an hour or two.

Culture and identification of isolates

Each water sample was centrifuged at 5000 rpm for 15 minutes. The water sediment and each skin swab was streaked directly onto blood agar (Oxoid) and incubated at 25 °C for 24 to 48 hours. Gram staining, oxidase and catalase tests were performed for preliminary bacteria identification.

Based on several properties of the *Aeromonas* spp., one to two colonies on blood agar that showed gram negative short rod, oxidase and catalase positive were subcultured onto nutrient agar and then incubated at 25 °C for 24 hours. The pure culture was then inoculated into Triple Sugar Iron (TSI) agar, Sulfide Indole Motility (SIM) agar, urease and citrate agars and incubated at 25 °C for 24 hours, for identification up to genus level. For further *Aeromonas* species identification, biochemical tests such as arginine dihydrolase, lysine decarboxylase, Voges-Proskauer, esculin hydrolysis, acid production from arabinose, glucose, mannitol, sorbitol and sucrose were performed.

Presumptive *Streptococcus* showed transparent pin-point colonies on blood agar, with variable haemolytic activity. Gram positive cocci and catalase negative were the confirmative characteristic of *Streptococcus*spp. One to two *Streptococcus* colonies were subcultured onto blood agar and incubated at 25 °C for 24 hours. For further *Streptococcus* species identification, biochemical tests such as soluble

hemolysin, 6.5 % NaCl, bile esculin, lactose, trehalose and sorbitol fermentation were performed.

Antibiotic susceptibility test

All *Aeromonas* and *Streptococcus* isolates were subjected to antibiotic sensitivity test using the disc diffusion method. The antibiotic discs used in this study for *Aeromonas* spp. were tetracycline 30 µg, erythromycin 15µg, nalidixic acid 30 µg, sulfamethaxalone and trimethoprim 25 µg, ampicillin 10 µg, and enrofloxacin 5 µg. For *Streptococcus* spp., instead of nalidixic acid, amoxicillin and clavulanic acid 30 µg were used. The Mueller Hinton agar plates were incubated at 25 °C for 24 hours. Each zone of inhibition was measured using a caliper to determine the susceptibility of the bacteria towards the antibiotic based on Clinical and Laboratory Standards Institute (CLSI) guidelines, 2011.

RESULTS AND DISCUSSION

The rate of occurrence of *Aeromonas* spp. in aquarium water was 30 % (Table 1). The occurrence of *Aeromonas* spp. in aquarium water was higher in water from aquarium shops (37.5 %) compared to water collected from home aquarium (21.4 %). The high occurrence of *Aeromonas* spp. shown in this study is suggestive of *Aeromonas* spp. being a natural flora in ornamental fishes. The occurrence of *Aeromonas* spp. on fish skin was 8.3 % (Table 2), which was lower compared to aquarium water.The low occurrence of bacteria on fish skin also may be due to different fish species and inconsistent fish size used in the sampling. Sufficient bacteria may be difficult to obtain on the fish surface swab especially from small sized fishes. In addition, bacteria may be injured or died during transportation to laboratory.

Table 1: Occurrence of *Aeromonas* spp. in aquarium water

Water sample	No. of samples	No. of positive samples	Occurrence rate (%)
Home aquarium	14	3	21.4
Aquarium shop	16	6	37.5
Total	**30**	**9**	**30.0**

Table 2: Occurrence of *Aeromonas* spp. on fish skin

Fish skin swab	No. of samples	No. of positive samples	Occurrence rate (%)
Home aquarium	17	2	11.8
Aquarium shop	43	3	7.0
Total	**60**	**5**	**8.3**

Of the 21 *Aeromonas* spp. isolates identified in this study, 76 % were likely to belong to the zoonotic motile mesophilic group (*A. hydrophilia, A. caviae, A.*

veronii), while the remaining were non-motile psycrhophilic group (*A. salmonicida)*. A high occurrence of mesophilic aeromonad shown in this study was also reported by Trust and Bartlett (1974).

A high number of the *Aeromonas* isolates showed resistance to ampicillin and tetracycline (Figure 1). It was reported that *Aeromonas* is intrinsically resistant to ampicillin; however, this observation was made on clinical isolates and since selective constraints in natural environment may differ (Dias *et al.*, 2012), thus isolates from such environment may differ in their susceptibility to ampicillin. Ninety percent (90 %) of the isolates in this study showed resistance to ampicillin. A high resistance of *Aeromonas* spp. to tetracycline was also reported by Dias, *et al.* (2012) due to the widely disseminated resistant *tet* gene in the farming environment.

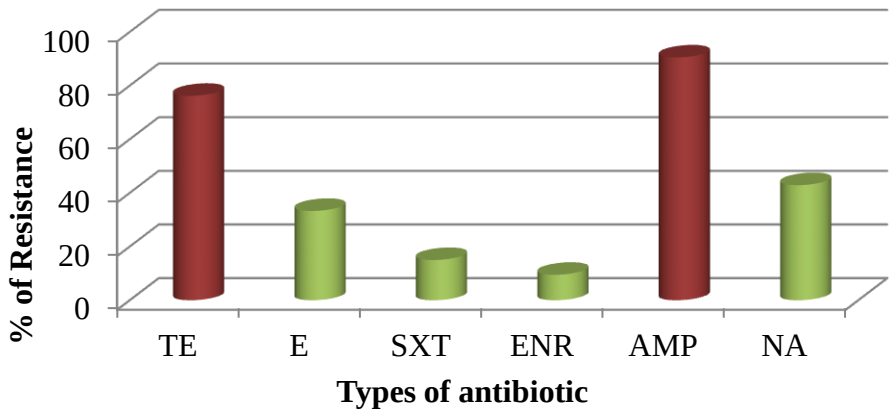

Figure 1**:** Antibiotic resistance profile of *Aeromonas* isolates
(TE: Tetracycline; E: Erythromycin; SXT: Sulfamethaxalone and trimethoprim; ENR: Enrofloxacin; AMP: Ampicillin; NA: Nalidixic acid).

One third of the *Aeromonas* isolates showed multiple drug resistance, that is, resistant to four or more antibiotics tested. Verner-Jeffreys *et al.* (2009) concluded that ornamental fish and their carriage water act as a reservoir for both multi-resistant bacteria and resistance genes.

The occurrence of *Streptococcus* spp. in aquarium water and fish skin were 3.3 % and 1.7 % respectively (Table 3 and 4). The low occurrence of *Streptococcus* spp. in a commercial fish farm was also reported by Shoemaker, *et al.* (2001). The bacteria infection typically occurred in overcrowded fish culture. Therefore, in this study, with no evident of introduction of infected feedstuff or new fish, the low *Streptococcus* spp. occurrence rate was as expected.

The two *Streptococcus* spp. that was isolated in this study belong to *S. viridans.* The definitive species identification is difficult using the conventional method.

As for the antibiotic resistance pattern of *Streptococcus* spp., one isolate showed resistance to all antibiotics tested which may indicate the emergence of multiple drug resistant bacteria.

Table 3: Occurrence of *Streptococcus* spp. in aquarium water

Water sample	No. of samples	No. of positive samples	Occurrence rate (%)
Home aquarium	14	1	7.1
Aquarium shop	16	0	0
Total	**30**	**1**	**3.3**

Table 4: Occurrence of *Streptococcus* spp. on fish skin

Fish skin swab	No. of samples	No. of positive samples	Occurrence rate (%)
Home aquarium	17	0	0
Aquarium shop	43	1	2.3
Total	**60**	**1**	**1.7**

Hence, it can be concluded that ornamental fish and its carriage water do play an important role in public health as they carry potential zoonotic *Aeromonas* spp. and *Streptococcus* spp. that are multiple drug resistant. Fish hobbyist, particularly immunocompromized individual, should be advised on the risk of zoonosis.

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BACTERIOLOGICAL AND CLINICAL FINDINGS IN CANINE AND FELINE BACTERIURIA CASES AT UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Bacteriological and clinical results of both canine (n=23) and feline (n=46) cases of bacteriuria presented to University Veterinary Hospital, Universiti Putra Malaysia for the period of 3 years (2010-2012) were descriptively analysed to determine the most commonly isolated bacteria in the urine samples as well as their antibiotic resistance patterns. The clinical abnormalities observed in these patients were also investigated. In dogs, 30 colonies were isolated. *Escherichia coli* and *Proteus mirabilis* comprised 23.3 % of the total isolates each, while *Klebsiella pneumoniae* comprised 20 % of the total isolates. In cats, 72 colonies were isolated. These same three species of bacteria, as for dogs, were the top three common isolates at different percentages (*Klebsiella pneumoniae* 20.8 %, *Proteus mirabilis* 16.7 % and *Escherichia coli* 12.9 %). They showed different degrees of resistance to the commonly used antibiotics in the settings. The most commonly observed clinical abnormality in both canine and feline bacteriuria patients was red urine. Comprehensive use of antibiotics following practical guidelines adapted for local settings is required to retain the efficacy of the antibiotics, as well as to reduce the occurrence of antibiotic resistance.

Keywords: Universiti Veterinary Hospital, bacteriuria, bacterial isolates, antibiotic resistance, clinical abnormality

INTRODUCTION

Through observation, cats and dogs with lower urinary tract disease are commonly presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). The true incidence of the disease is unknown. However, it has been reported that more than 20 % of cats that were presented to UVH (from year 2005 to 2008) had lower urinary tract diseases (Nurul Nadiah, 2010), while there is limited information on lower urinary tract diseases in dog patients.

Majority of urinary tract infections (UTIs) resolved with a 2- to 3-week course of antibiotics; however, some infections can persist or recur. Conventional antimicrobial therapy used may not be sufficient to treat refractory bacterial isolates or resistant bacteria that are present. Thus, greater understanding of common isolates and their local resistance patterns would be helpful in the selection of optimal antimicrobial agents, especially in terms of empirical therapy (Wagenlegner and Naber, 2006).

Recurrent or persistent bacterial UTIs are more difficult to treat compared to uncomplicated UTIs. Previous studies on the prevalence and antibiotic sensitivity profiles with respect to specific species of bacteria in different species of animal around Selangor and Serdang areas revealed that resistance rates might vary substantially among regions and species of animal (Seng, 2010; Joseph, 2011). Therefore, a local, hospital-based surveillance of the bacteria spectrum and their respective bacterial sensitivity for target animal species is paramount for a rational empirical therapy (Weese *et al*., 2011).

The clinical presentation varies among cats and dogs diagnosed with UTIs. Approximately 95 % of dogs diagnosed with UTIs did not show apparent correlation with clinical signs of stranguria, pollakiuria or pigmenturia (Seguin *et al*., 2003). In cats, however, bacterial UTIs were usually associated with haematuria, pollakiuria, dysuria, stranguria and inappropriate urination. Thus, this study was conducted to determine the association of bacteriuria and the clinical abnormalities of canine and feline patients in UVH setting. The aims of this study were to determine the common types of bacteria isolated and identified from urine samples of both cats and dogs with UTI presented to UVH, the resistance patterns of these bacteria to common antimicrobial drugs used and to investigate the clinical abnormalities (pertaining to urination) observed in patients with bacteriruia.

MATERIALS AND METHODS

Source of data

The bacteriological data for this retrospective study was obtained through a database search from the log book of the Bacteriology Laboratory, Faculty of Veterinary Medicine for the period of 1st January 2010 to 31st December 2012 (over the period of 3 years).

Those cultures with single or mixed colonies after incubation period of 48 hours were considered as positive growth. The isolated bacteria were subjected to antibiotic sensitivity tests. A pure culture from a single colony of each bacterium was streaked onto the Mueller-Hinton agar plate. Selected antibiograms (as requested by the clinician) were used. The zone of inhibition was checked after an incubation period of 24 hours to determine the susceptibility of the bacteria. The case numbers and laboratory reference numbers of canine and feline patients which had their urine samples submitted for bacterial isolation and identification (with or without antibiotic sensitivity tests) were cross-referenced to obtain their medical records from UVH.

From the medical records, the patient signalment and medical history were obtained for descriptive analytical evaluation. The clinical abnormalities observed during the hospitalization and/or during presentation were recorded.

Inclusion criteria

Suitability of cats and dogs for inclusion in the study were based on: (1) The urine samples submitted for bacteria isolation and identification had bacterial growth, and (2) The medical records of identified cats and dogs with bacteriuria were retrievable for evaluation.

Data Analysis

All descriptive analyses were performed using a commercial statistical software package (SPSS V21.0 for Windows, SPSS, Chicago, Illinois, USA). Reports of isolation and identification were analysed, retrospectively, to report the common bacterial uropathogens in dogs and cats presented. The antibiotic resistance patterns for the top three most commonly isolated bacteria were based on three common antibiotics prescribed from UVH. Information of the clinical abnormalities of the identified cats and dogs with bacteriuria were obtained and tabulated and expressed in percentages.

RESULTS AND DISCUSSION

Signalment

A total of 119 urine samples from both dogs (n=46) and cats (n=73) patients were submitted to the Bacteriology Laboratory, Faculty of Veterinary Medicine, UPM for bacterial isolation and identification. Fifty-five of these samples (17 dogs; 38 cats) had associated antibiotic sensitivity test results. Of the 119 cases, 106 files were retrievable (41 from canine patients and 65 for feline patients).

Bacterial isolates

Thirty isolates (pure bacterial growth, n=16; two or more colonies, n=14) were isolated from 23 dog urine samples. Both *Proteus mirabilis* and *Escherichia coli* represented 23.3 % (n=7) of the isolates each, *Klebsiella pneumoniae* comprised 20 % (n=6) of the isolates, while the other pathogens were *Streptococcu*s sp. (16.7 %; n=5), *Staphylococcus* sp. (10 %; n=3) and *Enterococcus faecalis* (6.7 %; n=2).

Seventy-two isolates were obtained from the urine of 46 cats. Urine samples from 27 cats (58.7 %) had a single bacterial growth while the remaining 19 (41.3 %) yielded mixed cultures. Of these 72 isolates, 20.8 % (n=15) were *Klebsiella pneumoniae*, 16.7 % (n=12) were *Proteus mirabilis*, 13.9 % (n=10) were *Escherichia coli*, 12.5 % (n=9) were *Enterobacter* sp., 11.1% (n=8) were *Pseudomonas aeruginosa*, 11.1 % (n=8) were *Acinetobacter* sp., 5.6 % (n=4) were *Staphylococcus* sp., 2.8 % (n=2) were *Klebsiella* sp., while *Burkhoilderia pseudomallei*, *Rhodococcus equi*, *Panteoea agglomerans*, and *Aeromonas* sp. comprised 1.4 % (n=1) each.

From the 5 isolates of *Escherichia coli* colonies tested for antibiotic sensitivity tests, 40 % (n=2) were resistant to amoxicillin/clavulanic acid, enrofloxacin, and/or cephalexin. For *Proteus mirabilis*, 3 out of 7 (42.9 %) colonies were resistant to enrofloxacin, 2 out of 5 (40.0 %) colonies were resistant to cephalexin, while 2 out of 7 (28.6 %) colonies were resistant against amoxicillin/clavulanic acid. For *Klebsiella pneumoniae*, all 3 isolates submitted for the procedure were resistant against amoxicillin/clavulanic acid. Approximately 50 % (n=1) were resistant to cephalexin, while 33.3 % (n=1) were resistant to enrofloxacin.

Approximately 88.9 % (n=8/9) of *Klebsiella pneumoniae* isolates were resistant to amoxicillin/clavulanic acid, 85.7 % (n=6/7) resistant to cephalexin, and 75.0 % (n=6/8) resistant to marbofloxacin. For *Proteus mirabilis*, 8 out of 9 (88.9 %) colonies were resistant to amoxicillin/clavulanic acid, 85.7 % (n=6/7) were resistant to cephalexin, and 60.0 % (n=3/5) were resistant to marbofloxacin. For *Escherichia coli*, 83.3 % (n=5/6) were resistant to amoxicillin/clavulanic acid, 81.8 % (n=9/11) resistant to cephalexin, and 75.0 % (n=3/4) were resistant to enrofloxacin.

Amoxicillin/clavulanic acid, enroflloxacin, and cephalexin were the top three antibiotics being requested for sensitivity tests. In dog urine samples, 7 out of 22 (31.8 %) isolates were resistant to enrofloxacin. Five of 16 (31.3 %) isolates were resistant to cephalexin and seven of 23 (30.4 %) isolates were resistant to amoxicillin/clavulanic acid. For cat urine samples, 33 out of 40 (82.5 %) isolates were resistant to cephalexin. Forty-two out of 53 (79.2 %) isolates were resistant to amoxicillin/clavulanic acid, and 24 out of 44 (54.5 %) resistant to enrofloxacin.

The most commonly observed clinical abnormality in both cats and dogs with positive bacterial growth was red urine.

In this study, the male dogs and male cats represented more than half the respective population, in which castrated males dominated. This could be due to the fact that male cats and dogs are more active and socially vivacious, thus increasing the incidence of harvesting or being infected with bacteria that may cause UTIs.

As Gram negative bacteria were the dominating bacteria in UTIs, clinicians could use narrow spectrum antibiotics in their first line therapy for UTI cases in dogs. A high percentage of *Klebsiella pneumoniae* isolated from cat urine samples could probably be due to contamination during urine sample collection, as majority of samples comprised of catheterised or spontaneously voided urine.

As regional variations in bacterial prevalence and antibiotic resistance are expected, thus quantitative bacterial culture and antibiotic sensitivity tests are necessary to confirm the pathogenic bacteria present in the urine samples from patients diagnosed with UTIs, as well as to determine the appropriate antibiotic treatment.

In this study, 52.2 % (n=12/23) of dogs with positive bacterial growth from the urine and 65.2 % (n=30/46) of that in cats had red urine. However, the red urine was not a clear indicator for the diagnosis of UTIs in patients. The presence of this sign might suggest the necessity of further diagnostic methods to reach a diagnosis.

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HAEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILES OF EGYPTIAN GOOSE (*ALOPOCHEN AEGYPTIACUS*)

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ABSTRACT

The study was conducted at Taman Wetland, Putrajaya, Malaysia. Blood samples were obtained from 18 clinically healthy adult Egyptian geese (9 males and 9 females) via the medial metatarsal and axillary veins for the study. Hematological parameters analysed were erythrocyte parameters and differential leukocyte counts and selected serum biochemical parameters. All values were recorded as mean ± SD except for eosinophil value, which was reported as median and range. The results showed no significant difference ($p>0.05$) in haematological and serum biochemical parameters between male and female geese. The data serve as a base for the establishment of the reference range for blood values of Egyptian geese.

Keywords: Egyptian geese, haematological, serum biochemistry.

INTRODUCTION

Egyptian Goose (*Alopochen aegyptiacus*) is classified as shelduck, which belongs to the class Aves, order Anseriformes, family Anatidae, subfamily Tadorninae and genus Alopochen. These geese are native to Africa, mostly found in Eastern and Southern Africa. Adult male and female Egyptian Geese share similar physical appearance. They have distinct chestnut eye patches surrounding yellow eyes, a brown chest patch and a brown strip that forms a collar around the nape of neck. Their legs and feet are pink. Similarly their bill is pink with a black tip and a dark base. On the other hand, juvenile geese lack the distinctive chestnut eye patch and brown chest patches and their plumage is duller, leg and beak yellowish. Haematology and serum biochemistry are important tools to aid in disease diagnosis and maintenance of population health status. This is especially true for avian because they rarely show clinical signs of diseases until at late stages of an illness. The normal range of avian blood parameters can be variable even among closely related species. Although there are numerous published reports on haematology and biochemistry status of domestic species, studies on haematology and serum

biochemistry of wild avian species are rather scarce and incomplete. The objectives of this study were to obtain haematological and selected serum biochemical baseline values and describe the blood cell morphology of Egyptian Goose.

MATERIALS AND METHODS

Animals

The study was conducted at Taman Wetland, Putrajaya, Malaysia. Eighteen clinically healthy adult Egyptian geese were used in this study. They were captured at different times and days using a net. Captured birds were collected and placed in quarantine area to facilitate blood collection. These geese were physically and carefully restrained during blood collection. Two milliliter blood samples were collected using 23G needles and 3 mL syringes from the medial metatarsal or axillary vein. One milliliter blood was transferred into ethylenediaminetetraacetric (EDTA) tube while another 1 mL into plain tube immediately after collection. The samples were then transported in an ice container to minimised deterioration.

Hematological and Serum Biochemical Parameters

All blood samples were processed within 24 hours of collection. The analysis was done at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Haematological parameters were analysed using the Cell-Dyn® 3700 (Abbott Diagnositcs, USA) haematology analyser. Pack cell volume (PCV) was determined using microhaematocrit method while plasma protein was estimated using a refractometer. Manual differential leukocyte counts were performed on 100 cells using the battlement method. Serum biochemical parameters analysed included calcium (Ca), inorganic phosphate (P), alkaline phosphatase (AP), γ-glutamyltransferase (GGT), total protein (TP) and albumin (Alb) levels were determined using automated blood biochemical analyser (Hitachi 902; Roche Diagnostic, USA). The A:G ratios were calculated using the formulae Globulin (g/L) = TP – Alb and A:G ratio = Alb / (TP – Alb).

RESULTS AND DISCUSSION

The haematology and serum biochemistry findings of Egyptian Goose are presented in Table 1 and 2 respectively. The blood cells of Egyptian Goose are shown in Figure 1. In general, the haematological and serum biochemical parameters were not significantly different between male and female Eygptian geese. This finding is similar to that of other studies on other wild birds (Fourie and Hattingh 1983; William and Trainer 1971; Shave and Howard 1976; Fair *et al*. 2007). For the Egyptian geese, the heterophils were shown to be the most numerous, which did not support the findings of other studies that showed that lymphocyte were the most

abundant circulating leukocytes (Samour, 2008). One of the factors that influence number of circulating heterophils and lymphocytes in avian species is stress (Scope *et al*. 2002).

Table 2: Serum biochemical parameters of males, females and mixed gender Egyptian Goose

Serum Biochemistry	Male	Female	Total
Calcium (mmol/L)	3.00±0.25	3.11±0.35	3.05±0.30
In.Phosphate (mmol/L)	1.36±0.41	1.25±0.37	1.37±0.46
ALP (U/L)	96±32.94	112.67±30.70	105.11±31.34
GGT (U/L)	< 3	< 3	< 3
Total protein (g/L)	49.36±5.48	49.89±4.18	49.36±4.74
Albumin (g/L)	21.40±5.65	21.44±4.61	21.37±2.68
Globulin (g/L)	27.96±1.88	28.44±1.54	28.00±4.95
A:G	0.80±0.22	0.78±0.17	0.79±0.19

Table 1: Hematological parameters of males, females and mixed gender Egyptian Goose.

Parameters	Male	Female	Total
Erythrocytes ($\times 10^{12}$/L)	3.40±0.42	3.38±0.36	3.40±0.42
Haemoglobin (g/L)	191.5±26.6	186.8±35.8	188.8±29.8
PCV (L/L)	0.47±0.07	0.44±0.06	0.45±0.06
MCV (fL)	134±9	129±10	131±10
MCHC (g/L)	405±101	430±109	420±100
Leukocytes ($\times 10^{9}$/L)	3.23±1.49	3.51±1.14	3.33±1.31
Heterophils ($\times 10^{9}$/L)	2.38±1.14	2.20±0.89	2.15±0.94
Lymphocytes ($\times 10^{9}$/L)	0.76±0.34	0.87±0.34	0.87±0.40
Monocytes ($\times 10^{9}$/L)	0.16±0.13	0.19±0.09	0.18±0.12
Eosinophils ($\times 10^{9}$/L)	0.00 (0.00-0.07)*	0.00 (0.00–0.07)*	0.00 (0.00–0.07)*
Basophils ($\times 10^{9}$/L)	0.10±0.10	0.12±0.08	0.10±0.08
Thrombocytes ($\times 10^{9}$/L)	3.13±3.43	3.69±2.88	3.45±2.94
Plasma Protein (g/L)	65.9±7.5	63.8±4.6	65.5±6.0

* means the value is reported in median (range)

Most of the parameters obtained in this study were in contradiction to that of a previous study on Egyptian goose (Fourie and Hattingh 1983). The most likely factor that resulted in higher haematological parameters in this study is nutrition, because, unlike the previous study, which was done on wild birds, the current study was conducted on birds in captivity. Leukocytes morphologies of the Egyptian geese were similar to other Anseriformes (Clark *et al.*, 2009). The greatest challenges in leukocyte differential count were to differentiate eosinophils from heterophils, and between small and medium lymphocytes from thrombocytes, large lymphocytes from monocytes, and immature erythrocytes from medium lymphocytes. The current data provides baseline values of blood profile of Egyptian Goose. Larger sample size from different geographic locations is necessary to ensure more reliable sets of data.

The data obtained in this study shall serve as a base for the development of haematological and serum biochemical reference values in Egyptian geese.

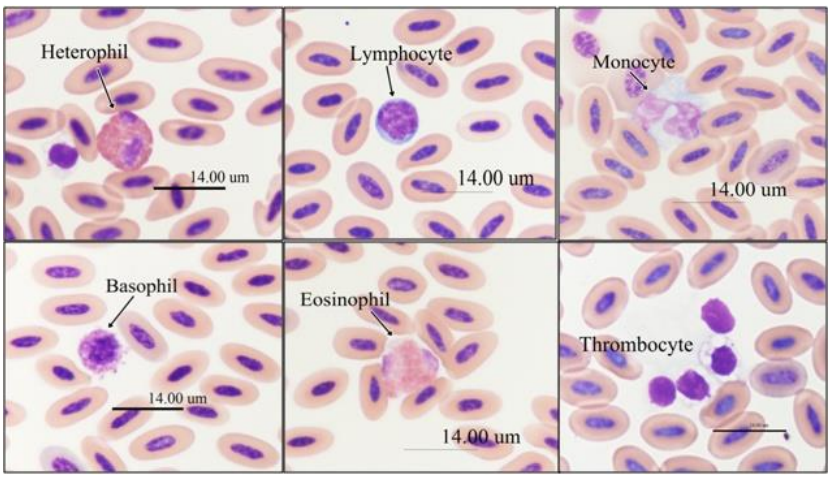

Figure 1. Blood cells of Egyptian Goose

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POTENTIAL OF FRESHWATER MUSSEL, *ANODONTA SP.* TO REDUCE PATHOGENIC BACTERIA IN AQUARIUM ENVIRONMENT

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ABSTRACT

Freshwater mussel, *Anodonta sp*. or locally known as *Lokan sungai* is an edible exotic food and easily found burrowed in the mud or soft substrate in rivers of Malaysia. On the other hand, this freshwater mussel has potential in the prevention of fish diseases. In this study, the potential of *Anodonta sp*. in reducing pathogenic bacteria concentration in the water was determined. Two treatment groups, i.e. T1 consisting of four freshwater mussels, three fishes and *Aeromonas hydrophila* type 2, was compared to T2, consisting of three fishes and *A. hydrophila* type 2. Three replications for each treatment group were also prepared. Types of bacteria present in the *Anodonta sp* before and after the experiment was also compared. The number of dead fish in the two treatment groups was recorded. At the end of experiment, the bacteria aerobic plate counts from the water collected from each treatment group were performed. In the current study, it was shown that the presence of *Anodonta sp* was able to reduce the concentration of pathogenic bacteria in the water and kept the fish healthy, and maintained good water quality. In conclusion *Anodonta* sp, apart from being sentinel species, could also be used as a biological control to prevent fish diseases from occurring by reducing the concentration of pathogenic bacteria in the water. Further studies are needed to determine the types of pathogenic bacteria that can be filtered by freshwater mussel while not causing harm to itself.

Keywords: *Anodonta sp.*, red hybrid tilapia, bacterial filtration, *Aeromonas hydrophila*

INTRODUCTION

Freshwater mussel is a family of Unionidae, which is under the class of Bivalvia in the phylum of Mollusca. It is locally known as *Lokan sungai* and naturally found burrowed in the mud and soft substrate areas of streams and rivers. Currently, *Lokan sungai* is used as an edible exotic food. It is also a sentinel species and can be used to determine the level of pollution in water bodies. *Lokan sungai* has a simple life-cycle where the fertilised eggs develop into glochidia, which is the only stage

where it becomes a temporary parasite and attaches to the gills of fish. The glochidia are released from the fish host and develop as juveniles before maturing into adult Lokan.

Figure 1: *Lokan sungai (Anodonta* sp.)

The *Anodonta* sp feed by filter-feeding, where they draw in water over their lamellibranchs, the modified gills, by pumping action of the cilia in the gills. The suspended food particles are collected on the food groove, bound with mucous before transportation to the mouth. In addition, the gills also serve as a gas exchange surface for breathing (Steele-Petrovic and Miriam, 1979). This feeding behavior filters particles, including bacteria and virus, from their surrounds, which accumulate in the body of the *lokan sungai*. A recent study showed that filter-feeding bivalves could remove avian influenza virus from the water and lower infectivity (Faust *et al*., 2009). In the present study, *Anodonta* sp. was selected as a model for bivalve species. Red hybrid tilapia, *Oreochromis niloticus x Oreochromis mossambicus* was selected as representatives of the fish species. During the experimental period, the fish and *lokan sungai* were kept in glass aquarium tanks as a model for fish ponds.

MATERIALS AND METHODS

Anodonta sp. collected from streams and rivers in Perak, Malaysia had sizes of 5 to 7.5 cm in broad shell length, while healthy red hybrid tilapias (*O. niloticus x O. mossambicus*) used in this study had total body length of 5 to 7.5 cm. *A. hydrophila type 2* was chosen, where there desired bacteria concentration was McFarland standard 2, viz., 6.0×10^8 CFU/mL. The bacteria concentration was based on the LD_{50} test.

In Experiment 1, to examine bacterila content in *Anodonta sp*, four pieces were randomly taken and using an aseptic technique, the siphon, gills and gastrointestinal tract were harvested. One portion was homogenized and streaked onto nutrient agar plate and incubated aerobically at 37 ºC, for 24 hours. For another portion, the squash smear was done and stained with May-Grunwald-Giemsa and Gram stains.

In Experiment 2, to study the filter-feeding behavior of *Anodonta* sp., two treatments group was used and labeled as T1 with three replicates, and also T2 with three replicates. One aquarium was used as control. A total of seven aquaria were used. The T1 aquaria contained four *Anodonta* sp., three fishes and *A. hydrophila* type 2. The T2 aquariums contained three fishes and *A. hydrophila* type 2, whereas the control aquariums contained three fishes only. Any clinical signs developed in the fishes during 10 days of experiment period were noted.

In Experiment 3, for aerobic plate count examination, at the end of the experiment, all the water from each aquarium (T1, T2 and control) were taken for analyses. Ten-fold dilution (10^{-1}) was done for each of the water sample. A 0.1 mL aliquot was used. All the plates were incubated at 37 ºC under aerobic conditions for 24 hours.

Experiment 1 and 2 were analyzed using descriptive statistic. For experiment 3, an independent t-test was used at 95 % confident interval. Statistical significance was determined at $P = 0.05$.

RESULTS AND DISCUSSION

In Experiment 1, it was found that the majority of the bacteria isolated were cream-coloured colonies, gram-positive and oxidase-positive coccus. The variety of bacteria commonly encountered in freshwater aquatic environments could be obtained from apparently healthy freshwater bivalves (Starliper, 2012).

In Experiment 2, higher fish mortalities observed were in the T2 than in T1 aquarium. Four of 9 fish died in the T2 aquarium whereas only one of 9 died in T1 aquarium. All fish in the control aquarium were alive. All *Anodonta* sp survived well throughout the experimental period. Bacterial cultures from the kidneys of dead fish suggested *A. hydrophila* types 1 and 2 when identified using the API20E test kit. Bacteria cultures from the *Anodonta sp* gills were identified as *Aeromonas hydrophila* type 2 and *Bacillus sp*. From the findings, it could be concluded that *Anodonta* sp. was able to reduce *A. hydrophila* type 2 population in water and prevent infection to the fishes.

In Experiment 3, the number of bacterial colonies in T1 plates was lower compared to T2 and control plates (Figure 2). The mean number of bacterial colonies in T1 was significantly lower at 19.9, compared to T2 with a value of 46.9. The number of bacterial colonies in the aerobic plate showed that presence of *Anodonta* sp. could reduce the concentration of *A. hydrophila* in water.

This novel discovery is very important with regards to fish diseases. In this study, the ability and potential of freshwater bivalve were elucidated, in which the *lokan sungai*, *Anodonta sp.,* was shown to be able to reduce the concentration of pathogenic bacterium in water.

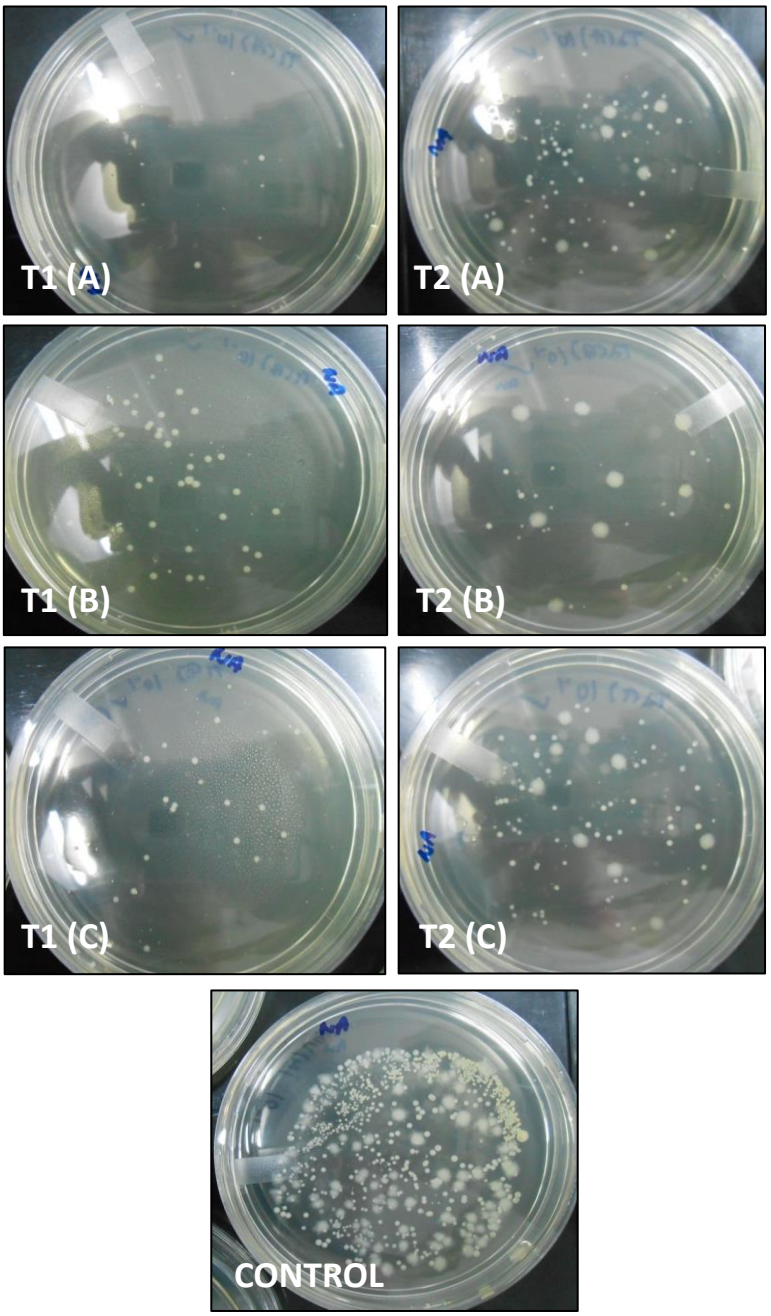

Figure 2: Aerobic plate cultureof *A. hydrophila* at 24 hours. Source of bacteria was from aquarium water of T1, T2 and control. Note the dense colonies grown from control tank's water.

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BLOOD PARASITES IN DOGS IN MANJUNG, PERAK, MALAYSIA

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ABSTRACT

This paper reports a study on blood parasites in dogs in Manjung, Perak, Malaysia. Blood samples were taken from 108 owned dogs of various breeds and aged more than 6 months old which were brought to Sitiawan Animal Clinic. The prevalence of blood parasites, association of blood parasite infection with tick fever-related clinical signs, association of blood parasite infection with presence of ticks and association of blood parasite infection with PCV, serum liver and pancreatic enzyme concentrations were studied. The prevalence rates of blood parasites found in this study were *Anaplasma platys* (13 %), *Babesia canis* (0.9 %), *Babesia gibsoni* (0.9 %) and *Mycoplasma haemocanis* (0.9 %). No significant association was found between current tick infestation and blood parasite infection. The presence of tick fever related clinical signs were significantly correlated with current blood parasite infection. However, the presence of blood parasites had no effect on PCV. Lastly, presence of blood parasites also had no effect on either the serum hepatic or pancreatic enzyme levels.

Keywords: Blood parasites, dogs, Manjung, ticks, clinical signs

INTRODUCTION

Southeast Asia provides an ideal environment for vector-borne disease transmission due to the tropical climate, large population of stray dogs and cats, and increasing popularity of pet ownership (Irwin and Jefferies, 2004). *Babesia canis*, *Babesia gibsoni*, *Ehrlichia canis*, *Anaplasma platys* (formerly *Ehrlichia platys*), *Dirofilaria immitis* and *Hepatozoon canis* are commonly reported vector-borne parasites in Malaysia.

The brown dog tick, *Rhipicephalus sanguineus*, is the vector for the transmission of *B. canis*, *B. gibsoni*, *E. canis* and *H. canis*. There is limited epidemiological information available but *R. sanguineus* is presumed to be a vector for *A. platys* (Beaufils, 2002). Babesiosis and ehrlichiosis (*E. canis*) are transmitted by the bite of an infected tick but *Hepatozoon canis* is transmitted via ingestion of an infected tick (Baneth, 2001). Mosquitoes are the vector and intermediate host for *D. immitis* (American Heartworm Society, 2007). A study was conducted to determine the prevalence of blood parasites, association of blood parasite infection

with tick fever-related clinical signs, association of blood parasite infection with presence of ticks and association of blood parasite infection with PCV, liver and pancreatic enzyme levels in dogs in Manjung Perak.

MATERIALS AND METHODS

Blood samples were taken from 108 owned dogs of various breeds and aged more than 6 months old that brought to Sitiawan Animal Clinic in Manjung, Perak, Malaysia. Blood samples were collected from both apparently healthy as well as sick dogs via cephalic venipuncture into EDTA and plain tubes. Physical examinations were performed on these dogs to determine tick fever-related clinical signs (fever, lethargy, jaundice, pallour and epistaxis) and presence of ticks. Blood in EDTA tube was used for direct wet mount and thin blood film to detect blood parasites. Packed cell volume (PCV) of each blood sample in EDTA tube was determined by the micro-haematocrit centrifugation method. Serum in the plain tube was sent to the laboratory for assessment of hepatic and pancreatic enzyme levels using Roche Hitachi 902® chemistry analyzer. The data were then statistically analysed using IBM® SPSS Statistics version 19.

RESULTS AND DISCUSSION

Anaplasma platys had the highest prevalence (13 %) among the blood parasites in the dogs. *Babesia canis*, *Babesia gibsoni* and *Mycoplasma haemocanis* had the same prevalence of 0.9 %. *Ehrlichia canis*, *Hepatozoon canis* and *Dirofilaria immitis* were not detected in this study. Occult infection of *Dirofilaria immitis* might be discounted since only direct wet mount was used in the study. Since these are all owned dogs, they do not harbored many ticks, thus there is low possibility of blood parasite infection, which may be the reason for the low prevalence of blood parasites in these dogs.

Fifty-two of 108 dogs (48.1 %) were infested with the common brown dog tick, *Rhipicephalus sanguineus* while 56 (51.9 %) were free of tick infestation. The majority (78.8 %) of tick infested dogs were negative for blood parasites (Figure 1). Nineteen percent of tick-infested dogs haboured *A. platys*. Other blood parasites were *B. canis*, *B. gibsoni* and *M. haemocanis*, found in 1.9 % of tick-infested dogs. Dogs without tick infestation were negative for blood parasites (89.3 %) while 10.7 % were infected with *A. platys*. However, blood parasite infection was not significantly correlated with current tick infestation. This finding suggests that arthropods other than ticks, which include lice and fleas, may be involved in transmission of blood parasites in dogs.

Fourteen of 108 dogs (13 %) showed tick fever-related clinical signs such as fever and/or pale mucosa and/or jaundice and/or epistaxis, while 94 dogs (87 %) had no such clinical signs. Of the 14 dogs with tick fever-related clinical signs 6 (42.9 %) were infected with blood parasites, which included one dog infected with *B. canis* (7.1 %), one dog infected with *M. haemocanis* (7.1 %) and four dogs

infected with *A. platys* (28.6 %). The remaining 94 dogs that did not show tick fever-related clinical signs (11.7 %) were infected with blood parasites. In this group of dogs, one had *B. gibsoni* (1.1 %) and 10 had *A. platys* (10.6 %). The presence of tick fever-related clinical signs was significantly correlated with current blood parasite infection. This finding suggests that blood parasites detected in this study caused acute illness and the infection caused rapid clinical signs in dogs.

The mean PCV of dogs with blood parasite infection were not significantly different ($P = 0.798$, $\alpha = 0.05$) from those without detectable blood parasites. Thus the presence of blood parasites which did effect on PCV may suggest low parasitaemia.

The mean liver and pancreatic enzyme concentration in serum is shown in Table 1. Dogs infected with *B. canis*, *B gibsoni* and *M. haemocanis* showed normal serum ALT, amylase and lipase concentrations. However, most dogs, either positive of negative for parasites had elevated serum AP and AST concentrations. Since these enzymes are not liver specific, their elevations in blood is the result of damage in other tissues to include the kidneys, placenta, intestinal mucosa, and bone (Syakalima *et al*., 1998). The study indicates that blood parasite infestation has no effect on the liver or pancreas.

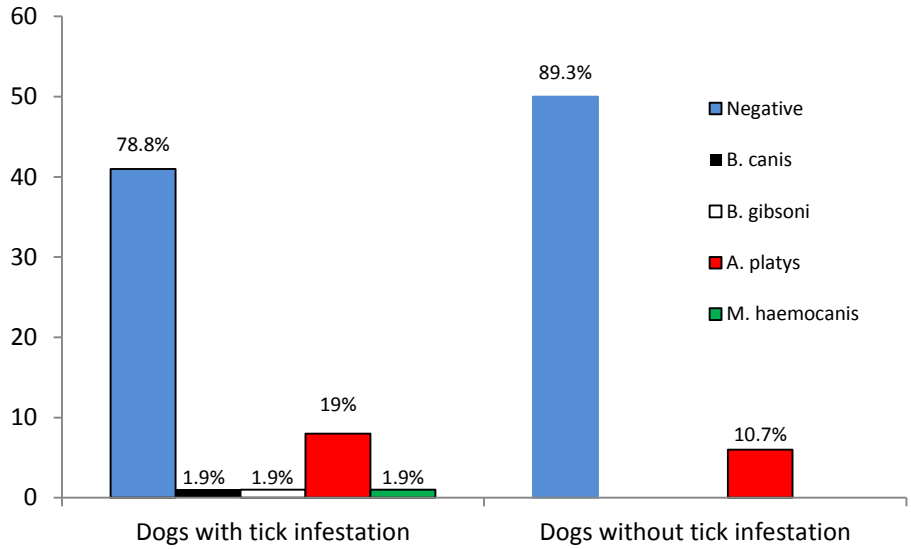

Figure 1: Prevalence of tick infestation and blood parasite infection in dogs.

Table 1: Serum liver and pancreatic enzyme concentrations in dogs

Enzymes (U/L)	Normal range (U/L)	Blood Parasites				
		Negative (N=91)	*A.platys* (N=14)	*B. canis* (N=1)	*B.gibsoni* (N=1)	*M. haemocanis* (N=1)
ALT	5-90	26.4±13.9	25.5±15.3	7	31.4	31.4
AP	40-100	152±71	140±57	120	104	342
AST	<60	202±129	185±144	159	100.9	68
Amylase	<1500	810±431	793±390	440	933	15
Lipase	13-200	178±106	186±86	180	23	81.3

Values are mean±SD.

ALT = Alanine transaminase; AP = Alkaline phosphatase; Aspartate transaminase.

CONCLUSION

In dogs in Manjung, Perak, *A. platys* was the most prevalent blood parasite, while *B. canis*, *B. gibsoni* and *M. haemocanis* had the same prevalence. No significant association was found between current tick infestation and blood parasite infection. Presence of tick fever-related clinical signs was significantly associated with current blood parasite infection. However, the presence of blood parasites had no effect on PCV or serum hepatic and pancreatic enzyme concentrations.

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DEVELOPMENT OF A VIDEO-BASED PRESENTATION FOR PAIN ASSESSMENT IN CATS: A PILOT STUDY ON UNIVERSITI PUTRA MALAYSIA VETERINARY STUDENTS

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ABSTRACT

Pain management is a rapidly emerging subject in the veterinary community. Studies showed that cats are often under-treated for pain. Attitudinal barrier, knowledge deficit and difficulty in pain recognition are major constraints in pain assessment and management. Since there is scanty reports available in Malaysia regarding this issue, this study aimed to: (1) examine UPM veterinary students' attitude, knowledge and pain assessment skills in cats and (2) develop a video-based training module for pain assessment in cats and (3) evaluate the training effect. A pre- and post-training survey model was used in this study, on DVM 4 and DVM 5 students of UPM in 2013. The questionnaire was designed to obtain information regarding veterinary students' attitude towards pain issues in cats, as well as their level of knowledge and skill in pain assessment. Upon completion of the questionnaire, students were subjected to a training module containing an interactive power point and case videos presentation, followed by discussion, and a post-training questionnaire. Students tended to underestimate the degree of pain associated with ovariohysterectomy and mistaken assumed that physiologic variables are good pain indicators in acute post-operative period. In pain assessment, students managed to recognise prominent expression of pain but failed to identify subtle changes in behaviour. Students' self-confidence in pain assessment had increased after the training. There was no significant improvement in their pain assessment skills as their responses matched those of the reference veterinarians. Further training and refinement of the training modules are warranted to improve students' knowledge and pain assessment skills in cats.

Keywords: cats, pain assessment, video-based training module, survey, veterinary students

INTRODUCTION

The International Association for the Study of Pain (IASP) defines pain as '*an unpleasant sensory and emotional experience*' and further states that, '*The inability to communicate verbally does not negate the possibility that an individual is*

experiencing pain and is in need of appropriate pain-relieving treatment.' In recent years, there is increased emphasis on pain prevention and management in veterinary medicine. Despite the heightened awareness of the detrimental effect of pain, studies suggested that pain is often under-diagnosed and under-treated in veterinary patients, especially cats (Raekallio *et al*., 2003; Williams *et al*., 2005). Pain assessment is the fundamental element for effective pain management in animals. Knowledge deficit and attitudinal barrier has been reported as barriers to pain recognition and pain management. Therefore, there is a need to develop training materials to educate veterinarians as well as veterinary students in pain assessment and pain management. This paper reports the development of a video-based presentation for assessment of post-operative pain in cats, and its training effect on veterinary students in Universiti Putra Malaysia.

MATERIAL AND METHODS

Case videos of cats following ovariohystectomy were incorporated into a power-point presentation. The 40-minute presentation utilized video footages to demonstrate the various behaviours and indicators of pain in cats. These included changes in demeanor, tensed and rigid posture, hunched posture, squinted eyes, vocalisation, head turn and attempt to bite.

For the purpose of testing the students' skill in pain assessment, video footages from another three cats that received acepromazine 0.1 mg/kg, SC, with or without tramadol 2 mg/kg, SC in the premedication were used (Table 1). Two experienced veterinarians, blinded to the type of premedication were asked to assess the level of pain and analgesic requirement based on the same footage. The average scores were used as reference to compare to the students' assessment.

A pre- and post-training assessment was used in this study. Year 4 and 5 veterinary students were invited to participate. A survey on students' perception and knowledge on pain in cats was conducted, followed by pain assessment on the three case videos. Students then listened to a 40-minute video-based presentation by the author (Lim Mei Yan), followed by discussion in a lecture-room setting. Following the training, students were again asked to assess the three case videos. Statistical analyses were performed using SPSS 20.0 statistical software. Simple descriptive statistics were produced for all variables in the dataset. For pain assessment, pain scores were expressed as mean ± SD. McNemar test was performed to examine the training effect.

RESULTS AND DISCUSSION

Participation rate

Ninety-two 92 students participated in this study with a participation rate of 60 %. The distributions of participants were comparable to the study population in terms of the gender, level of study and field of interest. The 60 % participation rate may

be a reflection that the majority of students are concerned about pain in cats, and are interested to improve their pain assessment skills.

Pain assessment skills

As illustrated in Table 2, students assigned the lowest pain score to the Cat 1, which was timid and quiet. Cats 2 and 3, which were more expressive and showed overt signs such as head turning and growling, were assigned higher scores. Vocalisation may be used to assess acute pain in cats (Brondani *et al.*, 2011). However, most cats in pain are usually depressed, immobile, silent, distant from their environment and tried to hide (Robertson, 2005). Students who are not familiar with cats' behaviour might expect cats to always vocalise and showed overt signs of pain. As this study showed, the majority of students erroneously assessed the pain level in Cat 3 and suggested pain relief, although this was the only cat that received analgesic in the premedication. However, both the experienced veterinarians were able to detect other subtle signs such as the demeanor, facial expression and mobility of the cat, and correctly identified that Cat 3 was experiencing lesser pain compared to Cat 1 or Cat 2.

Table1. Description of case videos

Case video	Pre-operative behaviour	Premedication	Post-operative behaviour
Cat 1	Timid, sitting at the corner, alert to surrounding, but not interactive when approached	Acepromazine only	Sitting on lateral recumbency, quiet, and immobile. Not interactive when approached, showed slight vocalization and squinted eyes when surgical site palpated
Cat 2	Relaxed, calm and confident; interactive when approached	Acepromazine only	Sitting on sternalrecumbency with head held low and squinted eyes. Growled and attempted to bite upon palpation of surgical site
Cat 3	Relaxed and calm, interactive	Acepromazine + Tramadol	Sitting on sternalrecumbency. Alert, no squinted eyes observed. Vocalized and attempted to bite when the flank area was palpated. Relatively mobile

Table 2. Comparison between reference veterinarians' and students' pain assessment

Case video	Pre-operative treatment	Comparison of Mean pain score on VAS (mm)			[1]Clinical opinion on the case (%)			p value
		RV	Students		RV	Students		
			Pre-training	Post-training		Pre-training	Post-training	
Cat 1	Acepromazine only	55	41±20	46 ±21	A	**A (12)** B (66.3) C (21.7)	**A (20.7)** B (60.9) C (18.4)	0.655
Cat 2	Acepromazine only	65	72 ±20	70 ±19	A	**A (83.7)** B (13) C (3.3)	**A (81.5)** B (16.3) C (2.2)	0.307
Cat 3	Acepromazine + Tramadol	20	64 ±21	59 ±20	B	A (43.4) **B (44.6)** C (12)	A (50.0) **B (41.3)** C (8.7)	0.388

RV = Reference veterinarian
[1]A = The cat is in pain, administration of analgesic is necessary.
B = The cat is having mild pain, need to reassess analgesic plan.
C = The cat is comfortable; no analgesic is needed for this moment.

Training effects

There was no significant improvement in the students' pain assessment skills following training (Table 2). This was likely due to the short period of training and limited case videos. Future training module will need to incorporate more videos to show how the cat's temperament and use of various drugs can affect its expression of pain.

CONCLUSION AND RECOMMENDATION

In short, veterinary students at Universiti Putra Malaysia were able to recognise prominent signs of pain in cats, but failed to identify subtle changes. Current training module did not improve students' pain assessment skills significantly. Future work should incorporate more videos of different case scenarios. Practical session(s) where students can practise assessment on real patients during clinical rotation would be the ultimate training and test, provided there are adequate teaching staffs.

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PREVALENCE OF MASTITIS AND ITS RELATION TO SELENIUM BLOOD LEVEL IN GOATS IN KLANG VALLEY, SELANGOR, MALAYSIA

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ABSTRACT

A study was carried out to determine the prevalence of subclinical mastitis and clinical mastitis in goats in Lembah Kelang, to investigate the pathogens in goats' milk from the subclinical group, and attempt to establish a relationship between the prevalence of mastitis and selenium (Se) status in goat. Forty five (45) lactating Jamnapari cross-bred does selected from five goat farms in Klang Valley, Selangor, Malaysia were group into clinical, subclinical, and normal group based on clinical changes of the milk, udder, and the goat, California mastitis test (CMT), and bacteria culture. Blood samples collected into heparinized blood tubes were analysed for Se-dependent enzyme, Glutathione Peroxidase (GSH-Px), using the NWLSS™ Glutathione Peroxidase Assay commercial test kit. The average prevalence rate of clinical mastitis in the study population was low with 10.20 % and 7.29 %, respectively, for the goat and quarter infection rates. The overall prevalence rate of subclinical mastitis was relatively high with 65.31 % and 50 %, respectively, for the goat and quarter infection rates. Normal goats had the highest mean GSH-Px level in blood, followed by goats with subclinical mastitis. Goats with clinical mastitis had the lowest mean GSH-Px level in blood. Descriptive study suggested that goats with high GSH-Px level in the blood are less prone to mastitis. However, statistically there was no significant difference for GSH-Px level in blood between groups. More samples are needed to prove there is statistically significant difference between mastitis groups for Se level in blood.

Keywords: caprine mastitis, selenium, prevalence, pathogens, Selangor

INTRODUCTION

Goat-farming in Malaysia is a rapidly developing industry. However, mastitis causes economic losses to the goat industry. High prevalence of subclinical caprine

mastitis in Malaysia was reported in 1997 and 2005 (Marina, 1997; Siti Zubaidah *et al*., 2005). In caprine mastitis, *Staphylococcus* sp. is the most prevalent pathogen responsible for intramammary infection in small ruminants. Selenium is a type of trace mineral that enhances immune response. In this study, glutathione peroxidase (GSH-Px) was used as the indicator of Se level in blood.

MATERIALS AND METHODS

Animals

Forty-five Jamnapari cross-bred goats were selected from five goat farms in Lembah Kelang, Selangor, Malaysia. Based on the findings of physical examination, California mastitis test (CMT) and milk culture, the goats were divided into clinical, subclinical and normal group. Clinical mastitis was diagnosed based on physical examination. Goats that had CMT scores of 2+ and 3+ and pathogenic bacteria isolated from the milk samples were diagnosed as subclinical mastitis, while goats that had CMT scores of negative, trace, and 1+ were considered free from intramammary infection (Feldman *et al*., 2000).

Determination of the pathogens in goats' milk from subclinical mastitis group

Quarter milk samples from subclinical mastitis group were collected and sent to the Bacteriological Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for bacteria isolation and identification.

Selenium

Blood sample collected into heparinised blood tubes were analysed for the activity of GSH-Px using the NWLSS^{TM} glutathione peroxidase assay commercial test kit (Northwest, Life Science Specialist, LLC, Canada).

Haematology and Serum Biochemistry

Blood samples were also collected into EDTA and plain tubes for basic haematology and serum biochemistry analyses.

Statistical Analysis

Data were analysed by one-way analysis of variance by using SPSS package version 20.

RESULTS AND DISCUSSION

The prevalence of subclinical caprine mastitis was 65 %, which is almost similar to the finding (58.3 %) of Siti Zubaidah *et al*. (2005) (Table 1). Subclinical mastitis is more difficult to detect than clinical mastitis, which had led to higher economic losses than clinical mastitis.

The present study found that the prevalence of clinical mastitis in goats was 10.20 % and the prevalence of quarter infection rates of clinical mastitis was 7.29

%. To the authors' knowledge, this is the first report of the prevalence of doe and quarter infection rates of clinical mastitis in Malaysia condition.

Table 1. Goat and quarter infection rates for clinical and subclinical mastitis

Types of mastitis	Infection rate (%)	
	Goat	Quarter
Clinical mastitis	10.20	7.29
Subclinical mastitis	65	50

Table 2. Type of bacteria in goats' milk in subclinical mastitis

Type of bacteria	*Prevalence (%)
Staphylococcus sp.	78
Streptococcus sp.	12
Corynebacterium sp.	4
Aeromonas sp.	2
Enterobacter sp.	2
Klebsiella sp.	2

*In goats' milk from subclinical group

Table 4. Glutathione peroxidase activity in goats with mastitis

Mastitis group	GSH-Px (mU/mL)
Normal	117.44 ± 75.81
Subclinical	100.51 ± 48.48
Clinical	97.53 ± 11.29

Values are mean ± SD
GSH-Px = Glutathione peroxidase.

Staphylococcus aureus and coagulase-negative *Staphylococcus* species were the most prevalent bacteria in goats' milk from the subclinical mastitis group (Table 2). *S. aureus* and coagulase-negative *Staphylococci* enhanced the zoonotic role by producing thermostable enterotoxins which are not destroyed during pasteurisation

(Contreras, 2007). This shows how important the prevention of subclinical mastitis in goats.

Descriptive study suggested that goats with high GSH-Px level in blood are less prone to mastitis (Table 3). However, statistically there is no significant difference in the GSH-Px level in blood between groups. The findings did not concur with those of Sanchez and co-workers (2007) controlled study which proved the significant difference in the incidence of mastitis between control group and Se-treated group. Controlled study can be used to minimise other confounding factors on the relationship between mastitis incidence and Se level in blood. There are no significant differences between control, subclinical, and clinical mastitis group in haematology and serum biochemistry parameters because blood parameters provide less diagnostic significance in goats with Gram-positive mastitis pathogens (Smith, 2001).

CONCLUSION

The study shows that there is high prevalence of subclinical mastitis, and *Staphylococcus* sp. is the most prevalent bacteria in the milk from subclinical mastitis goats. Descriptive study suggests that goats with high Se level in blood are less prone to mastitis. However, more extensive studies are needed to whether or not there is significant difference between normal, subclinical, and clinical mastitis group. Blood parameters are of poor diagnostic significance in goats with Gram-positive mastitis pathogens.

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COMPARISON OF FIELD EFFICACY USING FLORCOL PREMIX POWDER AND FLORFENICOL IN PORCINE RESPIRATORY DISEASE COMPLEX

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ABSTRACT

Porcine Respiratory Disease Complex (PRDC) is a costly and common problem encountered by the commercial pig farms. Disease control at the farm level is a crucial step to ensure good production and minimise the economic losses due to disease. This study was performed to compare the field efficacy between the use of Florcol premix powder (FCP) and florfenicol in treating the porcine respiratory disease complex in the commercially bred piglets. This study included 18 weaned piglets, from two pig farms located in Selangor, grouped into the control, florfenicol and FCP groups. A feed trial was carried out for 9 days and the clinical scores were recorded based on an alternate day basis. Twelve piglets were sacrificed at the end of this study for post-mortem examination. Piglets in the FCP group exhibited better clinical scores and reduced fever over time compared to the control group and florfenicol group, as well as better lung lesion scores. In conclusion, FCP showed good efficacy to treat PRDC.

Keywords: Swine, porcine respiratory disease complex, florfenicol, Florcolpremix powder.

INTRODUCTION

Porcine respiratory disease complex (PRDC) is a disease of major concern in the pig farms. It is a mixed infection with varied combinations from farm to farm (Bochev, 2007). Its primary agents include*Mycoplasma* spp., *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Bordetella* spp., and *Streptococcus suis* Type 2 (Lyutskanov *et al*., 2010; White, 2012), with *M. hyopneumoniae* being the most common isolate (Bochev, 2007). Cough is the main clinical sign in PRDC with other non-specific clinical signs of fever, inappetence, depression and difficulty in breathing due to pneumonia (Bochev, 2007; van Alstine, 2012; White, 2012).

Florcol premix powder (FCP) is a product produced by Y.S.P. Industry (M) Sdn. Bhd. as an off-white powder for in-feed medication. This product contains florfenicol, aspirin, and bromhexine hydrochloride. Florfenicol is a broad spectrumsynthetic antibiotic from thiamphenicol and it was found to be useful against many agents responsible in PRDC (Silva *et al*., 1999; Cipriánet *et al*., 2012;

Sacristán *et al*., 2012). Aspirin, on the other hand, is a non-steroidal anti-inflammatory drug (NSAID), which significantly reduced the fever caused by PRDC in a study (Vilalta *et al*., 2012). Conversely, bromhexine hydrochloride is used commonly as mucolytic expectorant by reducing the viscoelasticity of the mucus produced along the airway. Together, this product aimed to be alleviating the clinical signs and lesions caused by PRDC.

MATERIALS AND METHOD

Two farms in TanjungSepat, Selangor were enrolled into this study, i.e. Farm A, and Farm B. Clinically ill piglets were selected based on the criteria of depression, dyspnoea, coughing, and fever ($\geq$ 39.7 ºC).The selected piglets were subdivided into 3 groups, i.e. control, florfenicol, and FCP groups. All groups were fed commercial drug-free diet with corresponding treatment of 200 ppm florfenicol and FCP into the respective groups. The treatment was given for the first 5 days of the 9-days trial, after which all the piglets received the same commercial drug-free feed.

During the study, rectal temperatures, respiratory clinical scores, and general clinical scores were taken at every alternative day, while body weight was taken on day 1, 5 and 9 of the study.Respiratory and general clinical scores were assigned to each piglet based on the scoring systems adapted from the study (Vilalta *et al*., 2012). At the end of the study, post-mortem was conducted on 12 of the piglets for lung lesion evaluation and scoring using the Madec scoring system. The data were then statistically analysed using IBM® SPSS Statistics version 19.

RESULTS AND DISCUSSION

Throughout the study, the mean rectal temperatures of the piglets from the FCP group remained the lowest (Figure 1) and a noticeable drop was identified on day 3, with fluctuations noticed in both control and florfenicol groups. The respiratory clinical scores also produced the similar results where the scores of the FCP group remained lowest with very stable trend as compared to control and florfenicol groups (Figure 2). The decrease in the scores of the florfenicol groups on day 7 could be explained by the mortality of one piglet from the group; while the decrease in control group on day 9 could be due to the mortality occurring on that day.The percentage weight gain, on the other hand, was the highest in the FCP group during the first 5 days, with a drop observed after the treatment stopped, which could be due to several factors, including reinfection and other underlying diseases.

Post-mortem examination of the lung samples revealed numerically lowest lung lesion scores (12.25 ± 3.90) in the FCP group as compared to the control (16.75 ± 3.57) and florfenicol (18.75 ± 2.87) groups. The histological examination of the lung samples showed suppurative pneumonia and bronchopneumonia in the control and florfenicol groups, while interstitial pneumonia was noticed in the FCP group. The presence of interstitial pneumonia was suggestive of viral infection, which could propose the effectiveness of the FCP in eliminating the bacterial infection.

Due to the limited number of sick piglets enrolled, the results were not statistically significant ($p>0.05$).It is also arguable that sick piglets consume less feed, thus the actual dosage of the drug taken in was not known. However, the improvement of the disease in the piglets fed with FCP was noted by the farmers and observer.

CONCLUSION

FCP may be an effective premix powder in helping to reduce the severity of the clinical signs caused by PRDC. The use of antibiotic, anti-inflammatory drug and mucolytic expectorant may together result in the improvement of the general conditions of the piglets affected by PRDC, which is more superior to the use of florfenicol alone. Besides, the route of administration of feed premix powder is a practical way of administering medication for intensively reared animals.

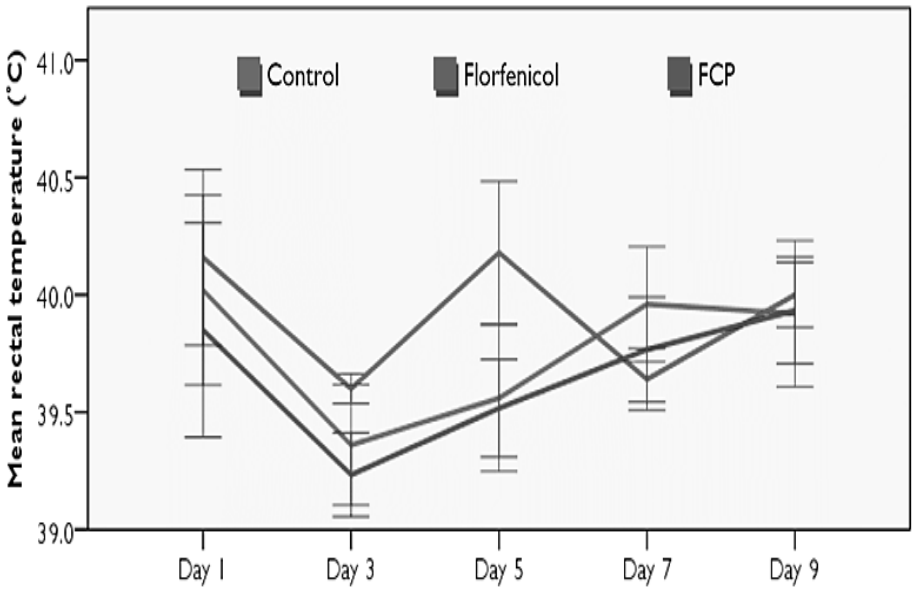

Figure 1. Mean rectal temperature of piglets over time.
Florfenicol = Florfencol treatment group; FCP = florcol premix powder treatment group.

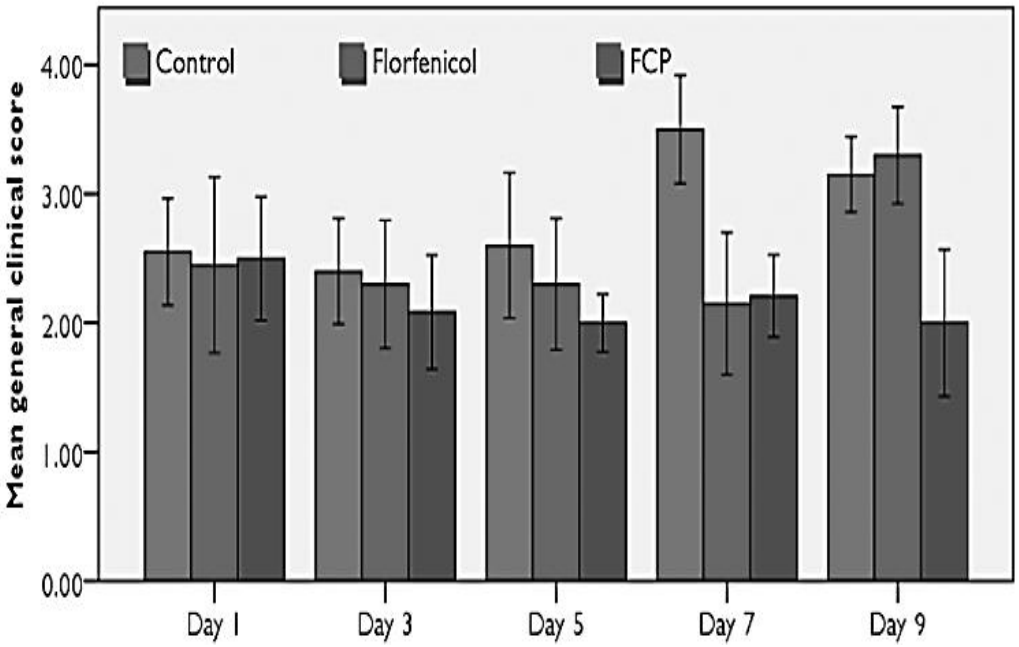

Figure 2. Mean general clinical scores of piglets over time.
Florfenicol = Florfencol treatment group; FCP = florcol premix powder treatment group.

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EMBRYOTOXICITY OF A MIXTURE OF FLUORANTHENE, PYRENE AND PHENANTHRENE IN EMBRYONATED CHICKEN EGGS

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ABSTRACT

Fluoranthene, pyrene and phenanthrene are members of polycyclic aromatic hydrocarbon (PAHs) which are ubiquitous and widespread environmental pollutant. PAHs are formed due to incomplete combustion of organic matter and are present as mixture in environment. The aim of this study was to investigate the *in ovo* effect of a mixture of fluoranthene, pyrene and phenanthrene on the hatchability of eggs and also as induction on immunotoxicity in chick embryos. A 18.75mg/kg mixture of fluoranthene, pyrene and phenanthrene was inoculated via allantoic route into 40 eggs while 10 eggs from control group were without inoculum. Hatchability of eggs was 5 % and 30 % for Flu-Py-Ph group and control group respectively. Yolk from dead embryos and serum from hatched chicks were collected for haemagglutination inhibition test (HI test) to determine Newcastle disease antibody titre. Thymus, Bursa of Fabricius, spleen and liver were collected for histopathology examination. Dead embryos showed generalized haemorrhages, stunted growth and curling of toes, pale and enlarged liver, thymus and bursa atrophy. Histopathologically, chicks from both control and Flu-Py-Ph group had severe lymphoid depletion in the Bursa of Fabricius and thymus. There was mild lymphoid depletion in spleen from both groups. Liver from both groups also showed inflammation and necrosis. Although comparable, lesions of chick embryos in Flu-Py-Ph group were more severe than the control group. Depletion of lymphoid tissues in lymphoid organs in Flu-Py-Phgroup led to decrease in antibody production as shown by lower HI Newcastle disease antibody titres compared to the control group. As evidence, a mixture of fluoranthene, pyrene and phenanthrene caused a mild degree of immunotoxicity in chick embryos.

Keywords: Fluoranthene, pyrene and phenanthrene, polycyclic aromatic hydrocarbons (PAHs), embryotoxicity, immunotoxicity, chick embryos

INTRODUCTION

Malaysia, as a developing country, has achieved rapid development in industrialization and urbanization. However, air pollution also occurs as consequence of increasing industrial factories, vehicles emissions, deforestation,

open burning and massive forest fires and land clearing. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutant which present as mixture and formed due to incomplete combustion of organic matters. Many PAHs are proven to be carcinogenic, teratogenic, mutagenic and immunosuppressive in human and animals (Anon, 1995). Fluoranthene, pyrene and phenanthrene dominate the atmospheric PAH profile (Arey and Atkinson, 1994). Latif *et al*. (2009, 2010, 2011) stated that inhaled PAHs can be disseminated systemically including the reproductive tract in broilers. This study was conducted to investigate the *in ovo* effect of a mixture of fluoranthene, pyrene and phenanthrene on the hatchability of eggs and immunotoxicity in chick embryos via assessment of Newcastle disease antibody titres using haemagglutination inhibition test and also via lymphoid tissue morphology.

MATERIALS AND METHODS

Fifty nine-day old embryonated chicken eggs were obtained from a local hatchery, divided randomly into 40 Flu-Py-Phgroup while 10 eggs acted as control. The 18.75mg/kg of mixture of fluoranthene, pyrene and phenanthrene was inoculated via allantoic route into 40 eggs while 10 eggs were without inoculum. Eggs were incubated at 37°C in an incubator at the Virology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Daily candling was done to determine embryonic mortality. Yolks were collected from dead embryos and serum sample was collected from hatched chicks via intracardiac route for haemagglutination inhibition test (HI test) of Newcastle disease antibody titre. Hatched chicks were euthanized via cervical dislocation. Post-mortem examination was carried out in all embryos. Thymus, bursa of fabricius, spleen and liver were collected for histopathology examination. Lesion scoring was done based on histopathology. Statistical analysis was performed.

RESULTS

Thirty eight out of 40 eggs from the Flu-Py-Ph group and 7 out of 10 from control group died in shell during day 18-21 of incubation. Hatchability of eggs from Flu-Py-Ph group was 5 % and 30 % for eggs from control group. Upon post-mortem examination, chick embryos from both groups showed stunted growth, generalized haemorrhage and curling of toes, pale and enlarged liver, thymus atrophy and bursa atrophy.

Newcastle disease antibody titre for Flu-Py-Ph group and control group was 2.9 and 3.7 respectively via haemagglutination inhibition test (HI test). Histopathologically for thymus, the morphology of the control group was within normal limits, vasculitis was present in the Flu-Py-Ph group. There was also severe lymphoid depletion and loss of demarcation between cortex and medulla in thymus of both groups. For bursa of fabricius, there was severe lymphoid follicles depletion

and loss of demarcation between cortex and medulla of bursa of fabricius in both the control and Flu-Py-Ph groups. Spleen of Flu-Py-Ph group had more loose space between splenocytes and less white pulps distribution compared to the spleen of control group. Liver had inflammatory cells in both groups. Inflammation in liver of Flu-Py-Ph was more severe than the control group. There were cell necrosis, vacuolation and lipidosis present in liver of both groups. Lesion scoring done for lymphoid depletion in thymus, lymphoid follicles depletion in bursa of fabricius, lymphoid depletion in spleen and necrosis and inflammation in liver showed no significant difference ($p>0.05$) in both control and Flu-Py-Ph group.

DISCUSSION

Polycyclic aromatic hydrocarbons (PAHs) generally occur as complex mixtures in the environment rather than a single compound. Mixtures of PAHs can interact in a variety of ways, and it is extremely difficult to accurately predict the toxicity of complex mixtures (Reeves *et al*., 2001). There are possibilities of synergistic, potentiation or antagonism effects of different PAHs mixtures. There is a lack of research concerning the effects of fluoranthene, pyrene and phenanthrene in inducing immunotoxicity in humans and animals. Complex mixtures of polycyclic aromatic hydrocarbons (PAHs) suppressed the splenic plaque-forming cell (PFC) response to T-cell dependent and independent antigens (Marquardt *et al*., 1999). The PAHs also suppressed cell-mediated immunity. T-Lymphocyte cytotoxicity and mixed lymphocyte responsiveness were impaired by most PAHs. PAHs can cause thymus atrophy (Holladay and Smith, 1995). Rodriguez *et al.*, 1999 also reported that PAHs can reduce specific T-cell subsets in thymus and spleen. Suppression of humoral immunity has been observed frequently upon exposure to a number of PAHs, including benzo[*a*]pyrene, DMBA, and 3-methylcholanthrene (Ward *et al*., 1984)

In this study, there were lower hatchability and Newcastle antibody titre in the Flu-Py-Ph group compared to control group. Using lesion scoring and statistical analysis, lesions in thymus, bursa of Fabricius, spleen and liver were more severe than the control group. This indicates that a mixture of fluoranthene, pyrene and phenanthrene can cause a mild degree of immunotoxicity in chick embryos, damage to lymphoid tissue in thymus, bursa of Fabricus and spleen which resulted in a decrease in antibody production.

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EFFECT OF COOLING METHODS ON EQUINE BODY TEMPERATURE UNDER MALAYSIAN FIELD CONDITIONS

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ABSTRACT

Endurance riding is gaining popularity worldwide as well as in Malaysia. Under Malaysian hot and humid climate, evaporative heat loss through sweating, which is the major heat dissipation method for horses, is limited thereby posing an increased risk of thermal stress and elimination from competitions. A cross-over experimental study using two FEI-sanctioned professional endurance horses and two non-FEI sanctioned amateur endurance horses was conducted under Malaysian field conditions to compare the effectiveness of ambient temperature water (29 °C), ice water (7 to 8 °C), and an alcohol-based cooling product (29 °C) treatment after routine endurance training exercises. Rectal temperature, regional skin temperatures (neck, triceps, gluteal), heart rates, respiratory rates, hydration status, cardiac recovery index, as well as muscle enzymes (CK, AST), serum lactate (LAC) concentration, and albumin (ALB) level measurements were analysed using One-way ANOVA followed by post-hoc test and Wilcoxon Signed Rank Test respectively. In general, ice water treatment reduced rectal temperature quickly for the professional horses and regional skin temperatures significantly for both groups of horses through the maintenance of a steep temperature gradient. Alcohol-based product and ice water cooling decreased respiratory rates significantly within 5 minutes of cooling probably because horses relied lesser on respiratory heat dissipation due to successful reduction of heat burden. In this study, no significant effect of cooling methods on heart rate and muscle enzymes were observed. Overall, ice water cooling is still the most effective cooling method for horses under our Malaysian climatic conditions compared to ambient temperature water and alcohol-based cooling products.

Keywords: Equine; Cooling Method; Body Temperature; Heart Rates; Muscle Enzymes

INTRODUCTION

Endurance riding is a relatively young but fast growing discipline worldwide and in Malaysia. Horses can be eliminated from an endurance ride due to many reasons, one of them was having higher than defined heart rates, usually more than 64 beats per minute. Meanwhile, failure to present the horses to the veterinarian gate within the stipulated time, which is usually 20 minutes, would also result in elimination. Therefore in endurance riding, competitors will always try to lower horses' heart rate to an acceptable level within the shortest time possible, normally by cooling down the horses.

Several studies comparing ice water cooling with normal ambient temperature water, hair coat clipping, and the use of cooling blankets have been conducted over the years to address heat stress problem in horses. There are several alcohol-based cooling products in the market, however there is insufficient information on the efficacy of these products. In addition to that, until today ice water cooling is still not completely accepted by the equestrian community as many still believed it would lead to muscle cramps and exertional rhabdomyolysis. The availability of sufficient ice also requires extensive preparation in terms of logistics and is labour intensive. Therefore, the need to search for a better cooling method is warranted.

This experimental study was conducted to compare the effectiveness of three cooling methods in horses, looking into speed of vital signs recovery and effectiveness of achieving successful cooling, as well as to investigate the efficacy of an alcohol-based cooling product.

MATERIALS AND METHODS

Animals and Sampling

Four healthy, fit, and physically sound endurance horses from the Selangor Turf Club (SLTC) consisting of one pure Arabian gelding, two Anglo-Arab mares, and a Part-bred Arab mare aged between 8 to 16 years old, were used in this study. Two of the horses had more than 5 years of endurance experiences, while the other two were new horses having less than 2 years of experiences. All these horses had acclimatized to the Malaysian climate, were conditioned for endurance rides, and were stabled together under a standardized housing environment with similar feeding, management, and training regime. These horses also had similar body surface area with body weight and height of 416.00 ± 18.58 kg and 151.63 ± 0.38 cm respectively.

Experimental Design

This study was conducted over 18 days (inclusive of a replicate study) using a cross-over experimental design. The sample units were divided into two equal groups according to years of endurance experiences. The FEI sanctioned professional horses (>5 years of endurance experience) covered a training distance of 20 km while the non-FEI sanctioned amateur horses (<2 years of experiences)

covered a training distance of 10 km. All horses were trained at an average speed of 12 to 13 km/hr on SLTC's sand track over flat and hilly terrain.

After the routine exercise, various cooling methods were used to cool the horses at the wash bay in the endurance stable for 20 minutes (10 minutes in replicate study). Throughout the study, passive cooling (control treatment without any form of external cooling), ambient temperature water (29 °C) cooling, ice water (7 to 8 °C) cooling, and alcohol-based product (29 °C) treatment were rotated among the horses. For active cooling, 200L of water was used for every horse in which the cooling was standardized by alternately having one personnel pouring water over one side of the horse's body while another personnel remove the water from the other side by using a horse sweat scrapper.

Microclimate

A portable thermohygrometer was used to measure the ambient temperature and relative humidity at the wash bay during cooling treatments. The ambient temperature ranged from 29.2 to 35.3 °C while relative humidity ranged from 40.9 to 68.6 % throughout the study period.

Physical Body Parameters

Heart rate, respiratory rate, rectal temperature, skin temperatures (neck, triceps, gluteal), and hydration status were measured before exercise, immediately after exercise, as well as 5, 10, 15, and 20 minutes during each cooling treatments. Every data collection took approximately 2 to 3 minutes. In the replicate study, data collection ended at 10 minutes of cooling treatment. The cardiac recovery index was calculated for each horse at the end of each cooling sessions.

Serum Biochemistry Test

Blood was collected via jugular vein puncture into plain serum blood tubes before exercise, immediately after exercise, and at the end of each cooling treatment. The sera were separated and stored in microcentrifuge tubes at -80 °C until analysis using Hitachi 902 Automatic Chemical Analyser for creatine kinase (CK) and aspartate aminotransferase (AST) concentration to detect for possible acute myopathy due to muscle cramping associated with ice water cooling, lactate (LAC) concentration; this was to characterize the horses' work load, and serum albumin (ALB) concentration as an adjunct to clinical evaluation of hydration status.

Statistical Analyses

The effects of various cooling treatments on equine physical and serum biochemical parameters were detected using One-way Analysis of Variance (ANOVA). Significant means were further investigated using Duncan's multiple range and Fisher's least significant difference (LSD) post-hoc tests. Wilcoxon signed rank test was used to detect for any significant changes in muscle enzymes concentration before and after cooling treatments. All statistical procedures were conducted at 95 % confidence interval using IBM® SPSS® analytical software version 21.

RESULTS AND DISCUSSION

Body Temperature

Based on the findings of this study, ice water cooling remained the most efficient cooling method to bring down both equine rectal and surface temperatures within a short period of time as compared to an alcohol-based cooling product and ambient temperature water. When ice water was applied over the skin surface of the horse, the temperature gradient is made steeper which allowed an effective conductive transfer of body heat directly from the horse's skin to the applied ice water. The efficiency of heat transfer depends on the size of contact areasand the steepness of temperature gradient (Marlin and Nankervis, 2002).

On contrary, alcohol based cooling depends on heat loss via evaporation and can be enhanced by convection. Heat is transferred from the horse body to the surrounding air based on the temperature gradient between skin surface and surrounding air (Foreman, 1996). A warmer boundary layer is formed quickly at the skin surface as heat is lost by evaporation of the alcohol in the cooling product which leads to a drop in the temperature gradient and thus reduces the rate of heat transfer. Heat loss by evaporation would be more efficient if the warmer air at the skin is continuously and quickly replaced by cooler air (Marlin and Nankervis, 2002), so that the temperature gradient is always maintained (Foreman, 1996).

Respiratory Rates

Based on the data obtained, both alcohol-based cooling product and ice water cooling decreased respiratory rates significantly for the professional horses. It is believed that alcohol-based product helped heat loss through evaporation and ice water cooling decreased the heat burden in the body by heat conduction, thus horses relied less on respiration to dissipate heat. As for the amateur horses, any form of active cooling reduced their respiratory rates significantly. This could be due to the different exercise intensity used in the study where horses are only allowed to train at the distance and speed not exceeding their physiological level, thus these amateur horses may not have been worked enough.

Heart Rates

From this study, cooling methods were found to have no significant effect on heart rateover time for all horses which differed from the findings of other researchers who reported significantly lower heart rates in horses bathed with cold water (Kohn *et al*., 1999; Foreman *et al*., 2006). This could be due to the different exercise intensity and the type of metabolism utilised by the Thoroughbred and Quarter horses in their studies. Thoroughbred breed are mainly used for racing while cutting horses are usually of Quarter horse breed. These activities require anaerobic metabolism such that horses go into oxygen debt and after exercise, their heart have to work harder to repay the oxygen debt. Thus, ice water cooling treatment is able to result in significant changes in this parameter. In this study, horses of the Arabian line were used and the overall work effort was characterized as low to moderate intensity aerobic work of long duration as evident by serum lactate data.

Although there was no significant effect of different cooling methods on heart rates in this study, the huge reduction in respiratory rates caused by alcohol-based product and ice water cooling could bring down heart rates indirectly through the reversed Bainbridge reflex (Greene, 1959). The reduction in respiratory rates lead to a decrease in intercostal muscles contraction resulting in reduced "milking" of blood back into the heart chambers, thus a drop in venous return. Consequently, there is a reduction in the stretch on atrial volume receptors leading to decreased heart rate.

Muscle Enzymes

There were no differences in muscle enzymes concentration before and after cooling among the professional horses. For the amateur horses, the muscle enzymes concentration was significantly higher after cooling, however differences were not significant across the treatment methods.

CONCLUSION

These findings reinforced that active cooling methods is safe and beneficial to horses. The conventional opinion that ice water cooling results in muscle cramps and injury did not occur during the entire study which is consistent with the works of other researchers (Williamson *et al.*, 1995; Kohn *et al.*, 1999).In this study, the increase of muscle enzyme concentration post-cooling in the amateur horses could be a carry-over effect from exercise and indicate that these horses are not as conditioned as the professional horses.

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SEROPREVALENCE OF *MYCOPLASMA MYCOIDES* CLUSTER INFECTION IN GOATS IN PULAU PINANG, MALAYSIA

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ABSTRACT

A pilot study was conducted from 14th to 24th January 2013 in Pulau Pinang, Malaysia, to determine the seroprevalence of *Mycoplasma mycoides* cluster infection and infection risk factors in goats. One of the most important members in the cluster is *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*), an agent that causes contagious caprine pleuropneumoniae. The study involved 163 sampled animals in 17 goat farms across five districts of Pulau Pinang. The study collected information on the history of the farm management and animal health, clinical examination, and laboratory analysis (competitive-ELISA) of the serum samples. The selection of farms was based on the recommendation by the Department of Veterinary Services, Pulau Pinang. Both healthy goats and those showing respiratory signs were sampled. Using competitive-ELISA, the seroprevalence of *M. mycoides* cluster infection in this study was 26 % (95 % CI = 19.46 - 32.86 %). The presence of *M. mycoides* cluster infection was associated with factors such as farm size, poor farm hygiene, and poor body condition of animals. The seroprevalence of animals was higher in smaller herd (<50 animals, 34 %) with an increase of three times likelihood for *M. mycoides* cluster infection. Goats of poor body condition (38 %) and reared under poor farm hygiene (42 %) were three times higher likelihood for *M. mycoides* cluster exposure or infection.

Keywords: *Mycoplasma*, contagious caprine pleuropneumoniae, competitive-ELISA, seroprevalence.

CLINICOPATHOLOGICAL STUDY ON PROLIFERATING CELL NUCLEAR ANTIGEN AND CD117 EXPRESSIONS IN FELINE OSTEOSARCOMA

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ABSTRACT

Feline osteosarcoma (OSA) is a rare tumour which accounts for 70-80 % of feline primary bone tumours. Proliferating Cellular Nuclear Antigen (PCNA) is a cell proliferation marker that has been described as a prognosticator for several human and animal cancers. CD117/ c-kit protein, a type-III tyrosine kinase receptor has been reported as prognostic marker in canine mast cell tumours where it is associated with uncontrolled proliferation. The prognostic value of CD117 and PCNA is not available for bone cancers in dogs and cats. The objectives of this study are to determine the immunoexpression of PCNA and CD117 in primary feline OSA and to evaluate their prognostic relevance in cats with this disease. Fifty formalin-fixed paraffin embedded feline OSA tissues were retrieved from the Veterinary Pathology of Utrecht University. Expression of PCNA and CD117 was evaluated using immunohistochemistry. Immunoexpression of PCNA and CD117 was evaluated for expression, localization and intensity. Normal feline bone and canine mast cell tumour was incorporated as control specimens. PCNA expression was localized within the nucleus while the CD117 expression was in the cytoplasm. Forty four percent of (n=22) tumours revealed a PCNA index of >0.75. PCNA index was significantly associated with mitotic index ($P<0.025$). Survival analysis revealed a poorer prognosis in feline OSA with PCNA index >0.75 ($P=0.024$, log rank score= 5.110). Nine feline OSA was positive for CD117 expression but no significant association was noted with the clinicopathological parameters. In conclusion, PCNA expression is a good prognostic marker however, CD117 expression is neither prognostic nor associated with any clinicopathological parameters in feline OSA.

Keywords: cat, bone cancer, immunohistochemistry, PCNA, CD117, prognosis

GROSS AND HISTOPATHOLOGICAL CHANGES IN MICE ORALLY INFECTED WITH *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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ABSTRACT

*Corynebacterium pseudotuberculosis*is, a causative agent of caseous lymphadenitis (CLA), is a facultative, rod-shaped, gram-positive bacterium. Caseous lymphadenitis a chronic granulomatous infectious disease characterised by the formation of abscesses, typically located in superficial lymph nodes and lungs. Difficulties in early clinical identification of CLA-infected animals resulted in limited effectiveness in controlling and eradicating this disease. This study was conducted to determine the pathogenesis and to compare severity of lesions in mice experimentally infected with *C. pseudotuberculosis*is. Forty healthy male mice were divided equally into 4 groups; the first group (control) of mice was orally inoculated with 0.4 mL sterile phosphate buffer solution (PBS), pH 7, the second, third and fourth groups of mice were orally inoculated with 0.4 mL of 10^3, 10^5 and 10^7 colony-forming-unit (cfu) of *C. pseudotuberculosis* respectively. Gross and histopathological changes in visceral organs were compared between the three diseased groups and non-diseased/control group over a period of 10 days of post-inoculation. Data were analysed using the SPSS version 19. The results from this study showed that there were significant ($p<0.05$) histopathological changes in the visceral organs (lung, liver and kidney) of the 3 diseased groups and non-diseased group. There was significant difference ($p<0.05$) between dose of 10^3 cfu from that of 10^5 and 10^7 cfu. However, there was no significant difference ($p>0.05$) between dose 10^5 and 10^7cfu. The severity of lesions at dose 10^3 cfu were normal to mild, while those given dose 10^5 and 10^7 cfu showed mild to moderate severity, that is, the visceral organs had severe congestion and increased vascularisation, micro-abscess formations, neutrophil and macrophage infiltrations, tubercular granulomas, necrosis and early signs of degeneration.

Keywords: *Corynebacterium pseudotuberculosis*, caseous lymphadenitis (CLA), mice, dose dependent, gross and histopathological changes.

CLINICAL RESPONSE AND DETECTION OF *PASTEURELLA MULTOCIDA* TYPE B FOLLOWING EXPERIMENTAL ORAL INFECTION IN MICE

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ABSTRACT

Hemorrhagic septicaemia is a fatal septicaemic disease caused by *Pasteurella multocida* Type B. The disease is economically important in Southeast Asia affecting cattle and buffalo. This study aimed to explore the use of mice as an animal model in relation to development of clinical signs caused by was *P. multocida* Type B, to determine the minimum infective dosage and the use of polymerase chain reaction (PCR) technique to detect the bacteria in various organs. The infection established was via oral inoculation with varied dosages. Thirty-two healthy ICR mice were grouped into 4 equal groups, 8 mice each. Mice of Groups 1, 2 and 3 were inoculated with 0.4ml of $10^{7,}$ 10^{5} and 10^{3} colony forming unit(CFU) of *P. multocida* Type B respectively, while the mice of Group 4 were inoculated with 0.4ml of phosphate buffer saline pH 7 as control. The mice were then observed for clinical signs throughout the 10 day-period post-inoculation. Almost all mice from treated groups showed clinical signs of ruffled hair, reduced activity, ocular discharges and laboured breathing at various rates and severity. All mice of the control group were observed to be normal. Mice with severe clinical signs and survived after 10^{th} day post-inoculation were euthanized, via cervical dislocation, for necropsy. Organs including heart, lung, liver, spleen, stomach, duodenum, jejunum, ileum, kidney and brain were subjected for bacteria isolation on blood agar and bacteria identification by using PCR technique. Results showed organs of all mice from infected groups were positive in PCR for *P. multocida* type B. No bacteria were isolated or detected from the organs of the control group mice.

Keywords: *P. multocida*, mice, PCR, orally inoculation, different doses, clinical response

INVESTIGATION ON USE OF TROPICAL ALMOND (*TERMINALIA CATAPPA*) LEAF EXTRACT AS AN ANTIMICROBIAL AGENT FOR TREATMENT OF SELECTED ORNAMENTAL FISH PATHOGENS

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ABSTRACT

The present study was undertaken to evaluate the *in vitro* antimicrobial property of Tropical almond (*Terminalia catappa*) leaf water extract against some fish pathogens isolated from the ornamental fish tank and to evaluate the efficacy of using different concentrations of the extract *ex situ* for antimicrobial activity in the ornamental fish tanks. Leaf water extract of 3-day preparation was used for the study. Evaluation of *in vitro* antimicrobial property was done by antimicrobial sensitivity test using disc diffusion method, minimal inhibitory concentration (MIC) using micro broth dilution method, and minimal bacteriacidal concentration (MBC) using triple soy agar (TSA) plate. Eight fish pathogens which were *Shewanella sp., Acinetobacter sp., Acinetobacter calcoaceticus, Cytophaga sp. VM1T, Flectobacillus sp. BF-1, Staphylococcus hominis, Staphylococcus saprophyticus* and *Bacillus sp. BVC27* were successfully isolated from the fish tank and used for the evaluation. Oxytetracycline, Tetracycline, Oxolinic Acid, Compound Sulphonamide and Chloramphenicol were used as standards for commercial antimicrobial agents. Antimicrobial activity was determined by measurement of inhibition zone around each paper disc for disc diffusion method, the lowest concentration which inhibited the bacterial growth for MIC and the lowest concentration which totally killed the bacteria for MBC. For each test, three replicated trials were conducted against each organism. *Ex situ* antimicrobial efficacy evaluation was done by total microbial plate count (TMPC). For each tank, three replicates were conducted for each concentration of the extract. The results showed the antimicrobial activity of the extract for disc diffusion method had an intermediate reaction for all types of bacteria isolated except for *Cytophaga sp. VM1T* and *Flectobacillus sp. BF-1* while most of the bacteria isolated were resistant towards one or more commercial antimicrobial agents. For MIC, most of the bacteria were inhibited at concentration of 3.1 mg/mL or higher and for MBC, most of the bacteria were totally killed at concentration of 6.3 mg/mL or higher. For TMPC, the lowest concentration that significantly inhibited bacterial growth in the water tank was at 60 ppm or 60 µg/mL. The results of this study showed that there is a potential of using Tropical almond (*Terminalia catappa*) leaves as a source of alternative antimicrobial agent and because of their presence in an abundant supply,

it is no doubt that these leaves can be developed for safer treatment of bacterial infection in fish and become a cheaper source of antimicrobial for water management of ornamental fish.

Keywords: tropical almond (*Terminalia catappa*), leaf extract, antimicrobial, ornamental fish pathogens

ISOLATION AND IDENTIFICATION OF *SALMONELLA* SP. FROM VILLAGE CHICKENS IN SELECTED FARMS IN JOHORE, MALAYSIA

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ABSTRACT

Salmonella sp. causes diseases in birds which lead to economic losses in the poultry industry, and food poisoning in human. There are several methods to isolate and identify *Salmonella sp*., but the conventional method was chosen for this study. Cloacal swabs, using sterile cotton swabs as per OIE standards (2010), were taken from 100 village chickens from seven backyard farms in five different districts of Johor, a state in southern West Malaysia. The swabs were placed in 10 ml of buffered peptone water (BPW), and kept at 37 ℃ for 24 hours. Then, 0.1 mL of the BPW was inoculated into 10 ml RV broth, and incubated at 37 ℃ for 24 hours. After that, one loopful of inoculated 10 ml RV broth was streaked onto XLD agar, and incubated at 37 ℃ for 24 to 48 hours. Colonies suspected to be *Salmonella sp*. were sub-cultured onto blood agar and then confirmed by the conventional biochemical tests and slide agglutination test with polyvalent-O antiserum. Positive *Salmonella sp.* isolates will be sent to One Point Health Lab Sdn. Bhd. for serotyping. Out of hundred samples, no *Salmonella sp*. was isolated. The reasons of the zero prevalence of *Salmonella sp.* in this study could be due to areas chosen for the sampling, low prevalence of *Salmonella sp.* in the farms, good husbandry management and biosecurity practiced in the farms, as well as the village chickens is already resistant to *Salmonella sp.* infection. This findings suggest that the village chickens in this state may be free from Salmonellosis, safe to be consumed, and has potential to become one of the export product to the neighbouring country.

Keywords: *Salmonella sp*, village chickens, Johore, cloacal swab

AETIOPATHOGENESIS OF KIDNEY DISEASE IN CATS PRESENTED TO VETERINARY LABORATORY SERVICES UNIT, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Kidney disease is one of the common diseases in cats. This retrospective study identified kidney disease in cats submitted to Veterinary Laboratory Services Unit, Universiti Putra Malaysia (VLSU-UPM) between year 2008 and 2012. The objective was to determine the prevalence of kidney disease in these cats submitted for post-mortem which includes the prevalence of infectious and non-infectious causes of kidney disease and the pathologic process. Results were based on histopathological examination of kidney sections. A total of 230 cats were submitted to VLSU-UPM for post-mortem examination; 180 cats had their kidneys sampled for histopathological examination and 155 of these cats had kidney lesions. The study showed the overall prevalence of kidney disease in this population of cats was 86 %; 70 % due to infectious causes, 30 % due to non-infectious causes. Of these, 84 % had evidence of a chronic process and 16 % for an acute process. Twenty-nine cats had kidney lesions suggestive of feline infectious peritonitis and 15 cats had cystic lesions and suggestive of polycystic kidney disease.

Keywords: kidney disease, infectious causes, non-infectious causes, pathologic process

CLINICAL RESPONSE AND REPRODUCTIVE PATHOLOGICAL CHANGES INDUCED BY *BRUCELLA MELITENSIS* and ITS LIPOPOLYSACCHARIDES FOLLOWING INTRAPERITONEAL INOCULATION IN FEMALE MICE

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ABSTRACT

Brucellosis is a bacterial disease that is caused by a large number of genus Brucella. Brucellosis in small ruminants is caused by *Brucella melitensis*, which is a gram negative, facultative intracellular coccobacillus bacteria. It is characterised by significant reproductive problems which lead to economical loses. The current prevention and control of Brucellosis in animals is through test and cull. Intervention by effective vaccine might help the farmer to overcome this condition. Little is known about the pathogenesis of Brucellosis in experimental animals particularly in the female mouse model. Hence, this study was designed to observe clinical responses and pathological changes in the reproductive organs of female mice via intraperitoneal inoculation. Twenty-four healthy female mice were divided into three different groups where each group was inoculated with 1.0 mL of 10^9 wild type *Brucella melitensis*,). One millitre of 10^9 *Brucella* LPS and 1.0 mL of sterile phosphate buffer was injected intraperitoneally. Mice were observed for clinical signs for 10 days post-inoculation. The reproductive organs were collected after 10 days for histopathological examination. Mice in group *Brucella* developed more severe clinical signs compared to mice infected with LPS. The clinical signs observed were ruffled fur, movement, responsiveness and eye condition. With regards to pathological changes in the reproductive organs, mice in the *Brucella* group showed moderate to severe presence of inflammatory cells, mild to moderate lesions of necrosis, degeneration, congestion and hemorrhage. The most affected reproductive organ when inoculated with the wild type *Brucella* was the ovary especially for lesions of infiltration of inflammatory cells and congestion/hemorrhage. The ovary was severely inflammed and congested with some evidence of necrosis. The LPS group of mice developed similar lesions as the mice inoculated with *Brucella* except that the mice in the LPS group developed normal to mild of lesions of necrosis and degeneration. The ovary of the mice infected with LPS developed moderate to severe lesions of inflammation and also

congestion/hemorrhage but it was less necrotized. In general, the oviducts were moderately inflamed with mild congestion. Some parts of the oviducts were also necrotized and degenerated. In addition the vulva of mice in the LPS group developed more lesions of necrosis compared to the *Brucella* group. This result suggests that the *Brucella* immunogen LPS was able to be a good candidate in the development of vaccine for Brucellosis.

Keywords*: B. melitensis*, female mice, intraperitoneal inoculation, clinical response, reproductive organs, pathological changes

OCCURRENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG DAIRY CATTLE FROM SELECTED FARMS IN SELANGOR, MALAYSIA

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a major human pathogen which causes severe morbidity and mortality worldwide. Additionally, the number of MRSA had been increasing in farm animals especially dairy cows. To analyze the occurrence rate of MRSA, 60 milk samples were collected from dairy cattle from four farms and subjected to bacteriology analysis. Identification of MRSA was done by performing a biochemical test and culture on Oxacillin Resistant Screening Agar Base (ORSAB). From 60 cows, 76.7 % (46/60) suffered from subclinical mastitis; 66.7 % (58/87) *Staphylococcus aureus* and 8.6 % (5/58) MRSA were recovered. Thus, the occurrence rate of MRSA in selected dairy cattle farms in this study was 8.3 % (5/60). All the five isolates were susceptible to vancomycin and resistant to oxacillin, methicillin and penicillin. It is known that the high resistance towards these drugs may be due to their frequent use over time for the drying off treatment. Thus, the detection of MRSA is essential for early prevention and control of this community- acquired infection.

Keywords: MRSA, milk, dairy cows.

SCREENING FOR ANTIBIOTIC RESIDUES IN LOCAL BEEF FOR NICHE MARKETS IN SELANGOR, MALAYSIA

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ABSTRACT

Antibiotic is known to be a most popular tool of drug in farming system for the purpose of treatment and as a growth promoter agent. Currently, the alarming concern particularly on antibiotic residues in food has gained attention from authorities around the world. Antibiotic resistant bacteria are on the rise creating a major concern in veterinary and human medical practice. Aside from antibiotic resistivity in microbes, evidence of allergic reaction and gastrointestinal disorder towards tetracyclines, sulfonamides, amino glycoside, ß-lactam derivatives and other antibiotics warrant attention to eradicate antibiotic residues in food. In this study, the presence of antibiotic residues was detected using microbial inhibition method, namely the Six-Plate Method in meat from selected abattoirs in Selangor. Basically, in this method each of the six agar media was seeded with different species of standard bacteria that were relatively susceptible and resistant towards specific antibiotics at certain pH level, meat pieces / extract were placed on the agar and incubated to detect the presence of zone of inhibition. Forty meat samples were collected from the three registered abattoirs in Selangor which comprised 14 samples from Department of Veterinary Services (DVS), Banting abattoir, 18 from DVS, Shah Alam Abattoir, and eight from H.S Nadar Rawang abattoir. All the samples tested were found negative for antibiotic residue. Taking into account the short duration of sample collections and limited number of samples, the present assumption of absence of antibiotic residues in the meat may not truly reflect the whole scenario. However, from the present limited study, it is found that the meat for the intended niche markets in Selangor is safe for the consumers.

Keywords: antibiotic residue, abattoir, local beef meat

CLINICAL RESPONSE AND REPRODUCTIVE PATHOLOGICAL CHANGES INDUCED BY *BRUCELLA MELITENSIS* AND ITS LIPOPOLYSACCHARIDES FOLLOWING ORAL INOCULATION IN FEMALE MICE

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ABSTRACT

Brucella melitensis is Gram-negative aerobic facultative intracellular bacterium that causes brucellosis and usually leads to abortion in sheep and goats. In this study, ICR mice were used as an animal infection model for *B. melitensis*. Three groups of eight healthy female were orally inoculated with 0.4 mL of phosphate buffered saline (PBS, as a control group), 0.4 mL of 10^9 cfu of *B. melitensis* or 0.4 mL of lipopolysaccharides (LPS) extracted from 10^9cfu of *B. melitensis*. Clinical signs exhibited by mice were observed for 10 days following inoculation. Surviving mice were then euthanized by cervical dislocation. Post-mortem was conducted and histopathology on the reproductive organs was carried out. *B. melitensi* inoculated group showed mild clinical signs compared to LPS inoculated group which showed generally normal behaviours except for mild ruffled fur during the first 14 hours and 34 hours post-inoculation respectively. Mice of the control group showed normal behaviours. Histopathology revealed that both *B. melitensis* and LPS inoculated groups showed mild to moderate infiltration of inflammatory cells in the reproductive organs, along with normal to mild findings of degeneration or necrosis. Mild to moderate congestions or haermorhages were found in mice of *B. melitensis* group, while LPS inoculated group showed normal to mild congestions or haemorrhages, except moderate to severe congestions in the ovary.

Keywords: *B. melitensis*, lipopolysaccharides, female mice, clinical signs, histopathology

SLEEPING PATTERN OF HORSES UNDER STABLE MANAGEMENT IN MALAYSIA

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ABSTRACT

Horses typically spend anywhere from 4 to 15 hours a day in standing rest, and anywhere from several minutes to hours lying down. Only part of that is actual sleep time, taken in brief naps that last a few minutes each. Horses are able to drowse by means of the unique passive stay apparatus of the equine fore- and hind-limbs. These enable them to be on their feet for long periods with a minimum muscular effort. This study was conducted in three different stables around Klang Valley. Three healthy adult gelding Thoroughbred horses with body condition score of three out of five were selected from each stable. The horses experienced the same daily activities and exercise for approximately one hour every day where they went for riding activity. Observational cohort study method was used in this study. Each horse sleeping patterns were observed for 72 hours where the time frame was divided into 12 hours a day for six days. The result revealed that the horses spent about 57 minutes in sternal recumbency with the standard deviation of 25 min. Besides that, the total mean time the horses spent on lateral recumbency was 8 min with standard deviation of 6 min. In short, the horses spent about 64 minutes with standard deviation of 26 minutes in recumbency position throughout 24 hour period and this behavior was only observed during the night observation throughout 24 hour period. On the other hand, the sleeping period of horses from the three stables revealed a bell curve like pattern where the peak time when the horses slept was at 1 am and 5 am. Finally, the total mean time for stay apparatus for all nine horses was 3.4 minutes with standard deviation of 0.7 minute. This means that, the horses can stay in the three leg passive stay apparatus for about 3.4 minutes before shifting weight to the other hind-limb.

Keywords: Thoroughbred horses, observational cohort study, sleeping patterns, sleeping period, stay apparatus

ANTIBACTERIAL PROPERTY OF SERUM FROM FALSE GHARIAL (*TOMISTOMA SCHLEGELII*)

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ABSTRACT

The False Gharial (*Tomistoma schlegelii*) serum was investigated for its potential antibacterial properties in this study. In addition, the association of these potential antibacterial properties with the serum levels of omega 3 (n-3) and omega 6 (n-6) polyunsaturated fatty acids were also determined. Blood samples were collected from five False Gharials through the dorsal tail vein and supravertebral branch of the internal jugular vein. Agar well diffusion method was used to determine the potential antibacterial properties of the False Gharial serum against various bacterial species by observing the zone of inhibition on Mueller Hinton agar. The remaining serum was then subjected to total lipid extraction method, transmethylated to fatty acid methyl esters and the serum fatty acid profile determined using gas liquid chromatography. Results indicated that the False Gharial serum showed potential antibacterial activities against *Escherichia coli* (clinical isolate), *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 700603 and *Salmonella typhimurium* ATCC 14028. These results pointed to the possibility of antibacterial properties from False Gharial serum samples. However, it was also concluded that the potential antibacterial properties were not directly associated with the PUFA n-3 and n-6 levels in the False Gharial serum.

Keywords: False Gharial, serum, antibacterial properties, fatty acids

COMPARATIVE STUDY OF VERTEBRAL FRACTURES AND LUXATION IN DOGS AND CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Vertebral fractures, luxation and subluxation commonly occur due to vertebral trauma leading to a vertebral dysfunction. This study was to evaluate species-related differences and clinical signs as well as to compare the common site and type of vertebral fractures and luxation. The medical records of cases with fractures and luxation referred to the University Veterinary Hospital of University Putra Malaysia between year 2008 and 2010 were viewed. The data that were taken from the records included the signalment, cause of injury, time elapsed before presentation (<24 h or >24 h), mode of treatment (conservative, surgical), abnormality in micturition and loss of deep pain perception were compared between groups. The radiographs of these cases were then reviewed. The type and site of lesions were then recorded. A cross tabulation on the various parameters and the associated factors were done and statistical significance were tested on the highest frequency data using Fisher's exact test were used in order to analyse the data. Forty one cats and 27 dogs were examined in this retrospective study. The most common site of vertebral fractures in both cats (20/41 or 49 %) and dogs (19/27 or 70 %) were at T3-L3. The least lesions seen were at the cervical region for both cats (4/27, 14 %) and dogs (3/41, 7 %). The most common type of lesions observed were vertebral subluxation in cats (24/41, 59 %) and dogs 20/27, 74 %). There were 2 times more cases of abnormal micturition in cats (21/41, 51 %) compared to dogs (7/27, 26 %). More lesions on the caudal segment of the vertebral were seen in cats compared to dogs. Almost all cases in both cats (100 %) and dogs (96 %) were brought in after 24 hours. Majority of cases were brought in with unknown causes and there were 1.4 times more hit by a car cases in dogs (7/27, 25 %) than cats (7/41, 17 %), 5.8 times more cases of high rise syndrome in cats (12/41, 29 %) compared to dogs (1/27,5 %). In dogs, the most common neurological status was Grade II (12/27, 44 %). This is because majority of the cases (8/12, 67 %) were treated with conservative treatment and the rest were treated surgically. However in cats, the most common neurological status were Grade III and IV, and both groups (10/13, 77 %) were treated conservatively. There were also 1.8 times more cases of Grade V neurological status in cats (9/35, 26 %) than dogs (3/21, 14 %). Most of the cases in cats (35/41, 85 %) and dogs (21/27, 78 %) were treated conservatively.

Keywords: vertebral fractures, vertebral luxation, cats, dogs

A PRELIMINARY STUDY ON HAEMATOLOGICAL AND PLASMA BIOCHEMICAL PARAMETERS OF CAPTIVE FALSE GHARIAL *(TOMISTOMA SCHLEGELII)*

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ABSTRACT

The False Gharial (*Tomistoma schlegelii*) is a freshwater, mound-nesting crocodilian with a distinctively long, narrow snout. It lives in dense secluded areas of the rainforest fringes, mainly near slow moving rivers, swamps and lakes. This once widespread species can be currently found only in Peninsular Malaysia, West Borneo, Java and Sumatra. Clinicopathology is a known method that can be used to monitor the health status of the false gharial but basic haematological and plasma biochemistry values need to be established. Blood samples were taken via the ventral caudal vein of five captive False Gharials and transferred into tubes containing anti-coagulant. Basic haematological and biochemistry values were analyzed from three males and two females. Morphology of erythrocytes, heterophils, eosinophils, basophils, lymphocytes and monocytes cells were determined by measuring the size and description of the appearances of shapes and colours. Compared previous reports, the values from this study revealed that the PCV was significantly higher and the erythrocyte count, plasma protein and haemoglobin concentrations were significantly lower. Plasma calcium and total protein concentratons were significantly higher but glucose and cholesterol concentrations were significantly lower than previously reported. Compared to *C. porosus*, only serum chloride chloride concentration was significantly higher in *T. schlegelii* while the calcium, glucose, alanine transaminase, alkaline phosphatase, total protein, albumin, globulin and albumin/globulin ratio were significantly lower. The results obtained in this study could not be used as reference values for the *T. schlegelii* and could be used as reference for future study.

Keywords*:* False gharial (*Tomistoma schlegelii*), haematology and plasma biochemistry

GROSS AND MICROSCOPIC LESIONS CAUSED BY TICK (*HAEMAPHYSALIS* SP.) AND MITE (*TROMBICULID* SP.) INFESTATIONS IN RED JUNGLE FOWL (*GALLUS GALLUS SPADICEUS*)

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ABSTRACT

A cross sectional study was carried out to compare the gross and microscopic lesions caused by ticks (*Haemaphysaliswellingtoni*.) and trombiculid mites (*Neoschongastia gallinae*) in the red jungle fowl. Ten red jungle fowls were caught from a farm in Jenderam Hulu, Selangor. Each bird was subjected to a thorough examination to locate the skin lesions caused by the Haemaphysalis tick and Trombiculid mite infestation. The lesions were evaluated using three gross parameters, which are the elevation from the skin, shape of the lesion and diameter of the lesion. Biopsy samples were taken after gross examination of the skin lesions. The biopsies were fixed in neutral buffered formalin and processed using the ususal histological technique. The tissue sections were stained with Hematoxylin-Eosin and Giemsa and examined for cellular identification and cell count. An eosinophil cell count was done under X40 magnification. Five different areas around the lesion/attachment sides were examined for each skin lesion sample and the average numbers of eosinophils were counted. A total of 12 skin lesions caused by ticks and 18 skin lesions caused by the trombiculid mite were found in the 10 red jungle fowls. The study showed that 8 % of the skin lesions caused by ticks had elevation characteristics while 100 % of the skin lesions caused by trombiculid mites have the elevated characteristics. All of the 18 skin lesions caused by mites showed crater-like shapes which resembled the volcanic crater while 92 % of the tick lesions did not have a specific shape and only 8 % had nodular shape. Ninety-two percent (11/12) of the skin lesions caused by ticks had diameters less than 0.1cm and only 8 % (1/12) had lesion diameters between 0.1 to 0.5 cm while 89 % of the lesions caused by mites had diameters between 0.1 to 0.5 cm while 11 % have lesion diameters of more than 0.5 cm. Microscopically the lesions with tick infestation have cement like structure, congested sinus capillaries around the necrotic area while in skin lesions cause by mite infestation had the presence of stylostome feeding tubes and thickening of epidermis layer near the attachment site. Both tick and mite lesions had massive cellular infiltration of mainly macrophages and eosinophils but the eosinophil numbers were much higher in the mite infestation compared to the tick infestation.

Keywords: Red Jungle fowl, ticks (*Haemaphysalis* sp.) and mites (*Trombiculid* sp.)

PATHOLOGICAL CHANGES ASSOCIATED WITH *AEROMONAS HYDROPHILA* INFECTION AND HEAT STRESS IN RED HYBRID TILAPIA (*OREOCHROMIS* SPP.)

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ABSTRACT

Aeromonas hydrophila can cause massive disease outbreak in fish in various aquaculture farms and in the wild worldwide. It is a short, Gram-negative bacillus with a single flagellum. *Aeromonas hydrophila* infection is characterized by the presence of petechial (pin-prick) haemorrhages on the body, haemorrhages of the eyes and distended abdomen. The objectives of this study were to determine the effect of different dose of *A. hydrophila* on histopathological changes in the kidney, liver, spleen, brain and eye and the role of heat stress in the development of *A. hydrophila* infection in Red hybrid tilapia. Experimental infection with *A. hydrophila* (isolated from a natural outbreak) in various concentrations were inoculated into the fingerlings of Red hybrid tilapia intra-peritoneally. The concentrations of *A. hydrophila* at 10^{12} colony forming unit (CFU), 10^{10} CFU and 10^{8} CFU/mL were inoculated into three different groups (each group consisted of 7 fingerlings) and heat stress was induced using aquaria heater at 31 °C which was attached inside the aquarium on day 8 to mimic the natural environment condition. Another three groups were inoculated with the concentrations as above, however no heat stress were induced to them and two control groups with and without the heat stress. The mortality rate in the groups with induced heat stress was higher; almost 43 % compared to non-heat stress groups which was 4.8 %. The histopathological lesions in the kidney, liver and spleen given the different concentrations were not significantly different ($p > 0.05$) except for inflammation in the kidney ($p < 0.05$) in all the experimental groups probably because of the high bacterial concentrations. In conclusion, the *A. hydrophila* of field strain was shown to be a potent pathogen, capable of causing disease and death in Red hybrid tilapia. Red hybrid tilapia reared in water with high temperature was more susceptible to *A. hydrophila* infection.

Keywords: Red hybrid tilapia, *Aeromonas hydrophila*, heat stress, histopathology

IDENTIFICATION OF METABOLIC PARAMETERS AS PERFORMANCE INDICATORS IN ENDURANCE HORSES

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ABSTRACT

One of the causes of elimination in endurance horses is metabolic problems and it can be detected by using of metabolic parameters at veterinary checkpoint. Metabolic problems in endurance horses lead to impairment of horse ability to perform well during the event. Therefore, the study was carried out to identify suitable metabolic parameters as performance indicators in endurance horses. Data from endurance horses that involved in 80-km event and above were selected. Twenty-seven horses that were eliminated due to metabolic problems and 98 horses that completed the event were included in the study. Metabolic parameters of individual horse were recorded. Data showed there were significant differences in heart rate, cardiac recovery index mucous membrane colour, capillary refill time and skin recoil parameters between the eliminated and completed groups. Therefore, combination of these data can be used to determine the performance of endurance horses during the event. These findings may assist veterinarian in detecting the metabolic crisis in endurance horses, at veterinary check point or as early as possible so that any injury can be prevented and safeguard the welfare of endurance horses.

Keywords: endurance horse, metabolic parameters, performance, metabolic crisis

PUBLIC PERCEPTION AND EVALUATION OF MALAYSIAN ZOOLOGICAL PARKS AS CENTRES FOR CONSERVATION AND EDUCATION

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ABSTRACT

Modern zoos worldwide are evolving towards becoming important conservation and education centres, with high commitment for animal welfare and visitor engagement. Zoos that do not subscribe to this progressive change risk public criticisms that may even threatened their very existence. In Malaysia, a similar trend towards an emphasis on conservation and education is being observed by many zoos. However, there remains a paucity of information on the effectiveness of these programmes. This study was therefore undertaken to gauge public perception and to evaluate the efforts taken by Malaysian zoos to fulfill their roles as centers of conservation and education. A total of 300 zoo visitors were interviewed in five Malaysian zoos (Zoo Negara, Sunway Wildlife Park, A'Famosa Animal World Safari, Taiping Zoo and Johor Zoo) over a period of three weeks following a standard questionnaire. The zoos' conservation and education efforts were evaluated through interviews with their respective zoo directors and through personal observations, and scored according to predetermined criteria. Most of the visitors (61.0 %) came to the zoo for recreation purposes although they acknowledged that zoos should fulfill their roles as conservation and education centres. Majority (69.0 %) were satisfied with the conservation and education efforts of the zoos, however, many (49.0 %) still held a rather simplistic view and were not well informed about the true conservation work that was executed. Evaluation of zoos revealed that many zoos were still in the early stages of evolving into effective conservation (30.0 %) and education (43.6 %) centres and as a consequence, there were still many areas that could be improved especially in the *ex situ* (32.6 %) and *in situ* (13.0 %) conservation programmes. Scheduled evaluation of zoos should be carried out regularly to determine the effectiveness of their education materials and programmes, in order for them to maintain their relevance in society and to ensure the optimal wellbeing of the animals under their care. Local zoos should devise more innovative and practical methods to integrate educational messages with interactive and entertaining exhibits.

Keywords: zoo evaluation, conservation, education, visitor perception

APHRODISIAC INFLUENCE OF *EURYCOMA LONGIFOLIA* (TONGKAT ALI) ROOT EXTRACT ON COURTSHIP BEHAVIOUR AND PLASMA TESTOSTERONE OF RAMS

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ABSTRACT

Eurycoma longifolia is a flowering plant that is available in Malaysia. It is well-known among human as an herbal plant that has aphrodisiac effects. This study aimed to determine the aphrodisiac influence of *Eurycoma longifolia* root extract (Tongkat Ali) and its effect on the plasma testosterone levels in rams. An experimental study was conducted on 10 three-year-old rams from the Veterinary Institute of Malaysia, Kluang, Malaysia. They were randomly allocated into treatment (n=5) and control (n=5) groups, respectively. The treatment group were drenched with 500 mg/kg *Eurycoma longifolia* root extract and the control group received 20 mL of distilled water for 12 consecutive days. Each ram were exposed to two ewes for 20 minutes in a test pen. The frequency of the courtship activities were observed and recorded each day throughout the treatment period. The plasma testosterone levels were also measured before, during and after treatment. The results showed that the aphrodisiac influence of *Eurycoma longifolia* root extract on the courtship behaviour between groups was significant ($p<0.05$). The *Eurycoma longifolia* treated group had a significant increase in frequency of pawing and nibbling at the flank region of the ewe ($p<0.05$) when compared to the control group. No significant difference in sniffing of the anogential region, flehmen response and grunting vocalization between groups were observed. Moreover, there was no significant effect of *Eurycoma longifolia* root extract on plasma testosterone levels between groups. External and internal factors may have affected the significance of the effect of *Eurycoma longifolia* root extract on plasma testosterone levels in rams in this experimental study. In conclusion, *Eurycoma longifolia* root extract at a dosage of 500 mg/kg has a significant aphrodisiac influence on courtship behaviour but did not elicit any significant effect on the plasma testosterone levels in rams.

Keywords: *Eurycoma longifolia* root extract, courtship behaviour, aphrodisiac influence, plasma testosterone levels, rams

GROWTH PERFORMANCE OF JUVENILE RED TILAPIA (*OREOCHROMIS* SP.) FED TUBIFEX WORM, EARTHWORM AND COMMERCIAL DIET

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ABSTRACT

A feeding experiment was conducted for 3 weeks to investigate the use of commercial diet, tubifex worm and earthworm as a utility in supporting the growth performance of juvenile red tilapia (*Oreochromis sp.*). The juvenile red tilapias (3.6 ± 2.0 cm; 5.7 ± 1.5 g) were fed three times (0800, 1200 and 1600 hours) daily at 5 % of the body weight for the duration of the experiment. The response of fish fed each of the treatment diets was determined by weight and length gain. The study revealed that red tilapias fed with tubifex worm showed significantly better growth performance than those fed with commercial diet and earthworm. The survival rates were 75.6 %, 55.6 % and 44.4 % among juvenile red tilapias on tubifex worm, commercial diet and earthworm respectively.

Keywords: *Oreochromis* sp., tubifex worm, earthworm, commercial diet, growth performance

EVALUATION OF SAFETY AND EFFICACY OF JAPANESE ENCEPHALITIS INACTIVATED VACCINE IN HORSES

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ABSTRACT

Japanese Encephalitis (JE) is a mosquitoe-born viral disease affecting human, pigs and horses in Asia. It causes stillbirths and abortions in pigs, and encephalitis in horses and man. In Malaysia, it is common practice to vaccinate all pigs and horses annually. University Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM), obtained their inactivated JE vaccine from a Japanese company (Nisseiken Co. Ltd.). However, the protective level of neutralising antibody produced by this vaccine is not known. Thus, the objective of this study was to evaluate the efficacy and safety of this vaccine in horses. Twenty-two healthy adult horses, with no history of vaccination against JE within the last 12 to 15 months were selected from two stables in Klang Valley, Malaysia. These stables had no history of JE incidence. Jugular blood was collected from these horses before they were vaccinated with 1 mL inactivated JE vaccine via intramuscular route. The horses were monitored daily for signs of side-effect or viral infection during the subsequent 10 days. The injection sites were also monitored for inflammation or allergic reaction. Blood was again sampled to obtain serum three weeks after vaccination. Serum was separated and frozen until use. All serum samples obtained before and after vaccination were analysed using an enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co., Ltd) to determine concentration of neutralising antibody. The results showed that the neutralising antibody concentration in serum before vaccination was not significantly different ($p>0.05$) from that after vaccination. Seroconversion rate was also low at 6.67 %. The result suggested that a single dose of inactivated JE vaccine is insufficient to produce desirable humoral response in the horse.

Keywords: Japanese encephalitis, virus, ELISA, vaccine, efficacy, safety, horse, pigs, neutralizing antibody.

IDENTIFICATION OF ECTOPARASITES AND DETECTION THEIR RICKETTSIAL ORGANISMS IN CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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ABSTRACT

Wild and captive elephants are commonly infested with external parasites. Amongst the most common ectoparasites are the lice (*Haematomyzus elephantis*), ticks (*Amblyoma tholloni*) and biting flies (*Cobboldia elephantis*). Presence of these ectoparasites normally do not cause a clinical disease however, they may be detrimental if present in a clinically ill elephant or even worst when they become vectors to rickettsial diseases. Many reports of ectoparasites in elephants have been published but they emphasized mainly on the therapy and not on the identification and the diseases they might carry. Therefore, this project aimed to investigate the prevalence of ectoparasites in Captive Asian elephants in Peninsular Malaysia as well as to carry out morphological identification of the ectoparasites and its potential as vector of rickettsial organisms. Out of 45 captive Asian elephants (*Elephas maximas*) examined from five zoological parks in Peninsular Malaysia, 12 were positive for ectoparasites (26 %). Based on the morphological features, the ectoparasites were identified as hair lice (*Haematomyzus elephantis)*. A total of 114 lice collected were examined for rickettsial organisms by polymerase chain reaction (PCR). Primers used were able to amplify the DNA of gltA genes of rickettsial organisms. Three of the 114 lice from a private zoo, were positive to rickettsial primers that yielded a 401 bp segment. DNA sequencing revealed the presence of *Rickettsia felis* in lice. In conclusion elephant hair lice could be a potential vector for *Rickettsia felis* and it may pose a public health threat to human.

Keywords: Asian elephant, *Haematomyzus elephantis, Rickettsia felis*

CHANGES IN MUSCULOSKELETAL PARAMETERS IN ENDURANCE HORSES ELIMINATED FROM RACES

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ABSTRACT

The endurance ride is gaining worldwide popularity as an equestrian sport. The purpose of this study was to determine change in musculoskeletal parameters and rate of elimination due to lameness during an 80 km endurance ride. The data on eliminated and completed horses collected from horse log book was according to year and race distance the horses participated. The elimination rate of horses in this study was 49 %. In eliminated horses, their muscle tone and gait differed significantly ($p<0.05$) between loops of race. The study showed that musculoskeletal parameters can used as a quick guide for the riders to decide on whether the horse is fit to continue with the race or to be withdrawn if the condition endangers the horse.

Keywords: horse, lameness, musculoskeletal parameters, endurance

EFFECT OF ESSENTIAL OIL OF *KAEMPFERIA PANDURATA* (SYN. *BOESENBERGIA PANDURATA)* ON BEHAVIOURAL MEASURES OF ANXIETY IN MICE

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ABSTRACT

Kaempferia pandurata (Syn. *Boesenbergia pandurata*) is a perennial herb of the family of *Zingiberaceae* and is known as "finger root" in China and "Temu Kunci" in Malaysia and Indonesia. The fresh rhizome of this plant is commonly used as food ingredient in cooking (as an appetite enhancer) and as aphrodisiac in folk medicine in Thailand, and also for the treatment of dysentery, as an anti-inflammatory agent, for the treatment of colic disorder and for promotion of health and wellbeing. In Malaysia, it is used as a stomachic and the decoction is given to women after childbirth. In Indonesia, it is used to relieve cough, treat thrush and enhance milk let down. Besides that, the essential oil of *Kaempferia pandurata* (KPEO) has a very strong and pleasant aroma similar to the orange or ginger-based oils. Although much research has been carried out to study the effects of the latter two oils on behavior and mood change in animals and humans (respectively), there was no scientific research to investigate the effect of inhalation of KPEO on emotion in man or behavioral effects in animals. Therefore, we investigated the effects of the essential oil of *Kaempferia pandurata* inhalation on behavioral measures of anxiety in mice. Thirty male ICR mice were randomly divided into 5 groups. Three treatment groups were exposed to KPEO inhalation at 1.0, 2.5 and 5.0 % w/w for 7 minutes while the negative control group was treated with 0.9 % NaCl (10 ml/kg) intraperitoneally and the positive control group was treated with diazepam (1.5 mg/kg intraperitoneally), 30 minutes before the behavioral tests. Three behavioral tests consisting of the open-field test, social interaction test and elevated plus-maze test were conducted. Results showed no significant effects of KPEO inhalation on the behavioral parameters, suggesting that there are no differences in the level of anxiety among the five treatment groups. This could indicate that KPEO did not activate receptors in the limbic system or release of inhibitory neurotransmitter to induce anxiolytic effect in mice. In conclusion, animals exposed to highest concentration of KPEO inhalation did not exhibit low levels of anxiety or high level of calmness and at this point of time, KPEO is not recommended as an agent for aromatherapy, either to induce sedation or relaxing effect in either humans or animals.

Keywords: *Kaempferia pandurata,* essential oil, anxiety

A SIX-YEAR TREND IN ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI, SALMONELLA* SP. AND *KLEBSIELLA* SP.

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ABSTRACT

This study was conducted to determine the trend of antibiotic resistance in *E. coli, Salmonella* sp. and *Klebsiella* sp. over a 6-year period (2007 – 2012). Results of antibiotic sensitivity tests for *E. coli, Salmonella* sp. and *Klebsiella* sp. were gathered from bacteriology laboratory log books of Faculty of Veterinary Medicine, Universiti Putra Malaysia. Data were categorized into species of animals, namely ruminant, avian, equine, porcine, small animal and wildlife and exotic pet. The pattern of microbial resistance against antibiotics was analysed according to species of animals. Resistance data of each bacterium towards tested antibiotics in each species were gathered throughout the 6-year period and the average resistance percentage was calculated to identify the most ineffective antibiotic to treat the bacterial infection in each species of animals. A total of 551 samples across all species were analysed. The study showed that there was an increased antibiotic resistance pattern for *E. coli* against forfenicol, amoxicillin/clavulanate, enrofloxacin and marbofloxacin. *Klebsiella* sp. showed an increased pattern of resistance towards amoxicillin/clavulanate and enrofloxacin. The most ineffective antibiotic in treating *E. coli* infection in ruminant, equine and wildlife and exotic pet was penicillin G with resistance of 10 0 %. *E. coli* isolates from avian were 100 % resistant against ampicillin. *E. coli* in small animals was most resistant to erythromycin. *E. coli* in porcine developed 100 % resistance towards streptomycin, erythromycin and ampicillin. The most ineffective antibiotic treating *Salmonella* sp. across all animal species was streptomycin. The most ineffective antibiotic to treat *Klebsiella* sp. infection in ruminant, equine and wildlife and exotic pet was penicillin G, avian was enrofloxacin, porcine was erythromycin and small animals was amoxicillin. The study revealed an increasing pattern of antibiotic resistance and penicillin G was the most ineffective antibiotic to treat *E. coli, Salmonella* sp. and *Klebsiella* sp. infection.

Keywords: antibiotic resistance, *E. coli, Salmonella* sp, *Klebsiella* sp.

EFFECT OF ESSENTIAL OIL OF *BOESENBERGIA PANDURATA* (SYN. *KAEMPFERIA PANDURATA*) ON SPATIAL AND NON-SPATIAL RECOGNITION MEMORY IN MICE

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ABSTRACT

Spatial recognition memory is a type of memory concerned with the location of object or place while non-spatial recognition memory is the memory which is not concerned with the location of the objects or items. In the Y-maze test, the learning and memory of mice will be evaluated based on the exploration behaviour of mice inside the maze, a parameter associated with novelty and memory of mice. *Kaempferia pandurata* (syn. *Boesenbergia pandurata*), belongs to the family Zingiberaceaea in Zingiberales order. This plant consists of leaves and flower but the common part of plant used in cooking and in folklore medicine are the rhizomes which contained 1 to 3 % essential oil. Based on previous studies, the rhizomes of the plant, known locally as *temu kunci*, exhibited analgesic, anti-inflammatory, anti-fungal, anti-bacterial effects and inhibited tumor growth. However, the effects of its essential oil on recognition memory have never been studied. Thus, the objective of this study is to evaluate the effects of *temu kunci* of the *Kaempferia pandurata* essential oil (KPEO) on spatial and non-spatial recognition in mice. In this study, 40 mice were randomly assigned to five (5) different groups (n=8) namely: 0.9 % normal saline (NS) control group (10 ml/kg, intraperitonelly), diazepam control group (1.5 mg/kg, intraperitoneally), and increasing dose of KPEO group at 30, 100 and 300 mg/kg, intraperitoneally. Each mouse was given an intraperitoneal injection of NS, diazepam or KPEO one hour prior to the training session. In spatial and non-spatial recognition memory test, each mouse was evaluated based on the percentage of total time spent and the frequency of novel arm or novel object exploration. Results from the behavioural tests demonstrated that TEKO did not induce significant improvement in the recognition memory of mice.

Keywords: *Kaempferia pandurata*, essential oil, Y-maze, spatial recognition memory, non-spatial recognition memory

IMMUNOHISTOCHEMICAL DETECTION OF *BRUCELLA MELITENSIS* ANTIGENS IN FORMALIN-FIXED TISSUES OF SMALL RUMINANTS

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ABSTRACT

Brucella melitensis is a Gram-negative facultative intracellular bacterium, the primary causative agent for caprine and ovine brucellosis and Malta fever in humans. The presence of *B. melitensis* antigen in 107 formalin-fixed, paraffin embedded lung tissue blocks of small ruminants were examined using immunohistochemistry method. Sample blocks from year 2007 to 2012 were collected from post-mortem archives in necropsy unit of Faculty of Veterinary Medicine, Universiti Putra Malaysia. Ten tissue samples with positive immunoreactivity of *B. melitensis* antigen were detected by immunohistochemistry method. The prevalence of presence of *B. melitensis* antigen in post-mortem cases for a six years period is 9.4 %. Statistical analysis revealed no breed, gender and age predilection among animals with positive immunoreactivity for *B. melitensis*.

Keywords: *Brucella melitensis*, small ruminants, immunohistochemistry

CLINICAL RESPONSE AND POLYMERASE CHAIN REACTION ANALYSIS IN MICE ORALLY INOCULATED WITH *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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ABSTRACT

Caseous lymphadenitis (CLA) is a chronic granulomatous infectious disease of sheep and goats that is characterized by abscessation of one or more lymph nodes, either superficial or visceral, that is caused by *Corynebacterium pseudotuberculosis* which is an intracellular gram-positive, facultative anaerobe small curved rod bacterium. However, the proper identification of the disease is based principally upon clinical signs or isolation of the agent from discharging abscesses. Little is known about pathogenesis of CLA in experimental animals particularly in a mouse model. Hence, this study was designed to observe clinical response and polymerase chain reaction (PCR) detection in mice following dose dependent infection of *C. pseudotuberculosis* via the oral route inoculation. Forty healthy mice were divided equally into 4 groups, where the first, second and third groups of mice were orally inoculated with 0.4 mL of 10^3, 10^5 and 10^7 colony form unit (cfu/mL) of *C. pseudotuberculosis*, and the fourth group of mice was orally inoculated with 0.4 mL of phosphate buffer solution (PBS), pH 7. Clinical signs were compared between the treatments and control group for the period of 10 days of post-inoculation. Seven organs were collected during post-mortem for detection of *C. pseudotuberculosis* using PCR. Results of this study for clinical signs showed no significant differences in all the treatment groups and control group. However, there were positive detection by PCR in all the samples taken especially in the intestine, stomach, heart and lung for all the doses used. For conclusion, the minimal dose of 10^3 (cfu/mL) was able to cause the disease via oral route inoculation in mice.

Keywords: *C. pseudotuberculosis*, mice, PCR, oral inoculation, different doses, clinical response

RETROSPECTIVE STUDY ON FELINE HIGH-RISE SYNDROME CASES PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

A retrospective study on feline high-rise syndrome (HRS) presented from year 2008 to 2012 was conducted. Records were obtained from University Veterinary Hospital, Universiti Putra Malaysia. The fall ranged from level 2 to level 15 with the highest incidence at level 4 (23 %) and the mean fall was from level 4.9. There was a correlation ($p<0.05$) between age and incidence of HRS in which in which <3 year old cats were more at risk of having HRS than older cats. There was no correlation ($p>0.05$) between gender and incidence of HRS. Limbs injury was the most frequently observed sequelae of HRS at 73 %; followed by orofacial injury (30 %), abdominal injury (16 %), thoracic injury (14 %), spinal injury (10 %) and shock (7 %). There were a correlation ($p<0.05$) between sustaining thoracic injury and orofacial injury in relation to level of fall. Chances of sustaining thoracic injury and orofacial injury increased with level of fall and peaked at level 5 and 6. There were also a correlation ($p<0.05$) between body weight and occurrence of limbs injury, abdominal injury and orofacial injury. Cats that were <3 kg were more at risk of sustaining limbs injury and orofacial injury, but less at risk of sustaining abdominal injury.

Keywords: high rise syndrome, feline, cat, limbs injury, thoracic injury, abdominal injury, orofacial injury, spinal injury, shock

NORMAL AEROBIC INTESTINAL FLORA OF CAPTIVE FALSE GHARIAL (*TOMISTOMA SCHLEGELII*)

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ABSTRACT

Tomistoma schlegelii, the "False Gharial", is one of the largest yet least-known of the world's 23 crocodilian species, restricted to Indonesia and Malaysia. They inhabit peat swamps in Southeast Asia. The threats affecting the species are mainly associated with habitat loss and illegal hunting which lead to difficulty in obtaining sample from this species. Bacterial cultures were performed on cloacal swabs collected from five captive False Gharials, captured at Taiping and National Zoos. Eleven species of Gram-negative bacteria from 22 isolates were obtained. Most of the genera belong to family Enterobacteriaceae and several had been implicated in cases of septicaemia in crocodilians. Each individual crocodiles yielded 1 to 4 bacterial species and no crocodile had a single species. The most commonly isolated bacteria were *Proteus vulgaris* and *Salmonella enteric* spp *arizonae*. Knowledge of the normal intestinal flora is a first step towards the development of crocodile-specific probiotic for use in farmed or captive crocodiles.

Keywords: False Gharial (*Tomistoma schlegelli*), intestinal flora, aerobic bacteria

SEROLOGICAL AND MOLECULAR PREVALENCE OF *EHRLICHIA CANIS* IN STRAY DOGS IN KUALA LUMPUR, MALAYSIA

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ABSTRACT

A molecular and serological prevalence study of *Ehrlichia canis* in stray dogs in Kuala Lumpur was carried out. Fifty-eight stray dogs from Dewan Bandaraya Kuala Lumpur (DBKL) dog pound were included in this study. PCR was carried out on both blood and splenic aspirate samples using *E.canis* species - specific primers, CANIS and GA1UR, which amplified a 409 bp fragment of the *E.canis* 16S rRNA gene. The serological prevalence of *E.canis* in stray dogs in Kuala Lumpur was determined using Biogal ImmunoComb® Canine Ehrlichia Antibody Test Kit. One out of fifty eight dogs (1.72 %) was tested positive for *E.canis* via PCR using blood, while none of the dogs were positive using the splenic aspirate. The total molecular prevalence of *E.canis* in stray dogs in Kuala Lumpur was 1.72 %. Whereas, 37 out of 58 dogs were seropositive using ImmunoComb® Canine Ehrlichia Antibody Test Kit. The serological prevalence was therefore 63.79 %. All of the dogs were negative for *E.canis* morulae under light microscopic examination. This is the first time splenic aspirate PCR to detect *E.canis* was attempted in Malaysia.

Keywords: *Ehrlichia canis*, splenic aspirate, PCR, Kuala Lumpur, molecular prevalence, serological prevalence, light microscopic examination

CRYOPRESERVATION OF GOAT SPERMATOZOA USING CITRATE EXTENDER CONTAINING OMEGA-3 EGG YOLK

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ABSTRACT

Egg yolk which contains lecithin and phospholipids is one of the most commonly used components of extenders to provide protection and nutrition to spermatozoa during cryopreservation. The aim of this study was to evaluate effects of different levels of omega-3 egg yolk (2.5, 5 and 10 %) in citrate extender and Bioxcell® as the standard extender, on goat semen cryopreservation. Semen from five adult goats was collected using artificial vagina. Semen collected was freshly evaluated before processed and extended using the four extenders. The extended semen was equilibrated by chilling at 5 ^{o}C for 3 h before packed into straws (0.25 mL), frozen and stored in cryogenic tank containing liquid nitrogen for 24 hours. Semen quality parameters assessed for comparison include: gross and progressive sperm motility determined by IVOS computer assisted semen analyzer, livability and morphological abnormality evaluated using eosin-nigrosin stain. The post-thaw quality of the semen was checked after 3 hours of chilling and 24 hours of freezing. The results of the experiment showed that 10 % egg yolk concentration provided the best result in cryopreservation of goat spermatozoa compared with 2.5 and 5 % egg yolk used. Moreover, the results obtained by using the 10 % egg yolk citrate extender were comparable with the commercial Bioxcell® and hence, it may be considered as cheaper alternative extender.

Keywords: goat, semen cryopreservation, omega-3 egg yolk, citrate extender, Bioxcell®

OCCURRENCE OF *SALMONELLA* AND MICROBIOLOGICAL PROFILE OF *HEMIDACTYLUS FRENATUS* FAECES

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ABSTRACT

The occurrence of *Salmonella* was determined in forty-five faeces/droppings of *Hemidactylus frenatus* (house geckos or lizards) as they are reported as one of the sources of *Salmonella* infection that causes gastroenteritis in human and animals. Twenty five faecal samples from house geckos were from human dwelling premises, eateries and water tanks around the Serdang areas in Selangor. These faeces were considered fresh since they were collected within 24 hours after excretion. Twenty dried lizard droppings of unknown age from various locations of housing premises and eateries were also sampled. Microbiological profile of the faeces was conducted. Each faecal sample underwent pre-enrichment and enrichment stages, followed by culture on Brilliant Green Agar (BGA) and Xylose Lysine Tergital 4 (XLT4) agar. Gram staining and routine biochemical tests were conducted to identify the bacteria, which includes Triple Sugar Iron, Sulfide Indole Motility, urease and citrate test. *Salmonella* was not detected in all the samples. The bacteria that could be isolated were *Klebsiella pneumoniae* (44.1 %), *Citrobacter freundii* (29.4 %), *Klebsiella oxytoca* (8.9 %), *Enterobacter cloacae* (5.9 %), *Staphylococcus* sp (5.9 %), *Bacillus* sp (2.9 %), and *Proteus penneri* (2.9 %). Antibiotic susceptibility test was carried out on predominant bacteria isolated which revealed antimicrobial resistance against amoxicillin and clavulanic acid and tetracycline. The occurrence of *Salmonella* in the faeces may be absent in the house geckos' faecal droppings in this study, however, these faeces still pose a risk to public health as they also contained opportunistic enteropathogens such as *Klebsiella pneumoniae* and *Citrobacter freundii*.

Keywords: house lizards (*Hemidactylusfrenatus*), Salmonella, enteropathogens, microbiological profile, faeces

SEROLOGICAL SURVEY OF ANTIBODY TITRES AGAINST CANINE DISTEMPER AND CANINE PARVOVIRUS IN VACCINATED WORKING DOGS

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ABSTRACT

Canine Distemper Virus (CDV) and Canine Parvovirus (CPV) infections have been known to cause high morbidity and mortality rate, and hence it is vital to evaluate the immune response following vaccination to assess if the vaccination conferred protection to the dogs. In Malaysia, dogs are routinely vaccinated yearly, however the response and antibody titre production following vaccination are often not documented. This preliminary study was conducted with the aim to determine the proportion of vaccinated dogs that will be tested positive for antibody against CDV and CPV and to determine if gender, age and breed have an effect on the immunity against CDV and CPV. Eighty eight working dogs were sampled from various governmental institutions, namely Jabatan Bomba dan Penyelamat Malaysia, Jabatan Kastam Diraja Malaysia, Jabatan Penjara Malaysia and the Polis Diraja Malaysia. Working dogs were selected for this study because these dogs were subjected to a defined health and preventive medicine programme since birth which allows for consistent monitoring compared to shelter or pet animal populations. Serum and plasma samples were collected and were subjected to TITERCHEK™CDV/CPV test kit. From this study, 61 % of vaccinated working dogs have retained sufficient antibody after the last vaccination and had sufficient antibody titres correlated well with protection against CDV and CPV and only a small proportion, 4 % of these dogs had insufficient titres to meet the cut off titres. This study supports previous findings that gender, age and breed do not influent the immunity against CDV and CPV. In conclusion, majority of the dogs with history of vaccination have had sufficient antibody titres to be considered protected from both viral diseases. However those dogs that did not have sufficient antibody titres do not equate to susceptibility to these infections, as presence of memory cells offers anamnestic response in subsequent challenge. Only dogs with zero titres, which could be detected by standard methods (Serum Neutralization Test for CDV and Hemaglutinin Inhibition test for CPV) are reckoned to be susceptible to infections and need revaccination.

Keywords: distemper, parvovirus, antibody, working dogs, TITERCHEK™

COMPARISON BETWEEN OVARIOHYSTERECTOMY AND OVARIECTOMY IN NEUTERING RABBITS

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ABSTRACT

With the growing popularity of rabbits as pets in Malaysia, there is a need to control their population through spaying. Two methods of spaying are ovariohysterectomy (OHE) and ovariectomy (OVE). Currently, no studies have been conducted to investigate the advantages of these two techniques of spaying rabbits. This study was a prospective experimental design which was conducted to compare the short term benefits between the two procedures but was limited to comparisons made during surgical procedure (estimated blood loss and occurrence of complications), cost, duration of surgery, post-operative behavioural pain and post-operative physiological pain. The objective of the study was to identify the best method that would benefit four parties, namely the animal, pet owner, veterinarian and researcher by providing information regarding both surgical techniques. Ten New Zealand White rabbits of which all were 6 months old, weighing between 2.0 kg to 2.5 kg were randomly allocated into in two groups namely OHE and OVE with 5 animals in each group. The results revealed that 1 out of the 5 subjects in the OHE group had complications while none occurred in the OVE group. Mean estimated blood loss in OHE group was 1.75 times higher than that in the OVE group. On the other hand, mean duration of surgery and mean total costs in the OHE group were 1.15 times higher than the OVE group. However, statistically, there were no significant differences for these four parameters measured. In addition, the post-operative behavioural pain and necessity to add analgesia 21 hours post-surgery also showed no significant disparity between the two groups. Meanwhile, physiological pain by measurement of serum cortisol level 20 hours post-operatively from the baseline showed that the increase in the OHE group was 2.62 times higher than the OVE group. Decrease in body weight post-operation for 7 days in the OHE group was also more significant when compared to the OVE group. Hence, from this study, it can be concluded that OHE is better than OVE in terms of short term advantages.

Keywords: spaying, population control, ovariohysterectomy (OHE), ovariectomy (OVE)

EFFECTIVENESS OF FIPRONIL FOR FLEA CONTROL IN CATS

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ABSTRACT

The main objective of this study was to determine the effectiveness of different dosages of fipronil for flea control in cats. An experiment was conducted in 12 cats of various breeds which were divided into three groups namely control group, 3 mL/kg fipronil-treated group and 6 mL/kg fipronil-treated group. Flea eggs were collected before treatment on day 0. Subsequently, egg collections were repeated on day 1, 3, 5, 7, 10, 13, 16, 19, 22, 25, 28 and day 30 post-treatment. Flea eggs count of each collection determined the percentage of flea control on the particular day. The results showed that there was no statistical significant difference between both treatment groups. In addition, both groups took a longer duration to achieve 100 % flea control post-treatment and showed earlier reinfestation of fleas compared to what the product claims. However, neither biochemical nor molecular assays wer conducted to confirm fipronil resistance in the fleas. In conclusion, fipronil showed a lower percentage of flea control and thus lower effectiveness in flea prevention as compared to previous studies and according to the product claims.

Keywords: effectiveness, fipronil, fleas, flea eggs, resistance

EFFECT OF ASCORBIC ACID IN ABATING PATHOGENICITY OF BOID INCLUSION BODY DISEASE VIRUS IN A MURINE MODEL

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ABSTRACT

This study is aimed at assessing the efficacy of ascorbic acid (vitamin C) in abating the pathogenicity of the elusive boid inclusion body disease (IBD) virus. Fifteen adult ICR mice were divided into five groups comprising of three animals each, viz; control (A), vitamin C only (B), virus only (C), prophylactic i.e., vitamin C then virus inoculation (D) and therapeutic, i.e., virus then vitamin C, two weeks post-inoculation (E). Virus and vitamin C was given at a dose of 1×10^{-6} pfu and 250 mg/kg body weight, respectively via the intraperitoneal route. Mice from groups C and D were euthanised on day 7 and the remaining groups were euthanised on day 14 post-inoculation. Standard neurological test were done on each remaining groups on days 7 and 14. Prior to euthanasia, blood sample was collected via intracardiac puncture for haematological and selected plasma biochemical analyses. Six organs namely spleen, brain, lungs, kidneys, small intestines and liver were taken for virus isolation and histopathological examination to verify Koch's postulate. Only a mild degree of leucopaenia was seen in mice from groups C and D, while those from group C had elevated ($p<0.05$) plasma ALT concentrations. Koch's postulate was verified since virus was present in all organs of the virus-treated group. Histopathological examination revealed varying degrees of on-going inflammation in the kidneys, liver and lungs in these groups. However, the changes seen in the vitamin C-treated groups were milder ($p<0.05$) than those of the untreated groups. In conclusion, ascorbic acid has potential abating effect on the pathogenicity of IBD virus.

Keywords: inclusion body disease virus, ascorbic acid, pathogenicity, murine

ENDOPARASITES AND ECTOPARASITES IN HAMSTERS IN SELANGOR, MALAYSIA

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ABSTRACT

In Malaysia, hamsters are becoming popular as pets beside cats and dogs especially among kids. Small and cute in appearance, it attracts people to keep them as pet. Besides that, hamsters are also being used in research. Just like other animals, they also have the potential to carry diseases that can be transmitted to human such as salmonellosis and hookworm infestation (Hymenolepiasis). The objectives of this study were to identify endoparasites and ectoparasites in hamsters from laboratory animal facility and pet shop in Selangor and to determine the potential zoonoses of the parasites, its public health impact and influence in research. Ten hamsters from two laboratory animal facilities and fifteen hamsters from three pet shops were selected in this project. Fecal and hair samples were taken to identify endoparasites and ectoprasites. Then, three hamsters were randomly selected as representative from each group and euthanized by carbon dioxide inhalation method. Necropsy on animals was done to remove intestines for detection of more endoparasites. Results showed that most hamsters were positive for both endoparasites and ectoparasites. Hamsters from pet shops were proven to harbour more ectoparasites and endoparasites compared to hamsters from laboratory animal facility. However none of the parasites were zoonotic. Based on this perspective, hamsters are safe to be kept as pet. However, the presence of those parasites in hamsters cannot be disregarded in animals intended for scientific research.

Keywords: hamsters, endoparasites, ectoparasites, zoonotic

ASSESSMENT OF STRESS LEVEL IN THOROUGHBRED FOALS UNDER MALAYSIAN CLIMATIC CONDITION

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ABSTRACT

Stress is defined as any negative condition that can cause changes either physically or mentally. This negative condition usually will lead to negative effects but at certain level it could actually bring positive desire effects. Most of the time, researchers will use cortisol level as an indicator of stress. This adrenal cortex release hormone can be detected from serum, plasma, urine and even saliva. Higher concentration of this hormone suggests that the animal is under stress. However, other parameters such as heart rate and respiratory rate, which will increase when blood cortisol level increased, can be used as stress indicator as well. In this study, one of the main interests on effect of stress towards physiological changes in yearling foals is to look on their growth rate. We believe that higher concentration of cortisol beyond normal level can retard normal growth of foal. The study was carried out using saliva to determine the cortisol level. Sampling saliva from foal will cause less stress to them hence will less likely to alter the actual cortisol concentration. Throughout the study, saliva was collected twice a day for a period of 1 month to avoid interpretation of result affected by the circadian cycle of cortisol release. Results showed that there are differences in stress level between morning and afternoon where the environmental temperature and the relative humidity are different respectively. However, statistical analysis revealed that correlation between growth rate and cortisol level is not significant ($p>0.05$) within the tested group even though the cortisol level difference between foal with highest growth rate and foal with lowest growth rate are practically obvious.

Keywords: Thoroughbred horse, stress, saliva, cortisol, growth rate

EXPRESSION OF 24-α-HYDROXYLASE AND ITS ASSOCIATION WITH CLINICAL PARAMETERS AND PROLIFERATING CELL NUCLEAR ANTIGEN IN CANINE MAMMARY GLAND TUMOURS

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ABSTRACT

The active form of Vitamin D (1, 25 Dihydroxyvitamin D3), has potential anti-tumour properties. 24-α-hydroxylase (CYP24A1) is responsible for the degradation of Vitamin D, thus limiting the anti-tumour properties at cellular level. Overexpression of CYP24A1 has been reported in several types of human cancer but only one report showed high expression in canine osteosarcoma that was associated with poor prognosis. Reports on CYP24A1 expression were associated with a more aggressive phenotype of cancer and it was suggested that inhibition of expression of this marker will induce apoptosis in cancer cells within the Vitamin D pathway. The aim of this study was to determine expression of CYP24A1 using immunohistochemistry in canine mammary gland tumours and its association with prognosis. Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue of mammary gland tumour (n=46), normal mammary gland (n=1), and metastasised tissues (n=6). Tissue of normal canine liver was included as positive control. CYP24A1 is expressed in 70 % of primary mammary tumours (adenocarcinoma, n=24 and squamous cell carcinoma, n=4) where the expression were all within the cytoplasm. A kidney metastasised tissue showed positive expression of CYP24A1 in the cytoplasm and nucleus. There was no significant association of CYP24A1 with age, breed, sex, neuter status, number of glands involved or histopathology status (proliferating cell nuclear antigen positive, histological subtypes). Kaplan-Meier survival analysis suggested, although not conclusive (p=0.146), that high CYP24A1 expression may be a prediction for poor survival (log rank test score 2.117). However, higher expression of CYP24A1 in small sized dogs (body weight < 15kg) predicts a poorer clinical course of disease, with a significantly shorter survival time (p=0.033, log rank test score 4.567). In conclusion, this is the first study to describe the CYP24A1 expression in canine mammary gland tumours where the expression is present in the majority of the tumours. The expression was prognostic for small sized dogs but was not a useful prognosticator in the general population of study; however, the expression alone may be useful to segregate dogs that may benefit from an interventional therapy against CYP24A1.

Keywords: 24-α-hydroxylase, mammary tumour, prognosis, survival, dog

EFFECT OF BIOCLEAN™ ON POST-HARVEST QUALITY OF WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) IN A FARM IN NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

This study aimed to evaluate the effects of Bioclean™, an organic food additive, composed of ascorbic, lactic and citric acid, on post-harvest quality of white shrimp (*Litopenaeus vannamei*). Standard plate count (SPC), coliform plate count (CPC) and organoleptic characteristics were used to assess the quality of shrimps. Three groups of white shrimp each weighing 1.5 kg was allocated into control, 500 ppm and 1000 ppm Bioclean™ treatment groups. The SPC and CPC were determined at day 0, 2, 4 and 6. Organoleptic assessments of raw and cooked shrimps were conducted at day 0, 2, 4, 6 and at day 0, 2, 4, respectively. Shrimps treated with Bioclean™ showed a significant reduction ($p<0.05$) in SPC and CPC when compared with the control, with 1000 ppm performing better than 500 ppm. However by day 6, the SPC of all groups exceeded 10 cfu/g limit recommended by International Commission for the Microbiological Speficification of Foods (ICMSF). Besides, CPC of all groups exceeded 10 cfu/g limit recommended by ICMSF on day 0 itself, indicating a high level of contamination of the shrimp rearing environment. As for organoleptic scores, there appears to be a significant bias among panelists ($p<0.05$), where treatment scores were grossly better in three parameters namely odour, appearance and aroma, when compared with the control. However, there was no significant difference ($p>0.05$) in the overall organoleptic scores between control, 500 ppm and 1000 ppm treatment groups. In conclusion, we could propose that a longer dipping time or a higher concentration of Bioclean™ at 1000 ppm or more could be considered to improve the post-harvest quality of white shrimp.

Keywords: Bioclean™, post-harvest quality, *Litopenaeus vanname*, Negeri Sembilan

IMMUNOHISTOCHEMICAL STUDY OF CD44, A PUTATIVE CANCER STEM CELL MARKER AND ITS CO-EXPRESSION WITH PROLIFERATING CELL NUCLEAR ANTIGEN IN CANINE AND FELINE SQUAMOUS CELL CARCINOMA

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ABSTRACT

Squamous cell carcinoma (SCC) is a malignant and locally invasive skin tumour of dogs and cats with poor sensitivity towards chemotherapy. CD44 expression was found to be a cancer stem cell marker and poor prognosticator for human SCC but this information is not available for the canine and feline SCC. The prognostic value of proliferating cell nuclear antigen (PCNA) in canine and feline SCC remained equivocal. The current study was designed to evaluate the prognostic value and clinicopathological association of CD44 and PCNA expression in canine and feline SCC. Fifteen canine and nine feline well-differentiated SCC paraffin embedded tissues were investigated for CD44 expression using immunohistochemistry. PCNA index was determined and CD44 expression was assessed by two individual assessors who were blinded from the clinicopathological data. Canine SCC (53.3 %) and feline SCC (55.6 %) were found to express CD44 protein. Fisher's Exact Test showed that there was no significant association between age, breed, sex, neuter status and tumour location with PCNA or CD44 expression in canine and feline tumours. Kaplan-Meier survival analysis also showed that there was no association between PCNA and survival time in canine SCC. However, there was a significant association between CD44 protein expression with survival among short surviving (< 365 days) dogs with SCC. Survival analysis was not performed on feline SCC due to poor clinical follow up. These results indicate that PCNA is not a suitable prognosticator for canine SCC and CD44 is a reliable prognosticator for a subset of canine SCC. Prognostic value of CD44 and PCNA in feline SCC was not able to be assessed in the present study because of the lack of clinical outcome data and follow up.

Keywords: prognosticator, dog, cat, CD44, PCNA, immunohistochemistry

SPRAY VACCINATION OF 18-DAY-OLD EMBRYONATED EGGS AGAINST INFECTIOUS BURSAL DISEASE IN BROILER CHICKENS

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ABSTRACT

Infectious bursal disease (IBD) is a contagious viral disease characterised by high mortality and immunosuppression that causes severe economical losses to the poultry industry. Control of IBD is mainly by practicing good biosecurity and vaccination programme. This study was conducted to determine the clinical signs, lesions and antibody titre of broiler chickens vaccinated against IBD through spray vaccination on embryonated eggs. Eighteen-day-old embyonated commercial chicken eggs were divided into control and vaccinated groups. The eggs in the vaccinated group were sprayed with an 'intermediate" strain of live attenuated IBD vaccine (10^3 EID_{50}/1.0 mL per egg). No treatment was given to the control group. Chicks that hatched were kept according to their group where feed and water were provided *ad libitium.* Five chicks each from the vaccinated and control groups were sacrificed on days 1, 7, 14 and 21. Before sacrificing, the body weight was recorded and serum samples were collected to determine the antibody titre using enzyme-linked immunosorbent assays (ELISA). On necropsy, gross lesions were observed and weight of bursa of Fabricius was recorded. The bursa was fixed in 10 % buffered formalin for histopathological examination. The study showed no difference ($p>0.05$) in body and bursal weight between the groups. There were also no differences ($p>0.05$) in bursal to body weight ratio except on day 1: the ratio was significantly lower ($p<0.05$) in the vaccinated group (0.0004 ± 0.0002) when compared to the control group (0.0013 ± 0.0002). The bursal lesions in both groups remained normal and mild, and no significant differences were recorded throughout the study. IBD antibody titre in vaccinated group (4095 ± 43) was significantly lower ($p<0.05$) than control group (9845 ± 28) on day 1 of age. However, the titre reduced in both groups ($p>0.05$) thereafter throughout the trial. It was concluded that spray vaccination on 18-day-old embryonated commercial chicken eggs was ineffective to induce of lesions in the bursa of Fabricius and IBD antibody titre. This could be due to the neutralisation of vaccine virus by high maternal antibody present in the chickens.

Keywords: Infectious Bursal Disease (IBD), spray vaccination, 18-day-old embryonated commercial broiler chicken egg, IBD antibody titre, maternal antibody

EVALUATION OF REPRODUCTIVE PERFORMANCE OF HOLSTEIN-FRIESIAN CATTLE IN SABAH, MALAYSIA

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ABSTRACT

The reproductive performances of Holstein-Friesian cattle in Desa Dairy Farm in Sabah, Malaysia were evaluated. Fifty cows from a population of 244 animals were selected and analysed for the pregnancy rate, calving interval and calving rate. These performance indices were then compared with published benchmarks for each index (pregnancy rate, 70 to 80 %; calving rate, 95.6 %; and calving interval, 360 to 390 days). This farm had recorded higher pregnancy rate of 88 %, lower calving rate of 91 %, and longer calving interval of 399 days compared to the benchmarks. The findings suggest that the reproductive performance of the farm studied could be related to the environmental temperature and farm management practices.

Keywords: Holstein-Friesian cattle, reproductive performance, pregnancy rate, calving rate, calving interval

DETECTION OF BLOOD PARASITES IN WATERBIRDS

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ABSTRACT

Blood parasites have the potential to infect various groups of avifauna. The prevalence might differ greatly among the different group and geographical distribution of the birds. This study was conducted to determine the occurrence of blood parasites in introduced water birds kept under Malaysia settings. Sixty one water birds consisting of 10 different species [Egyptian goose (18), Canada goose (4), Black swan (7), Mute swan (1), Greater flamingo (5), White pelican (2), Pink-backed pelican, Mandarin duck (4), Radjah shelduck (4) and domesticated Peking duck (14)] were sampled in their ground in Taman Wetland Putrajaya and were studied qualitatively for blood parasites. Thirty (49.2 %) of the sixty one birds in the study were infected by single infection with *Plasmodium* sp. and only three (5 %) of the total infected birds harbored double infection of *Plasmodium* sp. and *Hemaproteus* sp. Most species studied were infected with blood parasites except for negative detection in Radjah shelduck (*Tadorna radjah*). There was no evidence of *Trypanasoma, Leucocytozoon* and microfilaria infection in the birds studied. The study revealed that blood parasites could be found in introduced waterbirds in Malaysia settings. For this reason, exotic species should be screened for blood parasites before being introduced into chosen ground to prevent unintentional introduction of new strain of parasites that may be highly pathogenic to our local species or *vice versa*.

Key words: blood parasites, water birds, *Hemaproteus, Plasmodium*

ISOLATION AND CHARACTERISATION OF *ESCHERICHIA COLI* ISOLATED FROM BROILER CHICKEN WITH COMPLICATED CHRONIC RESPIRATORY DISEASE

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ABSTRACT

Complicated chronic respiratory disease (CCRD) caused by avian pathogenic *Escherichia coli* (APEC) is the main cause of a significant number of carcasses condemnation and economic losses of the poultry industry. The objective of this study was to isolate and identify *Escherichia coli* from broiler chicken with CCRD and to characterise the isolates for APEC. Post-mortem was performed on dead and live 23 and 25 day-old chicken from two farms for sampling. Clinical signs of respiratory distress, lethargy, distended abdomen, ruffled feather were observed in the affected chickens. On necropsy, pericarditis, perihepatitis, airsaculitis, conjunctivitis and generalized congestion of organs were recorded. Twenty samples included liver (7), heart (5), lung (1) and eye swabs (7) were taken for bacterial isolation and identification. Thirteen out of 20 samples (65 %) of the samples were positive for *E. coli*. Multiplex polymerase chain reaction was conducted to the *E.* coli isolates. The virulence genes selected for genotyping were *astA, iss, irp2, iucD, papC, tsh*, *vat* and *cva/cvi.* Nine out of 13 samples (69 %) collected were APEC. Six, five and four virulence genes among eight virulence genes were isolated from one (11 %), three (33 %) and five (56 %) samples collected, respectively. Multiplex polymerase chain reaction showed various distribution of virulence genes between the isolates collected from the same or different farms. Among the eight virulence genes, *iucD* was the highest genes isolated from the samples collected. Liver and heart samples were the ideal organs for APEC isolation.

Keywords: Complicated chronic respiratory disease (CCRD), avian pathogenic *Escherichia coli* (APEC), virulence genes, multiplex polymerase chain reaction, broiler chickens.

EFFECT OF *MORINDA CITRIFOLIA* FRUIT ON GLUTATHIONE PEROXIDASE ACTIVITY AND REDUCTION OF DEXAMETHASONE EFFECT IN RABBITS

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ABSTRACT

Rabbits are easily stressed due to their nature of being preys to other animals. Stress causes a stimulation of the release of adrenocorticotrophic hormone from the pituitary gland that stimulates the adrenal gland to release cortisol which causes many changes in the body like heterophilia and lymphopenia. Lymphopenia contributes to the immunosuppression subsequently causing the animal to be easily infected. In this study, pelleted *Morinda ctirifolia* fruits are used to determine their effects on rabbit health and to determine if *Morinda citrifolia* fruit can aid against harmful effects of stress. Animals were caged individually throughout the whole experiment. Pelleted *Morinda citrifolia* fruits were given for 25 days with dosage of 3 g/kg and dexamethasone injection was administered on day 27 and the protocol used was 2 mg/kg dexamethasone intramuscularly at 8 hours interval for three injections. The results revealed that there was an increase in the level of gluthatione peroxidase activity in the rabbits after they were fed with pelleted *Morinda citrifolia* fruits. On the other hand, once administered with dexamethasone, pelleted *Morinda citrifolia* fruits did not aid to reduce the effects of dexamethasone such as heterophilia, lymphopenia and hyperglycaemia.

Keywords: rabbits, *Morinda citrifolia*, stress, glutathione peroxidase, stress leukogram

EFFECT OF ENCLOSURE SIZE AND PRESENCE OF HUMAN VISITORS ON DIURNAL ACTIVITY OF CAPTIVE JUVENILE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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ABSTRACT

The present study was undertaken with six captive juvenile Asian elephants at the National Elephant Conservation Centre in Kuala Gandah, Pahang Darul Makmur, Malaysia, over a period of 21 days. Diurnal activities of the juvenile Asian elephants under two alternating housing types were assessed by instantaneous scan sampling method at an interval of 10 minutes in order to establish the impact of human visitors' presence on the elephants' behaviour. The relationship between the incidence of abnormal behaviours and time spent in captivity was also investigated. Daily behavioural data collection commenced at 0900 hours and terminated 8 hours later, at 1700 hours. Observations were conducted between 0900 and 1350 hours while the individuals were in the paddocks and between 1400 and 1700 hours, when the elephants were housed in their respective pens. Captive juvenile Asian elephants tend to display more maintenance, locomotive, vocalisation, reproductive and investigative behaviours in the large, enriched paddock while abnormal behaviours were more prominent in small, barren pens. The incidence of total abnormal behaviour was significantly higher with the decrease in the presence of visitors to the two enclosure types. Conversely, begging peaked when there was the number of visitors coming in contact with the elephants increased. The incidence begging was significantly higher in the pens (29.46 ± 6.18 %) compared to that recorded in the paddock (7.78 ± 6.14 %). This study concluded that the relationship between the frequency of total abnormal behaviour expressed and time spent in captivity was not statistically significant ($p>0.05$), therefore the duration in captivity did not inversely contribute to the mean frequency of abnormal behaviours expressed by the juvenile Asian elephants.

Keywords: Asian elephants, *Elephas maximus*, captive, juvenile, enclosure type, uman contact

OCCURRENCE OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* AND *SALMONELLA* SP IN CATS AFTER HOSPITALISATION

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ABSTRACT

In recent years, the number of pets has increased substantially, thus the use of antibiotics in pets has also increased. Pets treated with antibiotics may contribute to the rise of resistance of bacteria which is now an emerging phenomenon worldwide. Several longitudinal studies that were conducted at different veterinary hospitals have indicated that resistance to various antimicrobial agents has emerged. This study was conducted to determine the occurrence of antibiotic resistant bacteria in cats before and after hospitalisation at the University Veterinary Hospital, Universiti Putra Malaysia. Twenty-two hospitalised cats were sampled in this study. Two faecal swabs were taken for the isolation of *E. coli* and *Salmonella sp.* A series of biochemical test was done for identification of *E.coli* and *Salmonella sp.* The cultures were then subjected to the Kirby Bauer antibiotic sensitivity test. A total of 98 *E. coli* were isolated while no *Salmonella sp* were obtained. The results showed that only 2 % of the *E. coli* isolates were sensitive to all the antibiotics tested. Multiple antibiotic resistance of *E. coli* has increased from 66 to 71 % from the first to the last day, regardless of period of hospitalisation. There were significant differences in resistance to antimicrobial agents between before and after hospitalization. Multiple antibiotic resistances occurred as early as 4 days after hospitalisation.

Keywords: antibiotic resistance, cats, *E. coli, Salmonella sp.*

PLASMA CORTISOL LEVEL IN HORSES BEFORE AND AFTER SURGICAL PROCEDURES COMMONLY PERFORMED IN MALAYSIA

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ABSTRACT

Stress response due to surgery is one of the major concerns of the owner before making the decision for a surgical treatment. Prolonged stress has the detrimental effects of excessive protein breakdown with development of negative nitrogen balance as well as weight loss. Hence, this study was conducted to assess and determine the stress response due to different surgical procedures to result in utmost satisfying outcome. The surgery cases documented in Perak Royal Turf Club from the year 2008 to 2012 were put into different categories and the prevalence of each was then determined. Ten horses presented for various surgical treatments were included in this study and blood samples were taken before and after surgery to assess the plasma cortisol level using radioimmunoassay. The common surgical procedures in Malaysia included tie-back with/without ventriculocordectomy, arthroscopy and castration represented by 27, 26 and 22 % respectively. Arthroscopic surgeries had a non-significant higher magnitude of plasma cortisol increase compared to respiratory surgeries ($p=0.055$). Inhalant anaesthesia induced non-significant higher stress response in horses compared to total intravenous anaesthesia ($p=0.519$). Plasma cortisol level was increased due to surgery, especially in arthroscopic surgery where there was significant increase ($p=0.046$).

Keywords: stress, plasma, cortisol, surgery, horse

ASSESSMENT OF BACTERIOLOGY AND ANTIBIOTIC RESISTANCE OF MILK SAMPLES FROM SELANGOR *LADANG ANGKAT* DAIRY FARMS OF UNIVERSITI VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Milk is an optimum medium for bacterial growth of many microorganisms such as *Lactobacillus, Staphylococcus, Streptococcus, Escherichia, Bacillus, Salmonella, Pseudomonas, Corynebacterium, Klebsiella and Micrococcus sp.* which contribute to cases of subclinical and clinical mastitis in dairy cows. The indiscriminate use of antibiotics has led to the development of multi-drug resistant bacteria. This study was conducted to determine the prevalence of subclinical mastitis cases and determine the prevalence of antibiotic resistance for the most prevalent bacteria isolated through milk culture. Each quarter samples was tested with the California Mastitis Test (CMT). Milk samples were cultured and selected isolates were then tested for antimicrobial resistance. The prevalence of subclinical mastitis was 86.7 % out of 30 cows' quarter samples from three farms around Selangor. The most prevalent bacteria was *Staphylococcus sp.* (55 %), *Bacillus sp.* (21 %), *Corynebacterium sp.* (7 %), and others at below 5 % such as *Yersinia sp., Neisseria sp., Acinetobacter sp., Actinobacillus sp., Vibrio sp., Pseudomonas sp., E. coli, Klebsiella sp.,* and *Chromobacter sp*. Eighty three percent (83 %) of bacteria found in subclinical cases were from the Gram-positive group of bacteria. Selected *Staphylococcus sp.* showed 73.3 % resistance to Ampicillin, followed by 33.3 % to Compound Sulphonamide, 26.7 % to Methicillin and Penicillin, 20 % to Oxacillin, Amoxycillin, and Cefuroxime, 13.3 % to Polymyxin B, Erythromycin, Ceftriaxone, Azithromycin, and Tetracycline, and lastly 6.7 % to Streptomycin, Clindamycin and Lincomycin. However, *Staphylococcus sp*. showed the most resistance towards β-lactam group of antibiotics. This study indicated that more effective control measures are needed because there seemed to be an increase in the prevalence of subclinical mastitis cases along with antimicrobial resistance which is alarming.

Keywords: raw milk, subclinical mastitis, antibiotic resistance

DETERMINATION OF ESTROUS CYCLE LENGTH IN MALAYAN PORCUPINE (*HYSTRIX BRACHYURA)*

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ABSTRACT

The objective of the present study was to determine the estrous cycle length and to correlate the vaginal epithelial cells with plasma progesterone concentrations during the 32 days of sample collection in the female Malayan porcupines (*Hystrixbrachyura).* Ten healthy, adult Malayan porcupines with an average weight of 10.6 ± 1.6 kg and history of second and third parities were used in this study. Each female porcupine was placed in pairs with active males and was managed in semi-concrete and steel cells on smooth cemented flooring. For the determination of the estrous cycle length, blood samples and vaginal epithelial cells were collected twice weekly for 5 consecutive weeks. External genital appearances were inspected for vulva color, tumefaction of vulva and vaginal opening for signs of estrus during the vaginal epithelial cells collection. The mean duration of the estrous cycle length was estimated as being 18 days (n=8) in the Malayan porcupine. Circulating concentrations of progesterone during the estrous cycle varied from 0.2 to 8.8 ng/mL, with a mean of 2.2 ± 1.9 ng/mL and varied between females with a coefficient variance of 23.8 %. In vaginal cytology, the cellular morphology and staining properties were similar to those reported previously in the bitch. Vaginal epithelial cells were smeared onto a glass slide and stained with 10 % of Giemsa stain for 25 minutes. Cornified cells especially the enucleated superficial cells were predominantly (≥ 90 %) found during the estrus stage concurrent with reddish-pink vulva, tumefacted vulva, and full vaginal opening indicating the onset of estrus. Animals in diestrus or in the luteal phase indicates a mixture of parabasal, intermediate cells, superficial cells and abundant of leucocytes with purplish-pink to pale vulva color, non-tumefacted vulva and either with partial, collapsed or vaginal closure membrane. Plasma progesterone and superficial cells were significantly negatively correlated. Plasma progesterone was significantly positively correlated with color and swelling of vulva but not significantly correlated with vaginal opening. Superficial cells showed a significant negative correlation with color and swelling of vulva as well as the vaginal opening. Based on this pattern of association the superficial cells can be used as a useful indicator for estrus. In conclusion, it is suggested that plasma progesterone radioimmunoassay and vaginal cytology could be used as effective tools in improving the reproductive programs for the Malayan porcupine.

Keywords: estrous cycle, plasma progesterone, superficial cells, Malayan porcupine

GROSS AND HISTOPATHOLOGICAL CHANGES IN MICE ORALLY INOCULATED WITH *PASTEURELLA MULTOCIDA* TYPE B

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ABSTRACT

Haemorrhagic septicemia (HS) is an important disease of cattle and buffaloes, especially in Asian countries, including Malaysia, where cattle and buffaloes are abundant. This disease is caused by the *Pasteurella multocida* Type B and Type E. There were few studies of HS using mice as an animal model. This study described the gross and histopathological changes on organs of mice following oral route of inoculation with different doses of *Pasteurella multocida* Type B. Thirty two healthy mice were divided equally into four groups, where each group consist of 8 mice. Groups 1, 2 and 3 mice were inoculated orally with 0.4 mL of *Pasteurealla multocida* type B, but with different doses, which is 10^3, 10^5 and 10^7 colony forming unit (cfu), respectively. Group 4 was set as a control and was inoculated with 0.4 mL of phosphate buffer saline. All mice were observed for 10 days post-inoculation, and mice that showed severe clinical signs were euthanized. Surviving mice were also euthanised at day-10 post-inoculation. All mice were subjected for necropsy. Various organs collected and examined for gross lesions and histopathology. Histopathology lesions of each organ were scored and analysed using SPSS version 20. The results showed there was no significant different ($p<0.05$) in severity of lesions between any inoculated groups. However, lesion scores of inoculated groups were significantly different from the control group.

Keywords: Haemorrhagic septicemia, *Pasteurella multocida* Type B, mice, histopathology lesions.

EFFICACY OF A COMMERCIAL VACCINE AGAINST CASEOUS LYMPHADENITIS IN GOATS

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ABSTRACT

Caseous lymphadenitis (CLA) is an important emerging disease among goats in Malaysia. The disease has been observed following importation of goats and efforts must be made to control the disease. The aim of this study was to determine the efficacy of a commercially available vaccine [Glanvac® 6 Vaccine; Pfizer, Australia] in protecting goats against challenge by *Corynebacterium pseudotuberculosis*. The goats were divided to 3 groups based on their serological status against CLA and consisted of 10 goats per group. Goats of group 1 (seropositive) and group 2 (seronegative) were vaccinated with Glanvac® 6 vaccine while goats of group 3 (seronegative) remained as a control unvaccinated group. One month after the booster dose, all goats were challenged intradermally with 1 ml of the inoculum containing 10^9 cfu/ml of live *C. pseudotuberculosis*. On day 30 post-infection, surviving goats were killed and post-mortem examination was carried out immediately. The submandibular, prescapular, inguinal and mesenteric lymph nodes were collected for gross lesion, histopathological examination and bacteriological isolation. Approximately 30 % of goats of group 2 and 70 % of goats of groups 1 and 3 had abscesses in one or more of the lymph nodes. However, the differences were not significant ($p>0.05$). The gross and histopatholigical lesions were scored based on presence and thickness of abscess. There was no significant ($p>0.05$) difference between the groups on the gross lesion, bacterial isolation and histopathological parameters. Similarly, there was no significant ($p>0.05$) difference in the severity of the histopathological lesions between the three groups. The groups also showed no significant ($p>0.05$) differences on the measurement of the different layers of the abscess. In conclusion, the efficacy of this vaccine in this study was 70 %. Hence, vaccination with Glanvac® 6 did not fully protect the animals from CLA infection and future application of this vaccine needs to be revised.

Keywords: Efficacy, vaccine, caseous lymphadenitis, goats

EFFECT OF EXSANGUINATION UNDER KETAMINE PLUS XYLAZINE ANESTHESIA AS A MODE OF EUTHANASIA ON BLOOD PARAMETERS AND ORGAN PATHOLOGY IN SPRAGUE-DAWLEY RATS

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ABSTRACT

Euthanasia techniques can be divided into two groups; (i) chemical methods, which involves the administration of an overdose of drugs through injection or inhalation and (ii) physical methods such as cervical dislocation that is primarily employed in small laboratory animals. Despite the various methods that can be chosen, each will have an effect on the samples collected. Carbon dioxide inhalation and pentobarbital injection were the common methods used for euthanising laboratory animals. In this study, the effects of using anesthetic drugs Ketamine (100 mg/kg) + Xylazine (10 mg/kg) (Ketamine+Xylazine) followed by exsanguination in Sprague Dawley rats on the blood parameters and organ pathology were examined. Twenty (20) Sprague Dawley rats were divided into 3 groups; (i) 100 % carbon dioxide inhalation, (ii) pentobarbital injection (100 mg/kg) and (iii) Ketamine+Xylazine anaesthesia followed by exsanguination. Results obtained revealed that there were differences in blood parameters and organs pathology. The results showed that euthanasia using carbon dioxide and pentobarbital had more effects on the blood parameters and organ pathology changes compared with anaesthesia using Ketamine+Xylazine combination followed by exsanguination.

Keywords: euthanasia, carbon dioxide, pentobarbital, ketamine plus xylazine, blood parameters, organ pathology.

EFFECTIVENESS OF ENVIRONMENTAL ENRICHMENT IN PROMOTING ACTIVITY AND BEHAVIOURAL REPERTOIRE IN MALAYAN TIGERS (*PANTHERA TIGRIS JACKSONI*) HOUSED IN BARREN ENCLOSURES

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ABSTRACT

Zoological facilities worldwide are recognising the need for improved welfare standards and management of animals in their collection. Strategies adopted include providing naturalistic enclosures and instituting environmental enrichment programs. Captive tigers are a major feature in many zoos; however, there remains a paucity of information on their behavior and response to enrichment and novel stimuli. This study documented the diurnal activity budget of nine captive Malayan tigers (*Panthera tigris jacksoni*) housed in barren indoor enclosures. The effectiveness of olfactory enrichment in promoting naturalistic activity and increasing behavioural repertoire using natural (meat) and artificial (perfume) scented objectswere also evaluated. Activity budgets were compared during the pre-enrichment, enrichment and post-enrichment phase. The tigers spent a majority of the time resting during the pre-enrichment (63.3 ± 4.8 %), enrichment (66.4 ± 7.7 %) and post-enrichment (61.2 ± 8.5 %) phases. However, there was a significant increase (189.5 %) in investigative behaviour during the enrichment phase. The enrichment items also reduced the mean frequency of stereotypy by 20.5 %. The changes in behavioural pattern did not persist after the withdrawal of the enrichment items, indicating that the observed behavioural alterations were due to the stimuli provided. The results indicated that the provision of simple and inexpensive enrichment items can promote naturalistic behaviours and alleviate stereotypies in tigers housed in barren enclosures. Therefore, local zoological facilities are encouraged to use these inexpensive materials and olfactory enrichments to improve the welfare of tigers housed in non-stimulating captive environments.

Keywords: Malayan tigers, *Panthera tigris jacksoni*, activity budget, olfactory enrichment