

12th Proceedings of the Seminar on

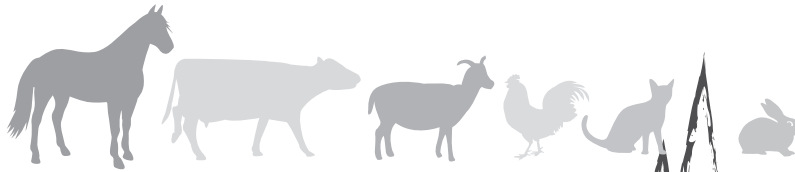


VETERINARY SCIENCES

Faculty of Veterinary Medicine UPM
20-22th February 2017



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Preface

The doctor of veterinary medicine (DVM) programme is not limited to lectures, practicals, and industrial attachments for the acquisition of skills and aptitude by students to eventually serve as competent veterinary practitioners. The programme could not cater for all eventualities faced by graduates in practice and in their interactions with society and the environment. In practice, veterinarians are often faced with challenges that would require innovations and occasionally also thinking outside the box, to overcome. Although new learning methods have been now introduced into the curriculum, to include problem-based learning and objective structured clinical examination, to ensure that graduates are better prepared to the challenges of the profession, the elements of creativity and acquisition of information through research, is still lacking. We hope the final year research project will at least partially fill the void.

The proceedings of the seminar on veterinary sciences compiles the final year projects as abstracts, not just as reference for veterinarians but also an archive of one of the activities of the DVM programme. This year in the 12th proceedings, we compiled 12 extended and 76 short abstracts.

The editors would like to congratulate students and supervisors for the successful completion and reporting of the projects. We also wish to express our gratitude to the Dean and management of the Faculty for facilitating publication of this proceedings.

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**ASSESSMENT OF BIOFLOC TECHNOLOGY FOR THE
MALAYSIAN GIANT PRAWN, *MACROBRACHIUM ROSENBERGII*,
JUVENILES CULTURED IN
RECIRCULATING AQUACULTURE SYSTEM**

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ABSTRACT

One of the options for sustainable aquaculture is Biofloc technology (BFT). Biofloc forms as flocculent aggregates of organic material, nitrogen-fixing bacteria, and algae in suspension, which serve as food for cultivated fish or crustaceans besides maintaining good water quality. Limited resource availability for pond production require a more intensive method such as Recirculating Aquaculture System (RAS) that enables recycling of water. *Macrobrachium rosenbergii* is one of the major freshwater crustaceans cultured in Malaysia. Thus, this study was conducted to develop a laboratory model for BFT in RAS and to assess water quality and growth performance of *Macrobrachium rosenbergii* juvenile cultured in RAS with the BFT. The study was conducted for 30 days using PL 30 of *M. rosenbergii*. There was no water exchange during this period. Six tanks were used with three control and three BFT tanks. Sucrose was used to maintain C:N ratio at 15:1 in the BFT system. The amount of total ammonia nitrogen (TAN), NO₂⁻, dissolved oxygen (DO), and pH and temperature were recorded for all tanks. Total ammonia nitrogen and NO₂⁻ were very low at 0 to 0.2 mg/L in BFT. Dissolved oxygen in BFT treatment tanks were lower at 6.00 ± 0.2 mg/L than in control tanks at 6.90 ± 0.19 mg/L. The pHs of BFT treatment tank were in the low range of 6.31 ± 0.13. The water temperature maintained at 26°C for the treatment and control tanks. Survivability of *M. rosenbergii* juveniles in control and BFT cultures was 81.67 and 100 %, respectively. Weight gain in BFT culture was between 0.8 to 1.0 g, while for the control it was 0.8 to 0.9 g. Biofloc aggregates was observed at a size range of 0.10 to 0.85µm. Planktonic organisms such as diatoms, phytoplankton, zooplanktons, and protozoa were observed in the BFT water. *Bacillus* sp. and *Aeromonas hydrophilla* were dominant in the culture with CFU of 1.7 and 0.7 × 10⁴/mL, respectively. Thus, it can be concluded that the biofloc community consisting of aggregates of bacteria, phytoplankton, zooplanktons, protozoa and diatoms while serving as additional food source for prawn larvae worked synergistically in maintenance of good water quality.

Keywords: *Macrobrachium rosenbergii*, Biofloc technology, Recirculating Aquaculture System

INTRODUCTION

Aquaculture is an alternative to open sea fisheries. It projected that by 2020 that aquaculture could contribute at least 40% of the total production of seafood (FAO, 2004). In aquaculture, a major challenge is to reduce water consumption in the maintenance of quality and quantity of effluent and the amount of dissolved solids generated during production. There is need to develop techniques to provide sustainable alternatives that would reduce environmental impact without affecting the health and growth of the crop organisms. One option for the development of sustainable practices in aquaculture is biofloc technology (Asaduzzaman *et al*, 2008). Biofloc forms in pond water as the flocculent aggregates organic material, nitrogen-fixing bacteria, and algae in suspension, which serve as food for cultivated fish or crustaceans and promotes direct use of toxic metabolites by degrading the activity of the bacteria and algae (Avnimelech, 2007). In situations where there is limited resource availability for pond production, a more intensive method such as Recirculating Aquaculture System (RAS) that enables recycling of water can be employed.

Freshwater giant prawn, *Macrobrachium rosenbergii*, indigenous to South and Southeast Asia, including Malaysia, has significant aquaculture potential. The sub-tropical climate and vast water bodies in Malaysia, provide a unique opportunity for commercial *Macrobrachium* spp. production. In fact, in this country, the production of freshwater prawn is on the increase, impacting the aquaculture industry significantly. To further fuel growth of this industry, more advanced technology such as use of biofloc that can to be integrated with the modified RAS system that can achieve optimum condition for the growth of giant freshwater prawns in nursery-rearing system.

MATERIALS AND METHODS

Biofloc technology model of Recirculating Aquaculture System

The experiment consists of six glass tanks with shade consisting of three tanks of biofloc technology treatment (BFT1, BFT2, BFT3) and 3 tanks of control RAS system (C1, C2, C3). Each tank (3.0 × 1.5 × 1.0 ft) with an elevated RAS filtration system was filled with water up to depth of 1.0 ft that was continuously aerated using an air-blower and each. The biofloc was first established by adding 50 g sucrose daily into the tank with tilapia until floc build up with C:N ratio of 15:1. BFT water was then transferred to each of the experimental tanks.

One hundred and twenty tails of post-larvae 30 Malaysian Giant Freshwater Prawns (*Macrobrachium rosenbergii*), weighing 0.5 ± 0.2 g were obtained from Hatchery from Sepang, Selangor, Malaysia, and allow to acclimatised for a week. Thirty post-larvae were stocked at a stocking density of 20 per tanks and the experiment was conducted over 4 weeks of rearing time.

Water quality and growth performance of *Macrobrachium rosenbergii* juvenile culture

Water quality parameters

Total ammonia nitrogen and NO_2^- contents were determined using the HACH colour disk test kits. Dissolved oxygen, pH, and water temperature were measured using YSI multi-probe YSI 556.

Water benthic organisms

Drops of water sample collected from experiment tanks were prepared on glass slides and examined under light microscopy (Nikon E500, Japan) to identify the microorganisms.

Bacterial isolation

Aerobic spread plate method using Tryptose Soy Agar (TSA) agar was used to obtain pure culture.

Growth morphometrics

The weight of each prawn after wiping with paper towel was obtained. Total body length of the prawns was measured from rostrum to telson using a ruler.

Statistical Analysis

Statistical Package for the Social Science (SPSS) 22 and One-way ANOVA were used to analyse data.

RESULTS AND DISCUSSION

Biofloc was successfully established with biofloc aggregates size ranging from 0.10 to 0.85 μm . The colour of water was brownish and under light microscopy (Figure 1). Planktonic organisms such as diatoms, phytoplankton, zooplankton and protozoa were identified in the BFT water. The dominant microorganisms in this culture were the zooplankton and diatoms that gave the brown appearance to the water. The microorganisms were of size suitable as feed for filter feeders such as the prawn juveniles.

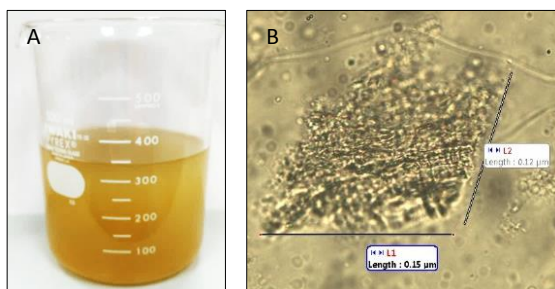


Figure 1. (A) Brown biofloc water. (B) biofloc particulates. Magnifications, 100 \times .

Bacillus sp. and *Aeromonas hydrophila* were identified to be the prevailing bacteria in the culture with CFU of $1.7 \times 10^4/\text{mL}$ and $0.7 \times 10^4/\text{mL}$, respectively (Tables 1 and 2). The TAN and NO_2^- values in BFT were low ranging 0 to 0.2 mg/L. Dissolved oxygen in BFT treatment tanks were also lower at 6.00 ± 0.2 mg/L compared to the control tanks at 6.90 ± 0.19 mg/L. The pHs of BFT treatment tanks were in the low range of 6.31 ± 0.13 and the temperature maintained at 26°C for both tanks. Survivability of larvae in control and BFT cultures was 81.67 and 100 %, respectively while weight gain was between 0.8 to 1.0 g as compared to the control at 0.8 to 0.9 g.

Table 1. Microorganisms identified in the biofloc tank at 25 days post culture.

Plankton	Phylum	Class	Genera
Phytoplankton	Chlorophyta	Chlorophyceae	<i>Chlorella</i> <i>Scenedesmus</i>
	Cynophyta	Cynophyceae	<i>Oscillatoria</i>
Zooplankton	Rotifera	<u>Bdelloidea</u>	<i>Rotaria</i>
		Monogonta	<i>Euclanis</i> <i>Collotheca</i>
Protozoa	Ciliophora	Vorticellidae	<i>Vorticella</i>
	Ciliophora	Ciliatea	<i>Ciliate</i>
	Ciliophora	Parameciidae	<i>Paramecium</i>

Table 2. Species of bacteria identified at 20 days of culture.

Colony	CFU ($\times 10^4/\text{mL}$)	Colony morphology	Gram Staining	Additional identification test	Species
1	0.7	Diameter: 0.5cm. Round-edged, white	Gram-negative, single small rods	API 20E	<i>Aeromonas hydrophilla</i>
2	1.7	Diameter: 1cm., rough surface, white	Gram-positive, large rod in chains	Catalase- positive BBL Crystal (not identifiable)	<i>Bacillus sp.</i>

CFU = colony forming unit

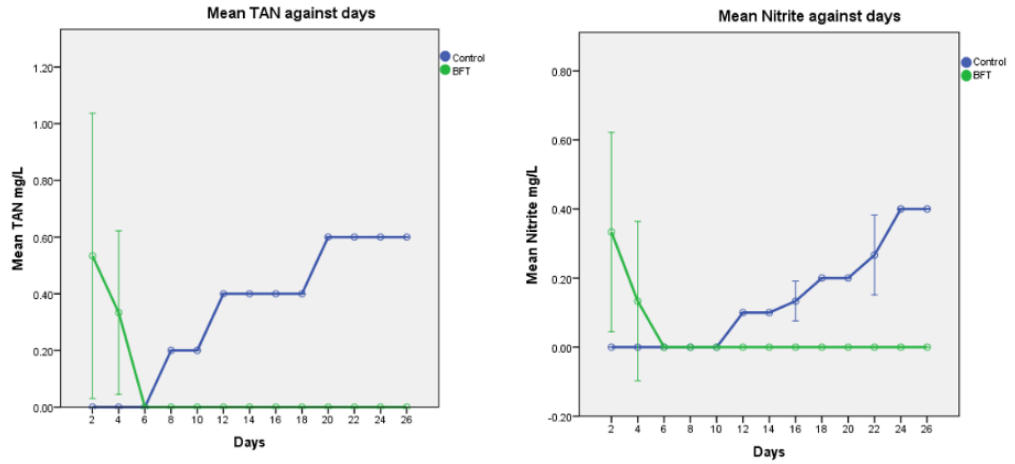


Figure 2. Means TAN & Nitrite values in BFT and Control tanks over a period of one month.

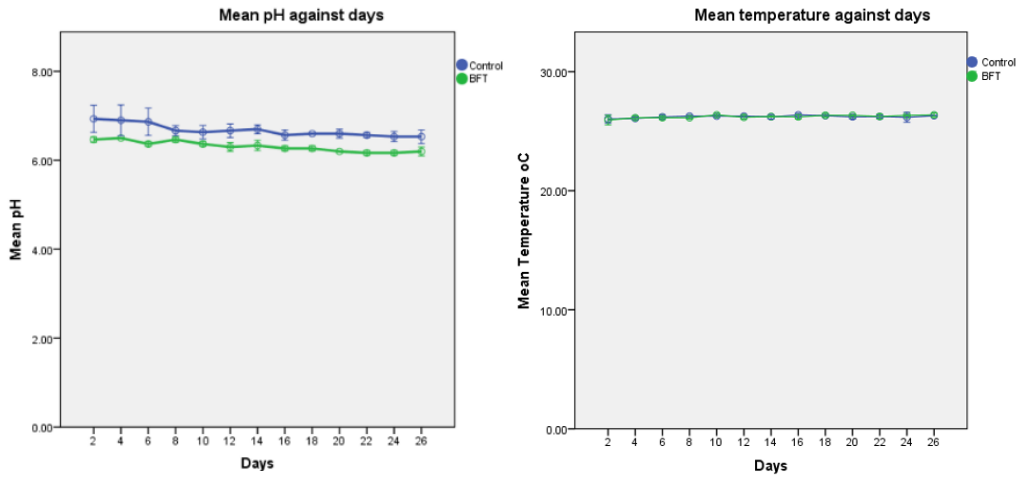


Figure 3. Mean pH and temperature in Biofloc technology treatment and Control tanks.

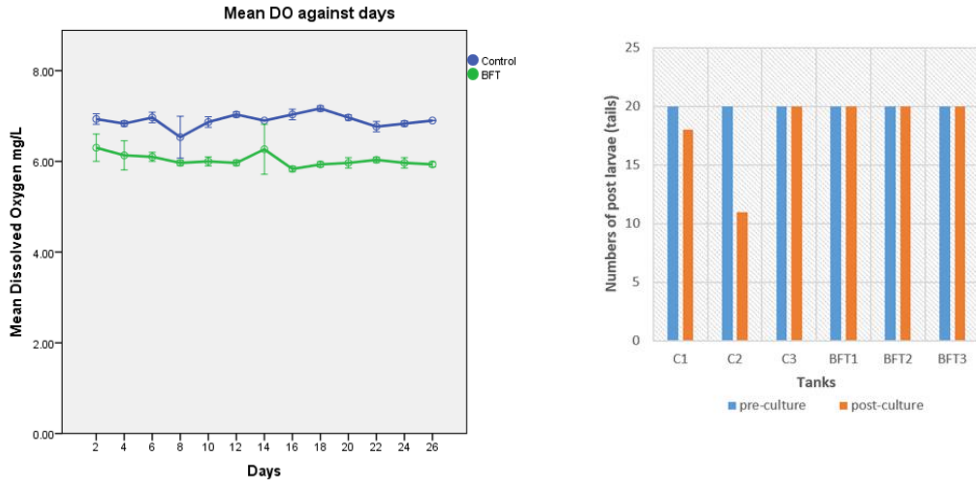


Figure 2. Dissolved oxygens in Biofloc technology treatment and Control tanks and prawn survival rate post-culture.

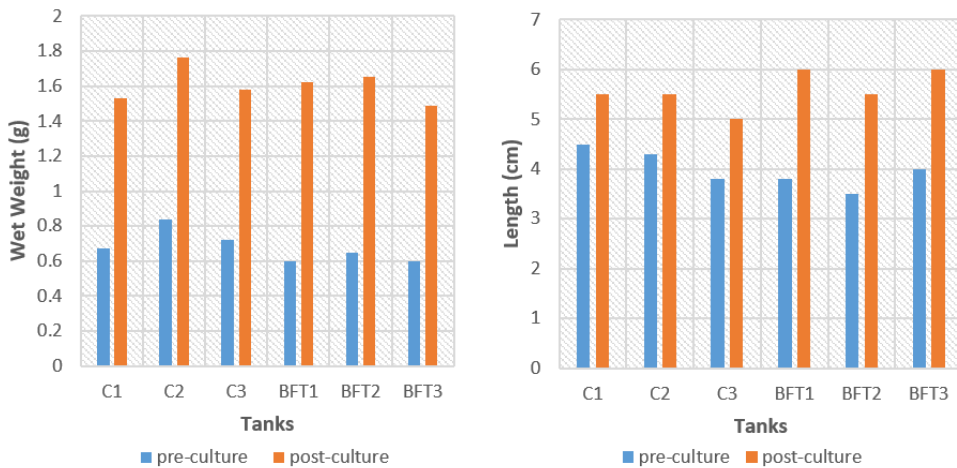


Figure 3. Weight and length of prawns in Biofloc technology treatment and control tanks.

CONCLUSION

Biofloc community, made up of microorganisms such as diatoms, planktons and bacteria, has been successfully cultured in the laboratory RAS model. Biofloc technology was shown to be able to stabilise and maintain water quality in RAS while reducing cannibalistic behaviour of the prawns. *M. rosenbergii* post-larvae cultured under biofloc technology in RAS system showed good growth performances.

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A SURVEY ON HEALTH PROBLEMS AND STEREOTYPIC BEHAVIOURS ASSOCIATED WITH MANAGEMENT OF STABLED HORSES

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ABSTRACT

The management of individual stabled horses in multiple equestrian establishments and the occurrence of health and behavioural problems were assessed in 131 animals. Based on interviews and direct observation of animals, it was found that 61 % of the horses spent less than 19 h/d in the stalls and 39 % spent more than 19 h/d. Eighty-five percent exercised regularly, whereas 15 % did not exercise at all. All horses were fed with mix feed which consist of commercial concentrates, fibres (hay and chaff) and some were fed with additional supplements. All horses were given four meals daily which the feeding frequency of the mix diet were twice per day (late morning and afternoon) and fibre diet (hay) twice per day (early morning and late evening). However, the timing of the meals was not precisely fixed. Lameness (30 %), skin diseases (16 %) and colic (9 %) were the most cited diseases in the interviews. Thirty percent of horses showed behavioural problems. Each of the management factors did not revealed any association on both occurrence of health and behaviour problems.

Keywords: abnormal behaviour, health problems, horse, management factors

INTRODUCTION

Horses in the wild spend most of the day in groups searching for a wide and safe place for food and rest while stabled horses are fed by humans have limited natural social interactions (Mills and Clarke, 2002). Despite horses kept in the stable can meet human's need, the stables contradict with the natural environment and many do not meet the horses' needs (Goodwin, 1999).

There are strong suggestions that equine stereotypies and occurrence of health problems are connected with poor welfare and a suboptimal management and/or stabling environment. Different forms of equine stereotypic behaviours described include crib biting, weaving, and box walking which are considered the most prevalent (Sarafchi and Blokhuis, 2013).

This study surveys the variety of stable management and evaluate the relationship of stabled horses between occurrence of stereotypic behaviour and health problems.

MATERIALS AND METHODS

Sample and data collection

Data were collected from 10 equestrian establishments that have individually stabled horses. A total of 131 individually stabled horses were observed and their behaviours recorded. Interviews with owners or caretakers were conducted to obtain information on the horses including their behaviours. The feeding regime was recorded for group of horses while data on work, duration of horses spent in the stable, weight of concentrate and health history were recorded for each horse or a group of horses. Abnormal behaviours of horses were according to the definitions described by Broom and Fraser (2007)

Statistical Analysis

Statistical Package for the Social Science (SPSS) 22 was used to analyse data. The categorical data were assessed in cross-tabulation analyses using the Chi-square and odds ratio tests to determine association between management factors and the occurrence of stereotypic behaviors and clinical problems.

RESULTS AND DISCUSSION

Based on interviews and direct observations of horses, it was noted that 61% of the horses spent less than while 39% spent more than 19 h/d in stalls. 85% exercised regularly, whereas 15% did not exercise at all. All of the horses were fed with mixed feed consisting of commercial concentrate, fibre (hay and chaff) and some of the horses were fed additional supplement. All horses were given four meals per day and twice per day (usually late morning to afternoon) fed mixed diet followed by fibre diet (hay) twice per day (early morning and late evening). However, the timing of the feeding was not precisely fixed. Coprophagia (13%), licking (5%), crib biting (2%), weaving (5%), aggression (5%) and multiple behaviours (1%) were among the stereotypic behaviours that were noted. A total of 30% of the horses showed behavioural problems. Lameness (30%), skin disease (16%) and colic (9%) were the most cited conditions. There was no association between occurrence of the stereotypic behaviours and health problems.

CONCLUSION

This study showed that common health problems and stereotypic behaviours under different management factors may be used to alert the local veterinarians and owners on the condition of the horse.

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ASSESSMENT OF SCHOOL ZONOTIC DISEASES AWARENESS PROGRAM AMONG PRIMARY SCHOOL STUDENTS OF SEKOLAH KEBANGSAAN SERI SELANGOR USJ4, SUBANG JAYA, SELANGOR, MALAYSIA

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ABSTRACT

Humans are prone to infection with zoonotic diseases from wildlife, domestic animals, and the environment. It is important for the public especially school students to be aware of zoonotic diseases in order to prevent transmission when handling animals. Thus, this study was carried out to determine the awareness on zoonotic diseases among primary school students of Sekolah Kebangsaan Seri Selangor USJ4, Subang Jaya, Selangor before and after the school zoonotic diseases awareness programme and to assess the effectiveness of the program in educating students. The programme consisted of eight modules with each module comprising of activities related to zoonotic diseases education. The programme was conducted in two phases; the first phase was conducted on 5th October 2016 at the school and second phase on 10th October 2016 at Universiti Putra Malaysia. A set of questionnaires on zoonotic diseases was designed to assess awareness and knowledge of students on zoonotic diseases. The same questionnaires were distributed to students before and after the zoonotic diseases awareness program. Forty students responded to the questionnaires. The Mann Whitney U-Test showed significant increases ($P < 0.05$) in awareness on zoonotic diseases after the awareness programme. It is concluded that school zoonotic disease awareness programme was effective in increasing the basic knowledge students on zoonoses and prevention of transmission of diseases.

Keywords: zoonotic disease, transmission, awareness programme, primary school students

INTRODUCTION

Zoonoses are diseases that are naturally transmitted between animals and humans. Zoonoses constitute a diverse group of viral, bacterial, rickettsial, fungal, parasitic, and prion diseases with a variety of animal reservoirs, including wildlife, livestock, pet animals, and birds (Nkuchia *et al.*, 2007). It was shown that several zoonoses

causing human illness, including brucellosis, leptospirosis, and Q fever, are common, but under-diagnosed, (Zhang *et al.*, 2016). In most cases, animals play an essential role in maintaining infection in nature and contribute to the distribution and actual transmission of infection in human and animal populations. These diseases have a variety of transmission mechanisms including via aerosol such as in rabies and anthrax, or indirectly by vectors, food, water and the environment, as in the case of bovine tuberculosis and cysticercosis. Many diseases also have multiple routes of infection.

Zoonotic diseases remain a genuine threat to health and survival for people, their livestock, companion animals and wildlife (WHO, 2005). Among zoonotic diseases prevalent in Malaysia are leptospirosis, rabies, influenza, Japanese encephalitis, toxoplasmosis, ornithosis, Q fever and monkeypox (Tan, 1981). The perception of the community and attitude towards zoonotic diseases plays an important role the life-cycle and transmission of these diseases. Thus, public education on the risk factors, routes of transmission, and life-cycle of zoonotic diseases is crucial for the development and implementation of appropriate disease prevention and control strategies (Tesfaye *et al.*, 2013).

The majority elementary and high school students acquire information on zoonotic diseases from their families. Currently, in Malaysia, there is no programme on zoonotic disease education to the public. However, there have been published reports on zoonotic disease such as leptospirosis and malaria in Malaysia (Thayaparan *et al.*, 2013; Tan, 1981; Lim, 2013). This information are rarely accessed by school children. In this study, an awareness on zoonotic disease programme was conducted for primary school students of Sekolah Kebangsaan Seri Selangor USJ4, Subang Jaya, Selangor. The objective was to determine the best methods to educate the students on the zoonotic diseases so that they have better understanding on the prevention of and exposure to zoonotic diseases by assessing the effectiveness of the school zoonotic diseases awareness program.

MATERIALS AND METHODS

Sampling and data collection

Forty standard 6 students from Sekolah Kebangsaan Seri Selangor, Malaysia comprising of 22 female and 18 male students from a multiracial background, were recruited in this study. The school zoonotic diseases awareness program was divided into two phases; the first phase was conducted on the 5th October 2016 at the school followed by the second phase on the 10th October 2016 in UPM. The programme consisted of 8 modules developed specifically to create awareness on zoonotic diseases for school students. Activities conducted at the school were based on Modules 1 to 4, while in UPM, activities were based on Modules 5 to 8. A questionnaire containing 35 questions on zoonotic diseases was designed to assess awareness and knowledge of students on zoonotic diseases. The same questionnaires were distributed to students before and after the zoonotic diseases awareness program.

School zoonotic diseases awareness programme

The programme consist of eight modules:

Module 1: Introduction to UPM-VET and What is a A Vet Doctor

The students were introduced to University Putra Malaysia (UPM) and veterinarian profession by viewing the UPM's Corporate video and slide presentation on veterinary medicine.

Module 2: Ice breaking session

The students were divided into smaller groups and one facilitator was be assigned to each group. This session guided by facilitators. The questionnaire was distributed to the students.

Module 3: What is a pathogen

Under the guidance of their facilitator, the students were introduced to different types of pathogens. In his module, the students construct different types of pathogens such as viruses, bacteria and parasite based on the information and materials provided.

Module 4: Introduction to zoonotic diseases

Students were introduced to the term 'zoonotic disease' and the different types of diseases that can afflict both animals and human. The discussions were guided by a slide presentation and interactions between the speaker and students.

Module 5: Dairy farm visit

Module 5 is the first session of the second phase of the programme. The students assembled in the early morning at dairy farm at Ladang 16, UPM. In this visit, students listened to a short talk by the attending veterinary officer on food safety and importance of drinking milk fresh. In this module student get to understand the term 'pasteurisation' and maintenance of personal hygiene after touching animals.

Module 6: Flashback activity – Wordsearch

In this module, students were required to recall the words that they have learned and heard from activities in phase one. This is an exercise in wordsearch particularly those term pertaining to zoonoses. A short flashback discussion with the speaker was also conducted.

Module 7: Be a zoonotic disease detective

Students were introduced to different types zoonotic parasites, mode of their transmission, life-cycles, and techniques in parasite detection. Students were divided into smaller groups and each group were given six specimens of parasites. They were required to view the specimens under the microscope and draw the parasites as they see them. The faecal floatation and proper hand washing techniques were also introduced to the students.

Module 8: My pet and I

Module 8 was a talk on keeping pets and precautions when handling animals. Talks were given by a small animal practice veterinarian via slide presentation and using residence University Veterinary Hospital, UPM cats, as subjects.

Statistical analysis

Data was analyzed using the IBM Statistical Package of Social Science (SPSS) version 22. Mann Whitney U Test was used to compare between pre- and post-programme awareness of zoonotic diseases among the students.

RESULTS AND DISCUSSION

Thirty-one of 35 questions showed significant ($P < 0.05$) differences in awareness score between before and after conduct of the programme (Tables 1 and 2). showed the types of questions that had significant different in awareness score and also type of of questions that had no significant different in awareness score between before and after the awareness program. Of all questions 89.1 % showed significant ($P < 0.05$) differences in awareness score between before and after conduct of the programmes, suggesting the programme was effective in education students of the importance of zoonotic diseases.

Table 1: List of questions that had significant different in awareness score between before and after the awareness program.

Questions	
Q1	I know what bacteria, viruses, and parasite are.
Q2	Animals can share bacteria, viruses and paasite with me.
Q3	Animal diseases can spread to humans.
Q4	I know what zoonosis means.
Q5	I have been infected with zoonotic diseases before.
Q6	When I am in an area with animals, I feel I am more at risk of contracting diseases.
Q7	I avoid big crowds because I fear disease.
Q8	I have been ill before with the symptoms such as a sore throat and flu.
Q9	I have been ill before with fever, diarrhoea, and vomiting
Q10	When I am sick I often consume antibiotic.
Q12	I have pet at home.
Q13	I need to wash my hands after playing with my pets.
Q14	I have recent contact with wildlife animals.
Q15	I wash my hands after visit to farms/zoos.
Q17	Stray animals and wildlife should be caught and translocated away residential areas.
Q18	Stray animals and wildlife, like rat and mosquitos, are pests.
Q19	I consider myself an animal lover.
Q20	I think feeding animals is fun
Q21	I like watching people feed animals but I dare not do it myself.
Q24	I am afraid that animals will transmit diseases to me.

Table 1 (Continued).

Questions	
Q25	Children should not be allowed to get close to the animals.
Q26	I have heard of the term of 'pasteurisation'.
Q27	I can drink freshly milked cows' or goats' milk.
Q28	I know the proper way to wash my hands to get rid of bacteria, viruses, and parasites.
Q29	I prefer to use hand sanitisers than washing my hand.
Q30	I routinely wash my hands after handling pets/animals before eating.
Q31	If I keep my hands clean, I reduce the chances of getting sick.
Q32	I am happy to learn new knowledge to avoid getting sick.
Q33	I understand why it is important to take care of my personal hygiene.
Q35	I know what a veterinarian is.
Q36	I know what a medical doctor is.

Table 2: List of questions that had no significant different in awareness score between before and after the awareness program.

Questions	
Q16	It safe to play with a stray animal
Q22	I want to see animals but do not want them to come close to me
Q23	I am afraid animals might bite or scratch me
Q34	I understand why it is important for a pet owner to be responsible for their animal' health

CONCLUSION

This study showed that the students had acquired basic knowledge on zoonoses and ways to prevent transmission of diseases after participating in the school zoonotic diseases awareness program. An overall total of 89.1% of questions that had significant ($P < 0.05$) different awareness score between before and after the programme was conducted. Thus, the study shows that students after the programme are more aware of zoonotic diseases and their importance to animals and humans.

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IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILE OF BACTERIA ISOLATED FROM CATFISH, *Clarias* spp. OBTAINED FROM SELECTED MARKETS IN SELANGOR, MALAYSIA

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ABSTRACT

Clarias sp. also known as catfish is a very popular freshwater fish in Malaysia. For human consumption, catfish species are normally reared in ponds and often get contaminated by a variety of microorganism because of the way they are being reared and the environment. Thus, these fish are highly likely to carry pathogens and can be unsafe for human consumption if the fish is not cooked well. This study was conducted to isolate and identify the types of bacteria found in catfish, *Clarias* spp. and to determine the antibiotic susceptibility pattern of the common bacteria found in the fish, *Clarias* spp. Twenty-Four *Clarias* spp. samples were purchased from 8 selected markets in Selangor, Malaysia for a period of two weeks. The gills and intestines were processed for isolation and identification of bacteria. The antibiotic susceptibility of the obtained isolates was also determined against the commonly used antibiotics in aquaculture. Twenty Gram-negative (41.67%) and 7 Gram-positive bacteria (14.58%) were isolated from the samples. The gram-negative bacteria isolated were *Aeromonas* species (37.5%), *Vibrio cholerae* (31.25%), *Escherichia coli* (16.66%), *Klebsiella pneumonia* (12.5%), *Vibrio parahaemolyticus* (10.41%), *Proteus vulgaris* (10.41%), *Pantoea agglomerans* (10.41%), *Proteus mirabilis* (10.41%), *Enterobacter aerogenes* (6.25%), *Photobacterium damsela* (6.25%), *Edwardsiella tarda* (6.25%), *Pleisiomonas shigelloides* (6.25%), *Citrobacter freundii* (6.25%), *Salmonella* species (2.08%), *Vibrio alginolyticus* (2.08%), *Chromobacterium* (2.08%), *Achromobacter* (2.08%), *Shewanella putrefaction* (2.08%) and *Alcaligenes fecalis* (2.08%). While, the Gram-positive bacteria species isolated were *Staphylococcus* sp. (6.25%), *Streptococcus viridans* sp. (4.16%), *Actinomyces* sp. (4.16%), *Staphylococcus hyicus* (2.08%), *Corynebacterium* sp. (2.08%), *Corynebacterium kutcheri* (2.08%), and *Listeria monocytogenes* (2.08%). Multidrug resistant traits were presented for 4 Gram-negative bacteria species and 1 Gram-positive bacteria species. In conclusion, this study suggested that the catfishes harbours pathogens that exhibited multidrug resistant trait which may bring risk to the consumers.

Keywords: aquaculture, catfish, gill, intestine, antibiotic sensitivity test, consumer

INTRODUCTION

Clarias sp belongs to the Phylum of Chordata, Class of Actinopterygii, Family of Clariidae, Order of Siluriformes, and locally known as catfish or *ikan keli* in Malaysia (Kottelat, 2013). These catfish are most commonly found in muddy or swampy water of high turbidity (Allen, 2011). It is considered as one of the delicacies in Malaysia. For human consumption, catfish species are normally reared in ponds and often get contaminated by a variety of microorganism because of the way they are being reared and the environment.

There has been a dramatic recent increase in the use of trash fish in aquaculture with the expansion of freshwater culture of catfish in cages and ponds (Edwards *et al.*, 2004). Consuming fish that are fed with trash fish made from marine fishes may also bring risk to the health of consumers as it may expose consumers to bacteria such as the *Vibrio* spp., which are commonly found and considered as human pathogens (Feldhusen, 2000). Therefore, these fish are highly likely to carry pathogens and can be unsafe for human consumption if the fish is not cooked and handled well.

As reported by FDA (2014), the judicious use of antibiotic in aquatic farming is part of good veterinary practice. Exposure of antibiotic residue from aquaculture exposes freshwater aquatic animals and may cause antibiotic resistance and it should be considered as a public health concern.

This study was conducted to isolate and identify the types of bacteria found in catfish, *Clarias* spp. and to determine the antibiotic sensitivity of the common bacteria found in catfish, *Clarias* spp.

MATERIALS AND METHODS

Sampling method

Twenty-four live catfish were purchased from 8 selected local markets at different locations in Selangor, Malaysia over a period of two weeks. Three live catfish were collected from each local market and transported in an ice-box to the Aquatic Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The samples were processed within 24 h of collection for bacterial isolation and identification.

Sample processing

Pithing method was performed for the collection of samples (gill and intestine) from catfish, *Clarias* spp. In the laboratory, samples were obtained using a sterile knife and a pair of sterile dissecting tools.

For isolation of bacteria other than *Vibrio* spp., the gills and intestines were placed separately in sterile plastic bags, labelled and homogenised with 10 mL of sterile peptone water using stomacher for 1 min. After homogenisation, a loopful of each gill and intestine sample was streaked onto blood agar and McConkey agar, respectively. The plates were incubated at 30°C for 24 h under aerobic condition.

For *Vibrio* spp. isolation, the gills and intestines were then separately placed in a

sterile plastic bag, labelled and homogenised with 10 mL alkaline peptone water using stomacher for 1 min. The homogenised sample was then incubated at 30°C for 24 h under aerobic condition. After incubation, a loopful of each enriched sample (gill/intestine) was streaked onto Thiosulfate-Citrate Bile Salt (TCBS) agar. Then the plates were incubated at 30°C for 24 h under aerobic condition.

Bacterial isolation and identification procedure

Primary culture

All samples were inoculated into Blood, McConkey, and Thiosulfate-Citrate Bile Salt (TCBS) agar, and incubated at 30°C for 24 h under aerobic condition.

Purification of culture

Visible colonies on the agars after 24 h of incubation were examined. The morphology of each visible colony was recorded based on their shape, size, color, surface texture, haemolytic activity (blood agar only), and odour. Each visible colony from the blood and McConkey agar was obtained using a sterile inoculating loop, streaked onto a blood agar before incubating aerobically for 24 h at 30°C. Typical colonies on TCBS are green and yellow. Individual colonies were selected and Gram-stained. Purification was done on Tryptic Soy (TSA) agar.

Gram staining

Gram-staining was done for identical bacterial colonies to differentiate between Gram-negative bacteria and Gram-positive bacteria. The stained smear was examined for Gram-staining reaction, and cell morphology and arrangement.

Biochemical tests

A series of biochemical tests were performed to identify the bacteria. Colonies presenting each bacterial species were identified and characterised using standard biochemical tests according to that described by Jang *et al.*, (2008). The biochemical tests were for oxidase, sulfur indole motility (SIM), triple sugar iron (TSI) test, urease test, citrate, catalase, and coagulase.

Antimicrobial susceptibility test

Testing for antimicrobial susceptibility was done using the Kirby-Bauer method. Two mL of sterile saline was dispensed into sterile test tube by using sterile pipette. Several isolated colonies from the subculture were collected using an inoculating loop and transferred into the test tube of sterile saline. The bacteria were diluted to obtain a turbidity equivalent to 0.5 MacFarland test standard. A sterile swab was dipped into the inoculum and streaked onto entire surface of the Mueller Hinton agar twice with the swab by turning the plate 60 degrees between streaking to obtain an even inoculation.

Eight commonly used antibiotics in aquaculture selected were Ampicillin (10µg), Penicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Gentamycin (10µg), Streptomycin (10µg), Sulfamethoxazole/trimethoprim (25µg). The antibiotic discs were placed onto the Mueller-Hinton agar using a disc dispenser

and then lightly pressed down with the sterile forceps to ensure contact with the agar surface. The plate was then incubated at for 24 h. Zones showing complete inhibition by gross inspection were measured in millimeters using calipers and the values obtained were compared to acceptable limits in Clinical and Laboratory Standard Institute (2010) to determine the susceptibility level of the antibiotic used.

RESULTS AND DISCUSSION

Isolation and Identification of bacteria

Out of 48 samples (24 gill and 24 intestine), 20 Gram-negative (41.67%) and seven Gram-positive bacteria (14.58%) were successfully isolated. The gram-negative bacteria isolated were *Aeromonas* sp. (37.5%), *Vibrio cholerae* (31.25%), *Escherichia coli* (16.66%), *Klebsiella pneumonia* (12.5%), *Vibrio parahemolyticus* (10.41%), *Proteus vulgaris* (10.41%), *Pantoea agglomerans* (10.41%), *Proteus mirabilis* (10.41%), *Enterobacter aerogenes* (6.25%), *Photobacterium damsela* (6.25%), *Edwardsiella tarda* (6.25%), *Pleisiomonas shigelloides* (6.25%), *Citrobacter freundii* (6.25%), *Shigella* sp. (4.16%), *Salmonella* sp. (2.08%), *Vibrio alginolyticus* (2.08%), *Chromobacterium* (2.08%), *Achromobacter* (2.08%), *Shewanella putrefaction* (2.08%) and *Alcaligenes fecalis* (2.08%).

The Gram-positive bacteria species isolated were *Staphylococcus* spp. (16.67%), *Corynebacterium* spp. (8.33%), *Streptococcus viridans* spp (8.33%), *Actinomyces* spp (4.16%) and *Listeria monocytogenes* (4.16%).

Gram-negative bacteria isolated from the gill were *Aeromonas* species (58.3%), *Escherichia coli* (16.67%), *Vibrio cholerae* (41.60%), *Vibrio parahemolyticus* (12.50%), *Klebsiella pneumonia* (29.17%), *Salmonella* species (4.16%), *Shigella* species (4.16%), *Pleisiomonas shigelloides* (0%), *Edwardsiella tarda* (0%), *Enterobacter aerogenes* (12.5%), *Vibrio alginolyticus* (4.16%), *Proteus vulgaris* (4.16%), *Proteus mirabilis* (8.33%) and Gram-negative bacteria isolated from the intestine were *Aeromonas* sp. (37.5%), *Escherichia coli* (29.17%), *Vibrio cholerae* (33.33%), *Vibrio parahemolyticus* (12.50%), *Klebsiella pneumonia* (8.33%), *Salmonella* sp. (0%), *Shigella* sp. (0%), *Pleisiomonas shigelloides* (12.5%), *Edwardsiella tarda* 12.5%), *Enterobacter aerogenes* (4.14%), *Vibrio alginolyticus* (0%), *Proteus vulgaris* (16.67%), *Proteus mirabilis* (4.16%)

Gram-positive bacteria isolated from the gill were *Staphylococcus* species (12.50%), *Staphylococcus hyicus* (4.16%), *Streptococcus viridans* sp. (8.33%), *Corynebacterium* sp. (4.16%), *Corynebacterium kutcheri* (0%), *Actinomyces* species (0%), and *Listeria monocytogenes* (0%) and Gram-positive bacteria isolated from the intestine were *Staphylococcus* sp. (4.16%), *Staphylococcus hyicus* (0%), *Streptococcus viridans* species (0%), *Corynebacterium* sp. (0%), *Corynebacterium kutcheri* (4.16%), *Actinomyces* sp. (8.33%), and *Listeria monocytogenes* (4.16%).

Antimicrobial susceptibility test

All Gram-negative and Gram-positive bacteria isolated were subjected to eight types of antibiotics and the results of the multi-drug resistant (MDR) bacteria (Figures 1 and 2). There are three Gram-negative multi-drug resistant bacteria and one multi-

drug Gram-positive bacteria were detected from this study.

	BL		TC	Q	M	AG	S		MDR
	Penicillin	Ampicillin					Streptomycin	Sulfamethoxazole / trimetoprim	
Gram-negative bacteria									
<i>Vibrio cholerae</i>	Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	YES
<i>Proteus vulgaris</i>	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Intermediate	Sensitive	YES
<i>Proteus mirabilis</i>	Resistant	Resistant	Resistant	Sensitive	Intermediate	Sensitive	Resistant	Sensitive	YES

Resistant
 Intermediate
 Sensitive

Beta- Lactam (BL), Tetracycline (T), Quinolone (Q), Macrolides (M), Aminoglycoside (AG), Sulfonamides (S).

Figure 1: Antimicrobial susceptibility for Gram-negative bacteria.

	BL		TC	Q	M	AG	S		MDR
	Penicillin	Ampicillin					Streptomycin	Sulfamethoxazole / trimetoprim	
Gram-positive bacteria									
<i>Staphylococcus</i> species	Intermediate	Resistant	Intermediate	Sensitive	Resistant	Resistant	Sensitive	Resistant	YES

Resistant
 Intermediate
 Sensitive

Beta- Lactam (BL), Tetracycline (T), Quinolone (Q), Macrolides (M), Aminoglycoside (AG), Sulfonamides (S).

Figure 2: Antimicrobial susceptibility for Gram-positive bacteria.

In this study, 20 Gram-negative and 7 Gram-positive bacteria were successfully isolated from the gill and intestine of the catfish, *Clarias* spp. The bacteria that may be pathogenic or potentially pathogenic and associated with fish include, *Vibrio* spp., *Aeromonas* spp., *Salmonella* spp. and others (Lipp and Rose, 1997). Humans most

often get infected either through contact with infected fish or orally by consumption infected/raw fish or related products or food contaminated with water or other constituents from water environment (Acha and Szyfres, 2003).

Aeromonas sp. (37.5%) were found to be the most gram-negative bacteria isolated followed by *Vibrio* spp, *Escherichia coli* (16.67%), and other Enterobacteriaceae. Aeromonads are ubiquitous in fresh water, fish and shellfish and it is commonly reflects the distribution of bacteria within the water environment (Isonhood and Drake, 2002). *Aeromonas* sp. also has been recognized as potential foodborne pathogens for more than 20 years.

The presence of *Vibrio* spp. and *V. parahaemolyticus* in samples of freshwater fish in this study suggests that foodborne illness could arise if uncooked or undercooked fish are consumed. Based on this study, three types of *Vibrio* spp. obtained were *Vibrio cholerae* (31.25%), *Vibrio parahaemolyticus* (10.41%), and *Vibrio alginolyticus* (2.08%). This high incidence probably reflects the nature of *Vibrio* spp. that is known to be a halophilic waterborne bacterium and commonly inhabits environmental water sources. It has been found that freshwater rivers as well as brackish water and marine environments may support the growth of these organisms, which are also pathogenic to humans (Janda, 1987). Previous study suggested that fish may act as reservoir and vectors of *Vibrio cholerae* (Senderovich *et al.*, 2010).

Other than *Aeromonas* sp and *Vibrio* spp, Enterobacteriaceae such as *Escherichia coli* and *Shigella* sp. are one of the potential source of bacterial pathogen to humans. *Escherichia coli* is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcus* spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. These organisms should not be present on fresh-caught fish (Chattopadhyay, 2000). Contamination of food of fish origin with pathogenic *Escherichia coli* probably occurs during handling of fish and production process (Ayulo *et al.*, 1994). The occurrence of *Shigella* sp. has nearly always been as a result of contamination of raw or previously cooked foods by an infected, asymptomatic carrier with poor personal hygiene.

The presence of *Listeria monocytogenes* in this study is highly likely due to contamination during processing. As reported by Huss *et al.*, (2000), *L. monocytogenes* is widely distributed in the environment including fresh and coastal waters.

Uncontrolled usage of antibiotics in aquaculture contributes to the emergence of resistant bacteria. Since most prescription in aquaculture sector is done by untrained salesperson of little knowledge regarding disease, the antibiotic prescribed may not be relevant for the disease, consequently causes the emergence of bacteria in the environment such as in the swamps. Resistant bacteria carried by food-producing animals can spread to people, mainly via consumption of inadequately cooked food, handling of raw food or cross-contamination with other food, and directly from the environment (Health Action International Asia Pacific, 2013)

The consumption of food contaminated with resistant bacteria can result in transfer of resistance gene to human pathogens. Human exposed to antibiotic resistant bacteria have higher chances of acquiring resistant bacteria infection. The

implications of antimicrobial resistance include increased frequency and severity of infections and treatment failure. (Heuer *et al.*, 2009).

CONCLUSION

In conclusion, this study showed that the catfish *Clarias* spp. harbours many pathogenic bacteria including *Vibrio cholerae*, *Proteus vulgaris*, *Proteus mirabilis* and *Staphylococcus* sp. that exhibited multidrug resistant trait. It is recommended that highly sensitive, rapid and specific methods such as polymerase chain reaction (PCR) be used for better and more detection of bacteria species. Good sanitation and hygiene during handling, processing, and proper cooking will reduce the possibilities of acquiring infections.

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COMPARISON OF TWO DIFFERENT DISINFECTANTS IN THE INACTIVATION OF NEWCASTLE DISEASE AND INFECTIOUS BURSAL DISEASE VIRUSES

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ABSTRACT

The poultry industry is currently threatened by either endemic or emerging viral diseases associated with major economic losses to the industry. Chemical disinfectants may help to disrupt transmission of viral pathogen; however, many of these disinfectants could pose harmful effects. Hence, there is need to test for alternative disinfectants that has less adverse health effect and do not compromise efficacy. Silver nanoparticles have been studied mainly for their antimicrobial potential; however, it has also shown to be effective against viruses. In this study, the virucidal efficacy of two nanosilver-based disinfectants, product A and product B, against the Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) were investigated. Quantitative analysis using embryonated eggs was used to determine the ability of these disinfectants to inactivate the viruses. Product A has greater virucidal efficacy against IBDV when used at manufacturer's recommended concentration compared to when to its 3-fold dilution. It was concluded that both disinfectants more effective at inactivating enveloped virus than non-enveloped virus and their efficacies are concentration-dependent.

Keywords: nanosilver disinfectant, infectious bursal disease virus, Newcastle disease virus, virucidal efficacy, qualitative analysis

INTRODUCTION

Poultry sector is the biggest component of livestock industry in Malaysia, supplying about 81% of the total meat demand and almost 111% egg domestic demand. The high demand of poultry birds had induced farmers to increase their poultry production by increasing the population of birds. High bird population with improper management may lead to various disease outbreak. In order to control the spread of the pathogens, an immediate control method, such as the use of disinfections, are needed. The objective of disinfection is to prevent disease transmission; thus, an outbreak can be either avoided or controlled. While there is

considerable published information and disinfection efficacy data regarding bacteria and fungi, the efficacy of chemical disinfectants against viruses has not been well studied. The need for a standard disinfectant test against viruses has long been recognised (McDuff and Gaustad, 1976; Chen and Koski, 1983). Mehrbod *et al.* (2009) investigated the antiviral activity of nanosilver and suggested that it has potential as disinfectant in the prevention of viral transmission.

This study was conducted to compare the efficacy of two nanosilver-based disinfectants in the inactivation of Newcastle disease (NDV) and infectious bursal disease (IBDV) viruses and the optimal concentration to inactivate these viruses.

MATERIALS AND METHODS

Sample preparation

Samples from commercially two available nanosilver technology-based products (product A and product B) were obtained and its efficacy in the inactivation of NDV and IBDV was evaluated. Since the information on the exact concentration of product A was not readily available, the product was used at concentrations based on the manufacturer's recommended concentration (MRC). Two concentrations of the products were used; MRC and 3-fold dilution of MRC (diluted MRC).

Disinfectant test protocol

The NDV V4 strain and UPM0081 vvIBDV strain were titred using 50 % embryo infectious dose (EID₅₀). The infectivity titre of NDV has been calculated to be $10^{-9.833}$ EID₅₀/mL, while for IBDV it was $10^{-8.23}$ EID₅₀/mL. The virus suspensions were applied to marked circle areas on the petri dishes and left to stand for 15 min. The circled area was wiped with disinfectant using sterile cotton sticks and the petri dish was left to stand for another 3 hours. One millilitre of deionized water was added into each wiped petri dish and 0.1 mL of deionized water inoculated into each of the 10 eggs. The eggs were incubated at 37°C for 5 days for NDV and 8 days for IBDV.

Data Analysis

The percentage of virus-infected eggs was calculated and the disinfectant efficacy was measured by comparing the average percentage of total virus-infected eggs.

RESULTS AND DISCUSSION

Both products managed to inactivate the NDV inspite of variations in concentrations. However, the virucidal efficacy against IBDV was different between the two disinfectant products. It was shown that product A has the lowest percentage of infected egg (20%) at MRC, which indicates highest virucidal efficacy. In contrast, at MRC of product B the average percentage of infected eggs was 55%, while for diluted MRC it was 63.3%.

Table 2: Disinfectant efficacy against IBDV

Product	(HA+)		(HA-)		Infected eggs (%)		Average Infected eggs (%)
	Post-treatment		Post-treatment				
	R ₁	R ₂	R ₁	R ₁	R ₂	R ₁	
A (MRC)	0	0	10	10	0	0	0
A (Diluted MRC)	0	0	10	10	0	0	0
B (MRC)	0	0	10	10	0	0	0
B (Diluted MRC)	0	0	10	10	0	0	0
Deionized water (virus only)	10	10	0	0	100	100	100

Table 2: Disinfectant efficacy against IBDV

Product	Infected eggs		Non-infected eggs		Total remaining eggs		infected eggs (%)		Average Infected eggs (%)
	R ₁	R ₂	R ₁	R ₂	R ₁	R ₂	R ₁	R ₂	
A (MRC)	2	2	8	8	10	10	20	20	20
A (Diluted MRC)	6	7	3	2	9	9	66.67	77.78	72.22
B (MRC)	6	5	4	5	10	10	60	50	55
B (Diluted MRC)	6	6	3	4	9	10	66.67	60	63.33
Deionized water (virus only)	9	10	0	0	9	10	100	100	100

CONCLUSION

This study revealed that both disinfectant products could inactivate enveloped virus but there were reduced in efficacy against non-enveloped virus. The disinfectant efficiency depends primarily on concentrations.

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ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ROOT PASTE AGAINST BACTERIA ISOLATED FROM CATS WITH GINGIVITIS

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ABSTRACT

Thirty oral swabs were taken from cats with gingivitis from the private clinics in Selangor, Malaysia. The samples were then cultured on the blood agar for bacterial isolation and subjected to biochemical tests for bacterial identification. The highest isolation of Gram-negative bacteria were *Acaligenes faecalis* (22/69 =32%) and *Pasteurella multocida ss multocida* (16/69=23%) while for Gram-positive bacteria were *Actinomyces sp.* (8/69=12%) and *Staphylococcus pseudintermedius* (5/69=7%). *Acalypha indica* root paste was freshly prepared through process of cleaning, rinsing, and grinding. The antibacterial activity of the *A. indica* root paste against all isolated bacteria was determined by the antibiotic sensitivity test. The diameter of inhibition zone of *A. indica* root paste was compared with that of enrofloxacin and amoxicillin-clavulanate treatments. Metronidazole was not included in the analysis because it showed resistant towards all isolated bacteria. The antibacterial activity of *A. indica* root paste was significantly ($p<0.05$) different from that of amoxicillin-clavulanate and enrofloxacin. This study has showed that *A. indica* root paste has antibacterial activity towards bacteria isolated from cats with gingivitis.

Keywords: *Acalypha indica*, antibacterial activity, gingivitis

INTRODUCTION

Gingivitis refers to inflammation of the gingiva or gums and is the most common oral disease in cats and dogs (Craig, 2011). The three stages of gingivitis are mild, moderate and severe. It is considered as the earliest stage of periodontal disease. The oral cavity is rich with microflora that thrives plaque formation on the tooth surface. As food decay, bacteria begin to grow causing formation of pockets of infections within the gingiva sulcus. The infection and debris irritate and destroy the gingival tissue stimulating an inflammatory response leading to reddened and swollen gum (Keith, 2008).

Acalypha indica is a perennial herb from family of Euphorbiaceae and is widely distributed throughout Asia and Africa (Nurul *et al.*, 2016). In Malaysia, it is called

as *galak kucing* or *anting-anting*. It has been traditionally used in humans as an emetic, expectorant, laxative, purgative, anthelmintic and diuretic (Gerai *et al.*, 2011). Studies have showed that *Acalypha indica* plant possess several health benefits such as antibacterial, anti-inflammatory, hepatoprotective, antifungal, anthelmintic, antitussive and for wounds healing (Jagatheeswari *et al.*, 2013). The presence of nutrients and phytochemicals elements has supports the usage of *A. indica* in medicinal uses as an alternative treatment for certain diseases (Nurul *et al.*, 2016).

MATERIALS AND METHODS

Thirty (30) cats that were having gingivitis at the private clinics located in area of Serdang, Seri Kembangan and Puchong were selected for this study. Sterile swab was used to swab on the gingival area. All oral swab samples collected were then proceeded with process of bacterial isolation and identification. Besides, *Acalypha indica* plants were freshly collected from areas in Sri Serdang, Selangor and being identified by Department of Agriculture Malaysia. *Acalypha indica* root was then processed into a paste form before proceeding with antibiotic sensitivity test (AST).

All of bacteria isolated were then adjusted to 0.5 McFarland and were spread on Mueller Hinton (MH) agar for antibiotic sensitivity test (AST). Three holes were made for the placement of *Acalypha indica* root paste (~7 mg) together with commercial antibiotics (positive control) includes enrofloxacin, metronidazole and amoxicillin-clavulanate. Antibacterial activity of *Acalypha indica* root paste against all the three antibiotics were measured and compared. The results obtained were analyzed using two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Out of 30 samples collected, a total of 69 isolates were identified which was narrowed down to 15 types of bacteria. Fifty out of 69 isolates were Gram-negative bacteria while 19 were Gram-positive bacteria. This is supported by study conducted by Williams & Aller (1992) whereby the increase in number of Gram-negative anaerobic bacteria is associated with gingivitis in cats. The highest isolates for Gram-negative bacteria were *Acaligenes faecalis* (32%), followed by *Pasteurella multocida* ss *multocida* (23%). The highest isolates for Gram-positive bacteria were *Actinomyces* spp. (12%), followed by *Staphylococcus pseudintermedius* (7%). The other isolates are as shown in Table 1.

Table 1: Bacteria isolated from cats with gingivitis

Gram-negative bacteria	No. of isolates	Gram-positive bacteria	No. of isolates
<i>Acaligenes faecalis</i>	22	<i>Actinomyces sp.</i>	8
<i>Pasteurella multocida ss multocida</i>	16	<i>Staphylococcus pseudintermedius</i>	5
<i>Pasteurella septica</i>	5	<i>Streptococcus sp.</i>	2
<i>Pasteurella pneumotropica</i>	2	<i>Streptococcus canis</i>	1
<i>Acinetobacter lwoffii</i>	2	<i>Streptococcus pyogenes</i>	1
<i>Pasteurella stomatis</i>	1	<i>Streptococcus intermedius</i>	1
<i>Proteus mirabilis</i>	1	<i>Corynebacterium sp.</i>	1
<i>Chromobacterium sp.</i>	1		
Total isolates	50/69	Total isolates	19/69

Antibacterial activity of *A. indica* root paste against all isolates was determined by conducting an antibacterial sensitivity test (AST) in comparison with three commercial antibiotics includes metronidazole, enrofloxacin and amoxicillin-clavulanate (AMC). These antibiotics works well against both anaerobic and aerobic Gram-positive and Gram-negative bacteria and commonly used as a first and second line defense antibiotics against gingivitis (AIDAP, 2010). Findings have showed that all of the isolated bacteria were sensitive towards *A. indica* root paste. Metronidazole was excluded from the statistical analysis as all the isolates showed resistant towards metronidazole antibiotic (absent of DIZ).

There was significant difference on the diameter of inhibition zone (DIZ) on the isolated bacteria against *A. indica* root paste and commercial antibiotics at $p < 0.05$. *A. indica* root paste showed varying degrees of antibacterial activity against all the microorganisms tested. Among the Gram-positive bacteria tested, *Corynebacterium spp.* was the most susceptible to *A. indica* root paste with DIZ of 17.65mm. Among the Gram-negative bacteria tested, *P. pneumotropica* was the most susceptible to *A. indica* root paste with DIZ of 17.03mm.

The different of antibacterial activities of *A. indica* root paste against tested bacteria could be due to the composition of the bacteria cell wall. Antibacterial activity is more pronounce on Gram-positive due to presence of periplasmic space whereas Gram-negative bacteria is hardier due to the presence of lipopolysaccharide layer which impede the entry of active compounds (Vijayarekha *et al.*, 2015). However, calculated mean of DIZ for *A. indica* root paste (13.80 mm) is significantly different from enrofloxacin (29.20 mm) and amoxicillin-clavulanate (35.50 mm) across all isolates at $p < 0.05$. Therefore, it can be concluded that *A. indica* root paste showed to have antibacterial activity against all the isolates with certain degree of inhibition but could not win over both enrofloxacin and amoxicillin-clavulanate. Presence of phytochemicals compounds such as tannins, alkaloids, saponins, steroids and flavanoids may contribute to the antimicrobial effect of *A. indica* plant (Rajaselvam *et al.*, 2012).

CONCLUSION

Antibacterial activity of *A. indica* root paste was observed in this study against bacteria isolated from cats with gingivitis. Antibacterial activity of *A. indica* root paste is significantly different from enrofloxacin and amoxicillin-clavulanate at $p < 0.05$. However, all isolates showed resistant towards metronidazole antibiotic. Therefore, *A. indica* root paste can be used as an alternative treatment for cats with gingivitis.

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ESTIMATING THE COSTS OF VIBRIOSIS IN THE ASIAN SEABASS (*Lates calcarifer*) CAGE CULTURE IN MALAYSIA

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ABSTRACT

Cage culture of Asian seabass (*Lates calcarifer*) has contributed significantly to Malaysian economy and food security. However, its production has been affected by infectious diseases such as vibriosis that can incur additional operational costs. The objective of this study was to estimate the costs of vibriosis in cage culture of Asian seabass. In the study, it was assumed that vibriosis outbreaks occurred in 4-inch (30-g) fingerlings. Estimation was done in Microsoft Excel®, where total costs of vibriosis was the sum of production losses (mortality and body weight loss (BWL)), dispose cost and treatment cost. Mortality cost was the price of fingerlings (PF) multiply with infection rate (IR) and mortality rate (MR). The BWL cost was the multiplication of weight loss per day when sick, PF, IR, morbidity rate (MB) and sick days. Dispose cost was the time needed to dispose the mortality fish multiply with labour cost, MR and IR. Treatment cost was the drugs costs including labour multiply with number of treatment days, IR and MB. Sensitivity analyses were done for IR, MR, labour cost and PF. Inputs were from farmers, expert opinion and literatures. The results showed that the total cost of vibriosis was RM0.385 per fish, where the largest cost was from mortality at RM0.206. The total cost was sensitive to MR where +55% MR increased the total cost to RM0.495 per fish. This study indicated that the total cost of vibriosis was 2.9% of the total to produce 1kg seabass. This evaluation is hoped to improve the awareness of farmer on the costs of vibriosis in Asian seabass cage culture so that they can improve decisions related to herd health management in the prevent diseases such as vibriosis.

Keywords: Costs, vibriosis, seabass, cage culture

INTRODUCTION

Aquaculture is one of agriculture sectors that is rapidly growing in Malaysia. One of the common aquaculture system that is been practiced in Malaysia is cage culture. According to Kechik (1995), Asian seabass (*Lates calcalifer*) is the main species

being employed in cage cultures. However, in cage cultures, disease occurrence can reduce production of seabass. One of the bacterial diseases in aquaculture that could reduce profitability of cage culture is vibriosis (Thompson and Adam, 2004; Ransangan, 2012). Vibriosis may impact the production system of the level of resources, production, and output (McInerney, 1996). In the grow-out production system of seabass cage culture, the main resources are fingerlings, labour, net cages, feed, and water. Diseases such as vibriosis can cause loss of resources, for example through mortality of fingerlings. Vibriosis can have an impact on the production level by reducing efficiency, feed conversion rate, and farm output. The disease may also affect timely achievement of target bodyweight of the product. Thus, with vibriosis, additional costs, e.g. mortality, treatment, and feed costs, in producing the seabass, will be incurred. There is no estimation on costs of vibriosis in cage cultures yet available in Malaysia. Hence, the objective of our study is to estimate the cost of vibriosis in cage culture seabass to provide awareness to the farmer on the cost of production so that better decisions on herd health management in cage culture of seabass, can be made.

MATERIALS AND METHODS

The estimation of the total costs in vibriosis were done in the Microsoft® Excel software. In this study, we assumed that the infection occurs to the 30-g fingerlings in an average floating net cages of seabass. The estimation can be explained by a biological model (Figure 4).

Biological model

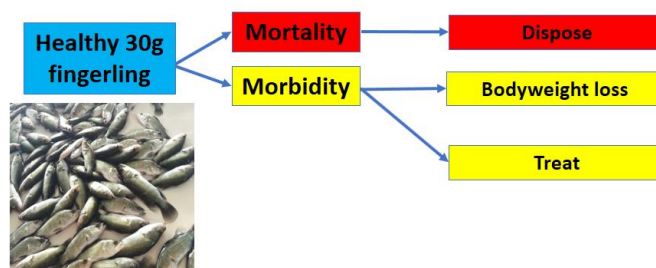


Figure 1: The biological model for seabass production. The assumption is that 30g fingerling are affected by vibriosis causing mortality. Dead fingerlings are disposed while morbid fingerlings assumed to be treated and lose bodyweight.

For the biological model, we assumed that mortality or morbidity occur in 30-g fingerlings when infected with fibrosis. Dead fish are disposed while morbid ones lose bodyweight and orally treated with antibiotic. In this model the 1-mortality rate was used for the estimation morbidity rate.

Economic model

In our study, to calculate the total costs of vibriosis, we included estimations of production-loss, disposal, and treatment costs in the following equations;

$$\begin{aligned} \text{Morality cost} &= \text{PF} \times \text{MR} \times \text{IR} \\ \text{Body weight loss(BWL) cost} &= \text{Weight loss/day} \times \text{PF} \times \text{IR} \times \text{MB} \times \text{Sick days} \\ \text{Dispose cost} &= t_d \times \text{LC} \times \text{IR} \times \text{MR} \\ \text{Treatment cost} &= (\text{O} + \text{LC}) t_t \times \text{TD} \times \text{IR} \times \text{MB} \\ \text{Total costs of vibriosis} &= \text{Morality cost} + \text{BWL cost} + \text{Treatment cost} \end{aligned}$$

Where PF = price of fingerling, MR = mortality rate, IR = infection rate, MB = morbidity rate, t_d = time needed to dispose a fry (minutes), t_t = Time needed to treat a fry (minutes), LC = Labour cost, O = Oxytetracycline used, TD = Number of treatment days.

Materials

Inputs used in this study were based on literature, expert opinion, and a survey made at the Asian seabass cage culture at Pulau Ketam, Selangor and Kerian, Perak, Malaysia. Information acquired were on the management of the farm and cost of feed, labour, and drugs. The inputs used in this study were biological and economical (Table 1). Treatment for vibriosis was by adding 50 g Oxytetracycline/kg fish in the feed for three days. The treatment regimen adopted was based on literature (Pillay and Kutty, 2005), information from the farmer, and the opinion of an expert.

Table 1: The inputs used to estimate the cost of vibriosis.

Item	Unit	Sources
Morbidity rate	1-mortality	Expert opinion
Mortality rate	0.40	Ransangan, 2012
Vibriosis infection rate	0.38	Wong and Leong, 1990
Average body weight loss when sick (kg) ¹	0.0027	Farmer interview,
Number of sick days	10	Pillay and Kutty, 2005
Treatment days	3	Expert opinion
Time (min) to dispose one dead fish	1	Expert opinion
Amount of time to treat (minutes)/fish	1	Expert opinion
Fingerling price/ tail (RM)	1.30	Farmer
Oxytetracycline 1.5gram (RM)	0.23	Pillay and Kutty, 2005
Labour costs per minutes (RM)	0.03	Farmer

¹100% loss* bodyweight gain per day. Assuming adult seabass reach 1 kg bodyweight in 1 year, thus weight gain is 2.7 g/day.

The sensitivity analyses for biological and economic inputs are shown in Table 2.

Table 2: Sensitivity analyses changes of inputs.

Inputs Change	Default Value	Sources
Mortality rate + 55%	40%	Ransangan,2012
Mortality rate - 40%	40%	Ransangan,2012
Infection rate +10%	38%	Expert opinion
Infection rate - 10%	38%	Expert opinion
Fingerling price/tail + 0.20 (RM)	1.30 per tail	Farmer interview
Fingerling price/tail - 0.20 (RM)	1.30 per tail	Farmer interview
Labour cost + RM600/month	1,300 per month	Farmer interview
Labour cost - RM300/month	1,300 per month	Farmer interview

RESULTS AND DISCUSSION

This is a first study that estimated costs of vibriosis in Asian Seabass cage culture in Malaysia. From our survey, the farmer estimated the cost to produce one kilogram of seabass at approximately RM13.00. In this study, it was shown that the total cost of vibriosis in cage culture of Asian seabass was estimated at RM0.385 per fish (Table 3). The cost of vibriosis accounts for 2.9% of the total cost of production. There were several previous economic studies on diseases in finfish aquaculture. In the 1989-1993 outbreak of diseases in grouper, snapper, and seabass cage culture in Malaysia, the estimated cost incurred was USD1.3 million for both the private sector and government farms (Wong and Leong, 1987).

Table 3: The cost component due to vibriosis in Asian seabass cage culture.

Variables	Costs (RM)
Mortality cost	0.198
Treatment cost	0.174
Body weight loss cost	0.008
Dispose cost	0.005
Total costs	0.385

Sensitivity analysis showed when the infection rate decreased by 10% from the default value and the total cost of vibriosis decreased by RM0.10 to RM0.28. When the mortality decreased to 0%, the total cost of vibriosis decreased to RM0.30 (Figure 2). One way to prevent vibriosis is by vaccination. In previous study, in Norway, the cost effectiveness of vaccination against vibriosis using three methods of vaccination was compared (Lillehaug, 1989). It was shown that vaccination by injection was more cost-effective than immersion or oral vaccination. However, this vaccine is to be used

in Malaysia and its cost and benefit in vibriosis is not known.

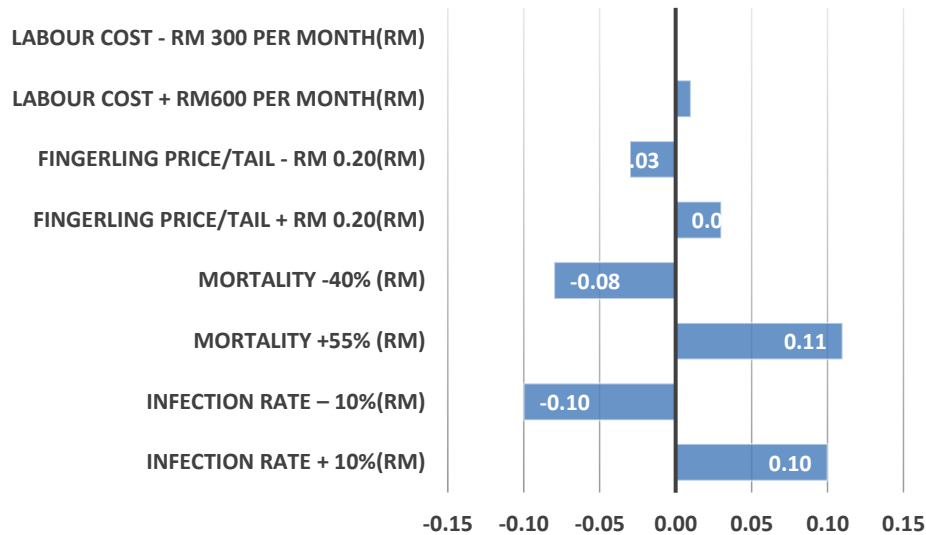


Figure 2: The sensitivity analysis of biological inputs and economical inputs towards total cost of vibriosis of cage culture Asian seabass.

In this study, we assumed that outbreaks occur to 4-inch fingerling. However, vibriosis affect older seabass. A previous study showed that the monthly mortality can range from 30% for 150g fish (medium-size) to 1% for 1 kg weight fish (large-sized) (Haenen *et al*, 2014). Older fish showed higher mortality than fingerlings when affected by vibriosis (Ransangan, 2012; Haenen *et al*, 2014). In this study we estimated the cost of vibriosis in 4-inch fingerlings because in Malaysia fingerlings started to be grow out from that size. It is expected that the cost of vibriosis in in fingerlings is lower than in older fish. The higher cost in older fish is suggested to be due to the higher price of fish, bodyweight loss, infection, mortality, and morbidity rates.

CONCLUSION

Assuming that cage culture produces 1 kg seabass, it is estimated that the total cost of vibriosis in fingerlings is RM0.38/kg seabass. The cost of vibriosis is 2.9% of the production cost (RM13) of 1 kg seabass. It should be noted that this estimation is only for one disease and it could increase with higher infection and mortality rates. The costs of seabass production can be reduced by improving herd health management and reducing the stress of the fish. Although vaccination can be applied to control vibriosis, the costs and benefits of the programme should be estimated.

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THE INFLUENCE OF RACING ON SERUM BIOCHEMISTRY PARAMETERS OF THOROUGHBRED HORSES IN SELANGOR, MALAYSIA

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ABSTRACT

Short duration high intensity thoroughbred racing causes elevations in serum lactate, glucose, creatine kinase (CK), aspartate transaminase (AST) and alanine transaminase (ALT) concentrations. This study was conducted to determine the influence of exercise to these serum parameters on 36 horses competed in 18 thoroughbred track races at distances of 1100, 1200, 1300, 1400, and 1600 m. Blood was sampled via jugular venepuncture, shortly after the races, from horses that won first and third places in the competitions. In the study, it was observed that the mean serum lactate concentrations increased substantially by approximately 30-folds while mean serum AST, ALT, and glucose concentrations increased by 2-folds. However, the mean serum CK concentration remain within the normal range. The substantial increase in serum lactate is due to the increased muscular anaerobic glycolysis to compensate for the high-energy demand of intensity racing in short duration. Serum CK concentration remained within normal range while serum AST and ALT concentrations increased slightly, which are normal for clinically fit horses at post-exercise period. Physiological hyperglycaemia was caused by insulin-antagonistic actions of catecholamines, glucocorticoids, growth hormone, and glucagon during intense physical activities. There was not significant difference in serum biochemical parameters between horses that won first and third places. Similarly, there no difference in value of serum biochemical parameters between race distances, gender, or ages.

Keywords: Serum biochemistry, thoroughbred racing, lactate, performance, anaerobic glycolysis

INTRODUCTION

Thoroughbred racehorses run at high speeds of approximately 64 km/h over distances ranging from 800 to 5000 metres. A large number of physiological and anatomical features act in concert to endow the horse with extraordinary athletic capacity. Maximal athletic performance is dependent upon integrated functioning of these physiological and anatomical features (Hinchcliff *et al.*, 2002; Evans, 1988). However, there are limits to maximal performance of horses, and there is evidence that these limits will be reached, particularly in thoroughbred racehorses (Denny, 2008; Pieramati *et al.*, 2011).

Fatigue is a complex chain of events, with central as well as peripheral contributions. Short-duration, high-intensity exercise performed by thoroughbred racing is not limited by availability of substrates but, more likely, by failure of energy production associated with an increase in protons and a decrease in adenosine triphosphate (ATP). Current studies focus on parameters that could be used to predict horse performances. The information will contribute to the optimisation of equine sports and racing.

Lactate and glucose are metabolic fuels for muscles in exercise. During short duration high intensity exercises, anaerobic glycolysis is the most dominant pathway for energy production and glucose regeneration. Lactate is the product of anaerobic glycolysis and it is also a precursor for further glucose production for muscle metabolism. There is a self-limiting nature in anaerobic power output, thus horse can only maintain maximal speed for distances between 600 to 800 m. At longer distances, the energy supply falls back to slower aerobic pathways, causing the horse to reduce speed or intensity of exercise (Hodgson *et al.*, 1985; McMiken, 1983).

This study was conducted to determine the serum biochemical parameters in horse that achieved first and third placings in thoroughbred track racing and the effect of race distance and gender and age of horses on these parameters. This information could be used to predict performance of horse in races.

MATERIALS AND METHODS

Animals and race

Blood were collected from 36 clinically healthy Thoroughbred horses that won either first or third placing in a two-day track racing at the Selangor Turf Club, Malaysia. The races began at 1:00 pm both on 29th and 30th January 2017 and ended at approximately 6:00 pm each day. The weather was sunny and partly cloudy on both days. In this study, 32 geldings, 4 mares, aged between 4 to 10 years that competed in thoroughbred track racing of which 10 horses participated in 1100 m, 4 in 1200 m, 8 in 1300 m, 8 in 1400 m, and 6 in 1600 m races, were recruited for the study.

Blood sampling

The ambient temperature and resting environment condition were noted. Blood was sampled by jugular venipuncture approximately 10 to 15 min after the completion of

racers. Sampled blood was allowed to clot for 20 min and was centrifuged at $3400 \times g$ for 10 min. Serum was separated and transferred into 1 mL Eppendorf™ tubes and frozen at -20°C before analysis.

Serum biochemical parameters

Serum samples were sent to Veterinary Laboratory Services Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for biochemical analyses. The parameters chosen for this study, lactate, glucose, creatine kinase (CK), aspartate transaminase (AST), and alanine transaminase (ALT) were analysed by chemistry analyser (Siemens Dimension Xpand Plus® integrated).

Statistical analysis

Mean concentrations serum biochemical parameters, race placings, age, gender and race distances recorded and statistically analysed using SPSS® 23 for Windows® Microsoft. The results are expressed as the mean standard error of the mean (SEM). Comparisons of means were made for race placings, gender, age, and race distances.

RESULTS AND DISCUSSION

The average serum biochemistry parameters obtained from the racehorses are as shown in the table below with their respective normal ranges (Table 1). Serum lactate increased substantially, which is the result of production during anaerobic respiration, the primary energy source during short duration high intensity exercises such as in Thoroughbred racing. A transient hyperglycemia could occur post-exercise as a result of insulin-antagonistic actions of catecholamines, glucocorticoids, growth hormone, and glucagon. Creatine kinase (CK) remains within normal range while AST and ALT were slightly elevated. Since, CK was normal, the slight elevations in AST and ALT is from liver cells. There seems no to be muscle insult during the races. This is presumable attributable to physically fitness of the horses. The no difference in serum parameters in horses of different ages and genders or among those participating in different race distances.

Table 1: Serum biochemical parameters in Thoroughbred horses after track races.

	Mean	Reference range	Fold increase
Lactate (mmol/L)	34.16	1.11 – 1.78	~20
Glucose (mmol/L)	11.056	3.3 – 5.5	~2
Creatine kinase (U/L)	383.22	100 – 500	No increase
Aspartate aminotransferase (U/L)	381.56	120 – 160	~2
Alanine aminotransferase (U/L)	17.167	< 10	~2

CONCLUSION

This study showed that lactate would increase significantly in Thoroughbred after track races. The serum glucose, AST, and ALT concentrations increased only slightly in post-race horses. Serum CK concentrations remained within the normal ranges in spite of high intensity race, suggesting that these horses were well-trained and physically fit. This study also showed that there is no significant association between the serum biochemistry parameters between first and third place horses. There was no difference in serum biochemical parameters among horses of different ages and gender, or the race distance they partook.

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**IN VITRO ASSESSMENT OF AQUEOUS AND ETHANOL BETEL
(*PIPER BETLE*) LEAF EXTRACTS ON
BROWN DOG TICK (*RHIPICEPHALUS SANGUINEUS*)**

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ABSTRACT

The brown dog tick, *Rhipicephalus sanguineus*, is found worldwide with high tendency to feed on dogs, other mammals and human and inevitably spreading many types of pathogens. Currently various synthetic chemicals are available to treat the infestation. The main objective of this study was to discover a natural alternative as a potential anti-tick agent. Betel leaves were chosen because it has many phytochemicals. The brown dog ticks were collected from stray dogs compounded at Unit Kawalan Vektor, Dewan Bandaraya Kuala Lumpur, Malaysia, using forceps and kept alive in modified specimen containers. The fresh *Piper betle* leaves were collected from the University's Agriculture Park, Universiti Putra Malaysia. The leaves were firstly dried in a hot-air oven and then grounded using bench-top grinder. The powder was then soaked in ethanol and water dilutions, filtered and dried using the rotary evaporator to obtain crude extract. Two experiments were conducted with ticks exposed to both extracts prepared in dilutions ranging from 5⁰ to 5⁻⁴. The results showed that betel leaves have a significant effect as an acaricide with the lethal dose at one to five dilutions of pure extract. The ethanol extract have higher efficacy killing 26 out of 50 ticks (52%), meanwhile for the water extract killed only 8 ticks of 50 (16%). In conclusion, the betel leaves ethanolic extract showed potential to be used an acaricides for *R. sanguineus*.

Keywords: *Piper betle* leaves, ethanol extract, water extract, *Rhipicephalus sanguineus*,

INTRODUCTION

The brown dog tick, *Rhipicephalus sanguineus* (Latereille, 1806) are arthropods of great significance to human medical and veterinary disease and medicine. The tick feeds on blood for survival, while causing direct damage and transmit various agents of diseases to the host (Oliver, 1989). The disease agents include *Babesia vogeli*, *B. gibsoni*, *Hepatozoon canis*, *Rickettsia conorii*, *R. rickettsii*, *Erlischia canis*, *Anaplasma platys* (Dantas-Torres, 2008; Fourie *et al.*, 2013). For the ticks to complete

life-cycle, they also feed on other mammals including domestic animals and humans, especially when no dogs or food source are available (Lord, 2008). Infestation could increase drastically with just a few ticks in the house or kennels (Figueredo *et al.*, 2006; Dantas-Torres, 2008).

There are various methods to control tick flare, which include chemical control using spot-on formulations, impregnated collars, shampoos, sprays, dips, powders, and various active compounds such as Fipronil, amitraz, carbaryl, and pyrethroids, the commonly used pesticides (Dantas-Torres, 2008; Fourie *et al.*, 2013). However, current trend in pesticides misuse is causing concerns because of environmental pollution and toxicity (Dantas-Torres *et al.*, 2006; Dantas-Torres, 2008). Abuse of pesticide have caused significant pesticide resistance (World Health Organization, 2006). As alternatives, common tropical herbs found in Malaysia have possibility to serve as new sources of cheap, effective, and environmental friendly alternatives to mitigate development of drug-resistant ticks. The betel, *Piperaceae betle*, leaves was selected for this study because it is proven traditionally to have anti-microbial, anti-oxidative, and anti-haemolytic, and anti-parasitic properties (Syahidah *et al.*, 2017).

MATERIALS AND METHODS

Sampling of parasites

One hundred adult ticks, *Rhipicephalus sanguineus*, were collected from stray dogs at the Unit Kawalan Vektor, Dewan Bandaraya Kuala Lumpur, Malaysia using forceps and kept in modified container with multiple small holes of approximately 1 mm size to allow for air-flow.

Extracts preparation

The herb, *Piperaceae betle*, was collected from University's Agriculture Park, Universiti Putra Malaysia (UPM). Fresh and healthy leaves were collected in the morning and evening. The leaves were then washed under running tap water to remove the dirt and debris, and dried in a hot-air oven. The dried leaves were grounded into powder using the mechanical grinder and then dissolved in ethanol and deionised distilled water. The extracts were then filtered, and filtrate dried by rotary evaporation to obtain crude extract.

Experiment

The ticks were divided into two groups to determine the efficacy of aqueous and ethanol *P. betle* leaf extracts. The extracts were first diluted with deionized double-distilled water in 5-folds to obtain concentration of 5^0 , 5^{-1} , 5^{-2} , 5^{-3} , 5^{-4} , and 5^{-5} . Experiment 1 was done by placing 5 ticks onto a petri dish containing a filter paper soaked with extract at various concentrations and the dish covered. In experiment 2, 5 ticks were placed in the Erlenmeyer flask and immersed for 3 minutes in diluted extracts at various concentrations, dried with filter paper and placed in a petri dish.

RESULTS AND DISCUSSION

Betel leaf extract caused 34 of 100 ticks (34%) to die. The ethanol extracts showed greater acaricidal effect, killing 26 ticks of 50 ticks (64%), while the aqueous extracts killed only 8 ticks of 50 ticks (16%). The acaricidal effect could be seen in the first 5 minutes, especially when pure and high extracts concentration were used. Tick mortality spiked during the 30 min to 4 hours of exposure to ethanol betel leaf extract with 22 ticks dead. Only 5 ticks died as the result of treatment with betel leaf water extract. Ethanol betel leaf extract caused 100% (5/5) mortality at 5⁰ and 5⁻⁵ dilutions, while only one of 5 ticks (20%) died with the aqueous betel leaf extract.

CONCLUSION

The study showed that *P. betel* leaf extracts is efficacious as as acaricides for dog brown ticks. This is especially true for the ethanol extract where 100% tick mortality was observed. The aqueous *P. betel* leaf extract is not as potent as an acaricide causing only 20% tick mortality.

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EVALUATION OF TOLL-LIKE RECEPTORS GENE EXPRESSION IN FELINE INFECTIOUS PERITONITIS VIRUS-INFECTED CELL LINE

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ABSTRACT

Feline infectious peritonitis (FIP) is one of the most infectious fatal diseases involving immune-mediated events caused by feline infectious peritonitis virus (FIPV), a highly virulent biotype of feline coronavirus. There are limited studies that characterise the innate immune component of FIP. Toll-like receptors (TLRs) recognition is one of the mechanisms used in innate immunity to detect repeated molecular pattern of pathogens. Thus, this study was conducted to evaluate the modulation of TLR3 and TLR7 genes expression in FIPV-infected cell line. Crandell-Reese Feline Kidney (CRFK) cell line was infected with FIPV 79-1146 at MOI = 0.01 and the expression of TLR3 and TLR7 were measured using Real-Time Polymerase Chain Reaction (qPCR) at 3 and 24 h post-infection (hpi). For both the TLR3 and TLR7 genes, the expression was upregulated at 3 and 24 hpi in comparison to control. In addition, there was a 5-fold significant increase of TLR3 gene expression at 24 compared to 3 hpi. The viral load at the different time points was not measured; however, the infectivity of the virus was confirmed using conventional RT-PCR assay. In conclusion, it appears that both TLR3 and TLR7 were upregulated in FIPV infection *in vitro*, but the correlation between gene expression and kinetics of FIPV infection warrants further examination.

Keywords: feline infectious peritonitis virus, toll-like receptors, Crandell-Reese feline kidney cell line, real-time polymerase chain reaction

INTRODUCTION

Coronavirus is a virus in the family of Coronaviridae of spherical particles of approximately 60–200 nm in diameter, surface projections of about 20 nm long, and estimated molecular mass of 400×10^6 Da. The name ‘coronavirus’ derived from the word “corona” which means crown, refers to the characteristic appearance of the crown-like morphology virions observed under electron microscope. Feline infectious peritonitis (FIPV) is one of the sub-groups under FCoV that have the ability

to develop the lethal disease feline infectious peritonitis (FIP), especially in young cats. Early detection of invading microorganism by innate immunity is crucial in viral elimination. Innate immunity acts as a front-line of the host defense against pathogen through germline encoded pattern-recognition receptor (PRRs) which includes Toll-Like Receptors (TLRs) and cytoplasmic receptors. To date, there is no study that determined TLRs gene expression in FIPV infections. Therefore, this study was conducted to determine and characterise a component of innate immunity in FIPV infection.

MATERIALS AND METHODS

Virus Infection

A total of 3×10^5 feline kidney (CrFK) cells were seeded into two 6-well plates containing MEM supplemented with 10% FBS and 1% penicillin-streptomycin-glutamine (PSG). Two time-points (t.p), 3 and 24 h were identified and three biological replicates used for each t.p. Uninfected CRFK cells served as control. The cells were incubated at 37°C, under 5% CO₂ for 48 h. Upon confluence, the cells were infected with 100 µL of virus at an MOI of 0.01. Complete media was added to the uninfected wells. The plates were incubated at 37°C under 5% CO₂ for 1 h to allow virus adsorption. Excess media was completely aspirated and the cells were washed excessively using 1X PBS to remove any traces of non-adhered viral particles. New MEM media containing 1% FBS and 1% penicillin-streptomycin-glutamine (PSG) was added to the remaining wells and the plates incubated at 37°C under 5% CO₂.

RNA Extraction and Real-Time PCR

Total RNA extraction was performed on the samples following manufacturer's recommendations (Machary-Nagel) and stored in -60°C freezer. 1 to 2 µg of total RNA was used to synthesise cDNA with a final volume of 20 µL. The reactions for real-time PCR were performed using SensiFAST™ SYBR No-ROX One Step kit (BioLine Ltd, UK). Then, 2 µL of cDNA was mixed with 1X Sensifast Probe No-Rox Mix, 400 nM of forward and reverse primer for TLR3 or TLR7 or GAPDH, 100nM of probe for TLR3 or TLR7 or GAPDH (Ignacio et al., 2005), and sterile deionized distilled water to a final reaction volume of 20 µL (BioLine). The reaction was done in triplicates for each biological replicate. The reactions were run on Bio-Rad CFX96™ Real-Time PCR, with a heat cycle protocol of 40 cycles of 95°C for 15 s for denaturation, 55°C (TLR3) or 54°C (TLR7 and GAPDH) for 15 s for annealing, and 72°C for 10 s for amplification.

Conventional PCR

Infectivity of the virus was confirmed using conventional PCR assay. The reactions were made by mixing 2 µL of cDNA with 2X Sensifast Probe No-Rox Mix, 10 µM of forward and reverse primer for 7b gene (Gut *et al.*, 1999), 10 µM of probe, and sterile deionized distilled water to reach final reaction volume of 50 µL (BioLine). The reactions were run on a thermal cycler (C1000 Touch™ Thermal Cycler,

California) and the PCR reaction conditions were as the following; 35 cycles of 95°C for 15s for denaturation, 52°C for 15s for annealing, and 72°C for 10s for amplification. The DNA fragment was determined using 2% agarose gel and visualized using Bio-Rad Gel Doc™ (USA).

Statistical Analysis

GraphPad Prism® software (GraphPad Software Inc, USA) was used to perform statistical analysis. The comparison of TLR3 and TLR7 gene expression at different time points was analysed using one-way ANOVA with $P < 0.05$ considered significance.

RESULTS AND DISCUSSION

Significant ($p < 0.0001$) upregulation of TLR3 gene expression at different time points was observed. There was significant upregulation in TLR3 gene expression between control and 3 hpi ($p = 0.0057$), control and 24 hpi ($p < 0.0001$), and 3 and 24 hpi ($p < 0.0001$) (Figure 1). Similarly, there is significant upregulation of TLR7 gene expression at different time points when compared to control ($p < 0.0001$) and between control and 3 hpi ($p = 0.0057$) and control and 24 hpi ($p < 0.0001$). However, no significance difference in TLR7 gene expression was observed between 3 and 24 hpi ($P < 0.0001$) (Figure 2).

Upregulation of TLR3 and TLR7 gene expressions began at 3 hpi and particularly the TLR3 expression became markedly elevated at 24 hpi with a 5-fold increase over the control. This is in agreement with a study conducted by Dewerchin *et al.* (2005). They observed appearance of viral antigen in positive-infected cells as early as between 3 to 6 hpi. In addition, there was a significant increase in infected cells between 12 to 24 hpi. In fact, 86% of the cells may show cytoplasmic expression of viral antigen and 75% surface expression at 24 hpi.

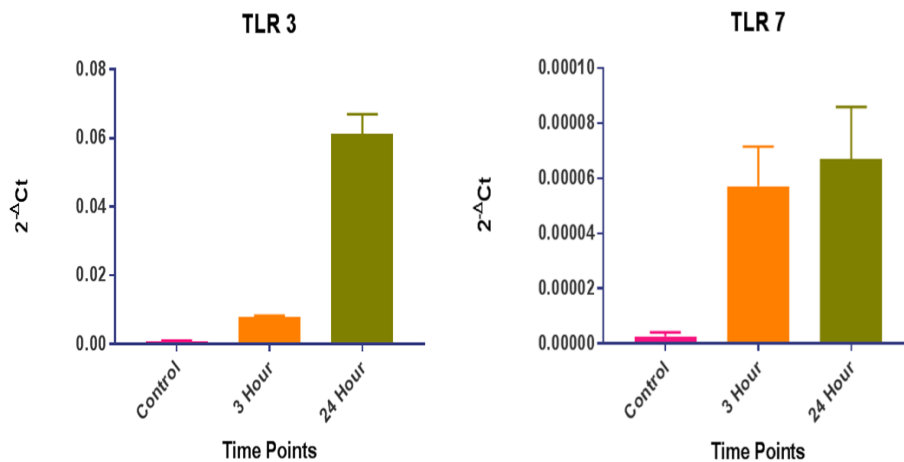


Figure 1; TLR3 and TLR7 gene expression in feline kidney (CrFK) cells

The TLR7 gene expression in CrFK-infected cell line was much lower compared to the TLR3 gene expression. This result is consistent with study by Ignacio *et al.*, (2005) that showed TLR7, not TLR3, gene was underexpressed by the CrFK cells. In addition, TLR3 and TLR7 were found to be highly expressed in the feline T-cell line, MCH5-4 and Mya-1.

Virus infectivity was confirmed by conventional PCR where presence of virus was detected at 3 and 24 hpi. This indicates the upregulation of TLR3 and TLR7 gene expressions were due to the FIPV infection. The intensity of the band was lower at 24 hpi than at 3 hpi, indicating there is reduction in viral replication at 24 hpi, a result similar to that of Law *et al.*, (2005), where the replication of SARS-CoV virus was shown to peak at 3 hpi and decline at 9 to 24 hpi.

CONCLUSION

In conclusion, this study showed that there is significant upregulation in TLR3 and TLR7 gene expressions in the FIPV-infected cell line.

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PREVALENCE OF GASTROINTESTINAL NEMATODES AMONG HORSES FROM VARIOUS ESTABLISHMENTS IN MALAYSIA

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ABSTRACT

A study was carried out to determine the prevalence and identify the types of gastrointestinal nematode (GIN) in horses from various establishment in Malaysia. A total of 100 horses of various signalments were selected for deworming programme. Fresh faecal samples were collected for McMaster faecal egg count (FEC). Positive samples were cultured to determine the genus of the nematodes. It was found that 38% of samples were positive for GIN eggs, most frequently strongyle eggs. There was significant ($p < 0.05$) differences in GIN prevalence among establishments. The species identified included *Trichonema spp* (53%), *Ascaris sp* (5%), *Trichostrongylus sp* (21%), *Strongyloides sp* (12%), *Strongylus sp* (2%), and *Poteriostomum sp*. (2%). Adult horses aged 16 ro 20 years were most affected with GIN. Female horses were more and highly affected by GIN than males. However, there was no significant ($p > 0.05$) association between GIN and gender. Horses treated with irregular deworming program and with Oxfendazole prior to the study were highly infected with GIN. There was significant association between age of horses, deworming program and gastrointestinal nematode infection.

Keywords: anthelmintic, faecal culture, gastrointestinal nematode, horses, helminth, McMaster faecal egg count

INTRODUCTION

Parasitism is the single most important impediment to successful horse rearing and horses are can be infected with many species of parasites. Large and small strongyles are the significant pathogens of horses. Ascarids, thread worms, hair worms, pin worms and tapeworms are found naturally in horses (Urquhart *et al.*, 1996). *Parascaris equorum*, *Trichostrongylus axei*, *Strongylus equinus*, the Cyathostominae, *Oxyuris equi*, *Probstmayria vivipara*, *Strongyloides westeri*,

Habronema microstoma, *H. muscae* and *Drascheia megastoma* are the some of the parasites also commonly found in horses (Foreyt, 2001).

Studies on prevalence of horse helminths in different parts of world have suggested that prevalence vary with different management and parasite control systems (Capewell *et al.*, 2005). Frequent and widespread application of the anthelmintics to horses at the intervals based of strongyle reappearance periods had substantially decrease incidence of clinical diseases, especially those associated with large strongyle species. However, this practice had lead to anthelmintic resistance, particularly by the cyathostomin species (Matthews, 2014).

There are very few studies on the occurrence of gastrointestinal parasites in horses in Malaysia. A field survey on ivermectin and mebendazole treatment against GIN in stable horses in Selangor, Malaysia showed that prevalence rate of GIN in horses from the two stables surveyed was very low at 3.2% and this is mostly by *Cyathostominae* spp. (Rohanizal, 2011).

The understanding of the epidemiology of horse helminths under local management and climatic conditions will help in devising effective and economically viable parasite control programmes. Therefore, this study determined the prevalence and genus of nematode and the strongyles shedding status in horses under various managements and deworming practices. It is expected that this study will help professionals in selecting more effective parasite control programmes for equine horses in the tropics.

MATERIALS AND METHODS

Horses and faecal examination

A cross-sectional study on 100 domestic horses from ten establishments in Selangor and Putrajaya, Malaysia with different deworming practice were used in the study. These stabled horses were of various age, sex, and breed. The horses that had dewormed a minimum of one week from the last deworming were selected. Faecal sample, freshly voided from rectum, was collected, sealed in sample bags with minimum air content, and kept refrigerated under 4°C until analysis. Faecal examination was done both quantitatively using modified McMaster faecal egg count (FEC) and qualitatively using faecal culture to detect infective stage of larvae (L3). The identification of the larval species was by gross morphology, number and shape of the intestinal cells. (Zajac and conboy, 2012).

Statistical Analysis

Chi-Square test was used to determine association between prevalence and breed, gender, and age at 95% confidence level. All statistical analysis was performed using SPSS 16.0 for Windows.

RESULT

Faecal Egg Count

The overall prevalence of GIN in horses in 10 different managements (A to J) around that 38 samples out of 100 samples were positive primarily for strongyle and ascarids. All the establishments were infected with GIN with low to moderate FEC (Figures 1 and 2). Among age groups, horses in 16 to 20-year age group showed the highest GIN prevalence while those in the 6 to 10-year age group showed the lowest prevalence at (Table 1). Between gender, female horses have higher prevalence than males (Table 2). Warmblooded horses shows highest GIN prevalence among breeds (Table 3). However, there were no significant ($p>0.05$) association between gender and breed and nematode infection. All the horses dewormed at irregular intervals were positive for nematode infection compared to those dewormed regularly at 3 and 6 months intervals (Table 4). Horses treated with Oxfendazole prior to this study show the highest GIN prevalence (Table 5).

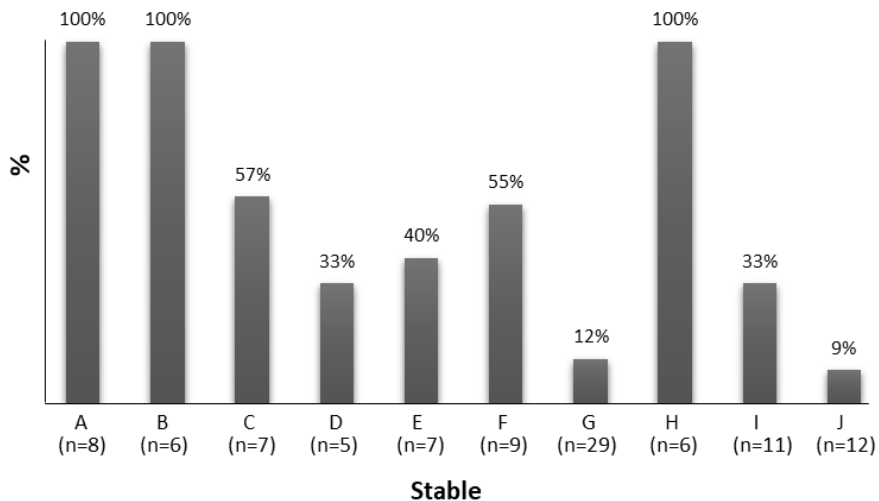


Figure 1: Prevalence of gastrointestinal nematode infection in horse establishments.

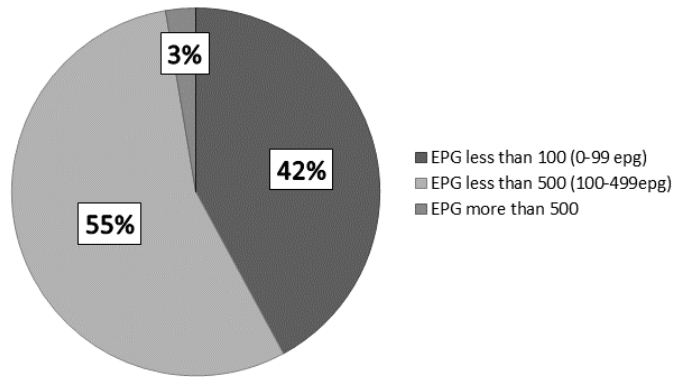


Figure 2: Distribution of Faecal egg count (FEC) in horses.

Table 1: Age-related prevalence of gastrointestinal nematodes in horses based on faecal egg count.

Age group (years)	Number of horses	Positive faecal samples	
		Number	%
1 to 5	12	4	33.3
6 to 10	31	6	19.4
11 to 15	29	13	44.8
16 to 20	28	15	53.6
(Chi-square) P-value		P=0.043	

Table 2: Gender-related prevalence of gastrointestinal nematode in horses based on faecal egg count.

Gender	Number of horses	Positive faecal samples	
		Number	%
Male	60	19	31.7
Female	40	19	47.5
(Chi-square) P-Value		P=0.257	

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Table 3: Breed-related prevalence of gastroneematode in horses based on faecal egg count.

Breed	Number of horses	Positive Samples	
		Number	(%)
Thoroughbred	39	10	25.6
Polo pony	34	16	47.1
Arab	13	6	46.2
Coriollo	4	1	25.0
Warmblood	10	5	50.0
(Fisher exact) P-value		P=0.29	

Table 4: Deworming regime-associated prevalence of gastrointestinal nematode in horses based on faecal egg count.

Deworming regime	Number of horses examined	Positive Samples	
		Number	%
3 months once	37	9	24.32
6 months once	56	22	39.29
Irregular	7	7	100.0
(Chi-square) P-value		P=0.001	

Table 5: Deworming program-associated prevalence of gastrointestinal nematode in horses base on faecal egg count.

Drug	Number of horses examined	Positive Samples	
		Number	%
Oxfendazole	11	8	72.7
Abamectin+Praziquantel	14	6	42.9
Pyrantel	18	8	38.9
Ivermectin+ Praziquantel	28	9	17.9
Moxidectin	12	5	35.7
(Chi- square) P-value		P=0.0001	

Identification of infective (L3) stage

Faecal culture revealed the prevalence of 7 types of genus in these stables (Figure 3). The identification of these larval species was based on gross morphology and number and shape of the intestinal cells (Zajac and Conboy, 2012). The L3 were *Trichonema spp* (53%), *Ascaris sp* (5%), *Tricostrongylus sp* (21%), *Strongyloides sp* (12%), *Strongylus sp* (2%), and *Poteriostomum sp.* (2%).

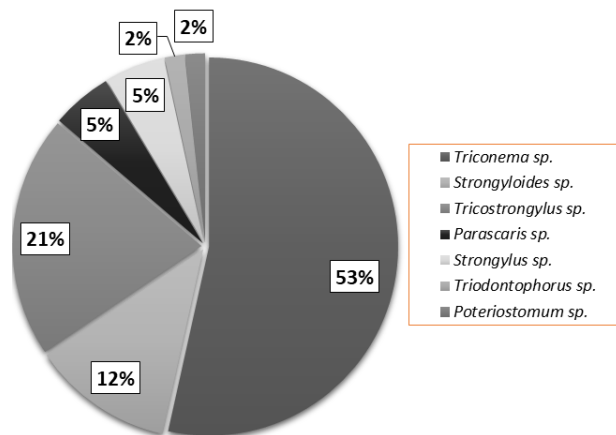


Figure 3: L3 in faecal culture from horses.

DISCUSSION

There were only a few studies on the prevalence of gastrointestinal parasitism in horses in Malaysia. One study reported the prevalence of gastrointestinal parasitism in local indigenous ponies in Kelantan was 43.97% (Mimi, 1999). All the establishment tested was positive for GIN; in fact, some establishment had 100% prevalence. This is probably due to poor sanitation in some stables where the contaminated manure remained cleared even after 3 days. Although all horses in this study were stabled, they are allowed graze a few hours on the same pasture each day. Therefore, they were at risk of acquiring infection from contaminated pasture and horse manure. It was shown that horses from herds using the same areas to grazing has high prevalence of nematode infection (Ionita *et al.*, 2013). Most horses in this study were low and moderate shedders. It is recommended not to deworm Low shedders. However, moderate shedders should be dewormed with higher doses of anthelmintics (Burk *et al.*, 2013).

Adult horses aged 16-20 years old were most affected by GIN among age groups. According to AAEP, in any group of mature horses age > 3 years, strongyle egg counts are high in certain horses only. Thus only 20 to 30% of adult horses usually shed approximately 80% of the eggs. Female horses showed GIN infection than males. However, there were no significant association between gender and prevalence of GIN. It seems that the physiological state of horses does not influence the worm egg production (Daniel, 2007).

Irregular deworming causes horses to be more prone to be infected than horses dewormed regularly every 3 and 6 months. The irregularity in dewormed results in large parasite residing in the intestines (Loving, 2014). Horses dewormed with Oxfendazole has highest GIN infection; this suggested to be due to resistance of nematodes, especially Cyathostomins, to anthelmintics. One study showed in horses in Selangor, Malaysia, that there was only 65% reduction of FEC with the use of mebendazole FECRT among (Rohanizal, 2011). Cyathostomin resistance towards Benzimidazole has been reported in several other countries (Herd, 1990). Even though the nematode resistance towards Macrocyclic lactones has never been shown, a few horses previously treated with Ivermectin and Abamectin were infected with GIN. This is probably due to reappearance of eggs after the effect of the drugs has worn off (Nielsen, 2010a).

In this study, faecal cultures showed that the most prevalence subfamily of nematode is the cyathostomins and primarily of the *Triconema* and *Poteriostomum* spp. This is also possibility that resistance to benzimidazoles and tetrahydropyrimidines had developed in the cyathostomin population (Herd, 1990; Kaplan *et al.*, 2004; Andersen, 2013).

CONCLUSION

Prevalence of GIN among horses in horse establishments in Malaysia is 38%. Cyathostomin is the most prevalent nematode in these horses. The prevalence of GIN in these horse is suggested to due resistance to dewormers frequently used in these

stables. There was significant association between deworming programme, age group and GIN infection.

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SERUM CORTISOL AND NEUTROPHIL TO LYMPHOCYTE RATIO BEFORE AND AFTER RECTAL PALPATION IN SWAMP BUFFALO COWS (*BUBALUS BUBALIS CARABANENSIS*)

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ABSTRACT

Rectal palpation is one of the veterinary procedures used to diagnose pregnancy in buffalo cows. However, this procedure is painful and stressful to the animal. Stress is one of the important factors in the welfare of animals. The objective of this study is to evaluate stress response to rectal palpation by comparing serum cortisol concentration and neutrophil to lymphocyte (N:L) ratio before and after rectal palpation in a buffalo cows. Blood was collected from the coccygeal vein before and directly after rectal palpation from 3 non-pregnant and 12 pregnant swamp buffalo cows aged between 5 to 8 years old and weighing 300 to 400 kg. The serum cortisol concentration and N:L ratio was determined via Radioimmunoassay and differential count, respectively to evaluate stress response to the procedure. The result revealed that there is significant increase in the serum cortisol concentration following a rectal palpation in pregnant and non-pregnant buffalo cows. However, there no significant different between pregnant and non-pregnant buffalo cow in stress response to rectal palpation. There is also no significant increase in N:L ratio in both groups of buffalo cows following rectal palpation. Thus, this study shows that rectal palpation in buffalo cows causes stress and the pregnancy status does not seem to influence the response to rectal palpation.

Keywords: rectal palpation, buffalo cows, stress, neutrophil to lymphocyte ratio, cortisol level

INTRODUCTION

Buffaloes are valuable species as their meat, milk and horns can be exploited (de la Cruz-Cruz 2014). Due to its multiple purposes, there is a high demand for buffaloes. Reproductive management is important in animal breed to increase population. In

reproductive management, rectal palpation is one of the methods that is used farms to determine the success of the breeding programme. This technique is frequently used to diagnose early pregnancy because it is the most convenient method and fast to perform. Although rectal palpation is a non-traumatic procedure but it can be painful and cause stress to the animals (Cingi *et al.*, 2012).

Stress in animals can be reflected through physiological changes such as increase in the stress hormone, cortisol, level in blood. Physiological stress can also be assessed through haematological parameters such as total and differential leucocyte differential counts. This is because cortisol affects the function and migration pattern of leukocytes that affects their blood counts. Increase in cortisol level during stress response will cause neutrophilia and lymphopenia. Therefore, the increase in neutrophil to lymphocyte (N:L) ratio can be used to determine stress level, corresponding to the increase in serum cortisol (Davis *et al.*, 2008).

Rectal palpation may cause pain and induce stress in buffalo cows and this is of concern to the welfare of animals. Thus, this study is conducted to determine serum cortisol concentration and the N:L ratio, as stress indicator, in swamp buffalo cows (*Bubalus bubalis carabanensis*) before and after rectal palpation, in the effort method to least stress procedure to determine pregnancy.

MATERIALS AND METHODS

Sample and data collection

This study was conducted at Telupid Buffalo Breeding and Research Centre in Sabah, Malaysia. The animal used were 3 non-pregnant and 12 pregnant buffalo Swamp buffalo cows, age 5 to 8 years and weighing 300 to 400 kg. The buffaloes were reared with free grazing and fed with supplement. To perform rectal palpation, the buffalo cow was first restrained with minimal stress, using the crusher. The rectal palpation was done for approximately 3 minutes for each buffalo cow. Blood samples were collected by coccygeal venepuncture using 18 G needles before and directly after the procedure. Blood samples in the EDTA tubes was used for make blood smears and in plain tubes to obtain serum.

Neutrophil to Lymphocyte Ratio

Thin blood smear was made on the histological slide at the farm and stained with wright's stain. The slides were examined under light microscopy under 400× magnifications and differential count performed on 200 leucocytes to obtain the neutrophil to lymphocyte (N:L) ratio.

Cortisol

The blood in plain tubes was centrifuged for 15 minutes at 300 × g, the serum for cortisol determination using the Cortisol Radioimmunoassay kit (Beckman Coulter).

Statistical Analysis

Data were analysed using SPSS version 22. Paired T-test was used to determine differences in data between pregnant and non-pregnant buffalo cows before and after rectal palpation at $\alpha=0.05$.

RESULT AND DISCUSSION

Before rectal palpation the mean serum concentration was 9.58 ± 2.31 and 7.45 ± 3.33 nmol/L in pregnant and non-pregnant buffalo cows, respectively. After rectal palpation the serum cortisol level increased significantly ($P<0.05$) to 23.33 ± 5.89 and 37.28 ± 14.26 nmol/L in pregnant and non-pregnant groups, respectively. By the increase in serum cortisol level, the results show that rectal palpation induces significant stress to both pregnant and non-pregnant buffalo cows. It is assumed that the stress in the procedure is due pain.

Non-pregnant buffaloes had insignificant ($p>0.05$) higher serum cortisol concentration than pregnant buffaloes group at 13.75 ± 5.36 and 29.82 ± 14.56 nmol/L, respectively. The study shows that the responsiveness to rectal palpation did not differ between pregnant and non-pregnant buffalo cows.

There was insignificant ($p>0.05$) increased in N:L ratio following rectal palpation in buffalo cows. The mean N:L ratio before rectal palpation was 1.37 ± 0.22 and 0.81 ± 0.11 in the pregnant and non-pregnant buffalo cows, respectively and rectal palpation the value increased to 1.66 ± 0.26 and 0.98 ± 0.18 , respectively. This increase was not significant. From this study, it is suggested that the acute stress induced by rectal palpation does not cause significant increase in N:L in blood samples taken immediately after the rectal palpation. This may be due to sampling being done too early after rectal palpation for stress to be reflected as change in leucocyte counts because in terms of response time, the N:L ratio tends to lag behind serum cortisol. Serum cortisol level is a good indicator of acute stress while the N:L ratio can be used as an indicator for acute stress if determined at the appropriate time.

CONCLUSION

Irrespective of pregnancy status, rectal palpation induces stress to the animals, as shown by the increase in serum cortisol concentration and possibly N:L ratio.

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INVESTIGATION INTO THE CONTRIBUTING FACTORS TO IMPROVE THE DAIRY AND MEAT CATTLE PRODUCTIVITY

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ABSTRACT

In Malaysia, both dairy and beef cattle industry are mainly operated by smallholder farmers with low input and output of the farm. Thus, importation of exotic cattle breed into the country was introduced. However, due to lack of improvement on animal husbandry and feeding system, increase in milk production did not fulfilled expectations. One of the factors in the improvement of livestock industry is by practicing proper farm management in the aspects of housing, health management, nutrition and reproductivity of the animals. Thus, investigations on the management system of dairy and beef cattle practiced by smallholder farmers should be done so as better plans and recommendations can be made for more systematic management practices for the improvement of farm productivity. This study was conducted on the Universiti Putra Malaysia foster and non-foster smallholder farms. A survey of 10 farms including dairy and beef farms was done through a questionnaire and interviews as well as obtaining relevant data from the University veterinary hospital (UVH). In general, foster farms and non-foster farms adopt almost the same management practices. However, most farms lack proper data keeping on the reproductivity and productivity, health management practices, and sanitation of the farms. The study shows that farms management practiced by smallholder farmers have weaknesses that can be improved through proper recording system and good animal husbandry practices.

Keywords: livestock, smallholder farmers

RELATIONSHIP OF SCROTAL CIRCUMFERENCE AND TESTICULAR VOLUME TO AGE AND BODY WEIGHT OF DORPER SHEEP

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ABSTRACT

This study was conducted to evaluate reproductive parameters that can be used as indicators to estimate fertility status of Dorper sheep. Scrotal circumference and testicular volume were the reproductive parameters measured to correlate with age and body weight. Thirty-Two Dorper rams were divided into two groups: Group A (< 12 months old) consisted of 6 rams and Group B (\geq 12 months old) with 26 rams. The scrotal circumference of each ram was measured. The length, width and height of left and right testes were also measured to calculate testicular volume. All parameters were positively correlated to each other. The scrotal circumference and testicular volume are more correlated to body weight compared to age. The scrotal circumference is positively correlated with age ($r=0.605$), body weight ($r=0.824$) and testicular volume ($r=0.925$). The testicular volume is positively correlated with age ($r=0.535$), body weight ($r=0.873$) and SC ($r=0.925$). This study shows that the Dorper rams >12 months old, with body weight at least 45 kg have higher scrotal circumference and testicular volume. Therefore, age, body weight, scrotal circumference and testicular volume can be used in selection of breeder sires.

Keywords: Dorper sheep, scrotal circumference, testicular volume.

RELATIONSHIP BETWEEN INTRAPELVIC AREA AND EXTERNAL MORPHOMETRY IN FRIESIAN CROSS CATTLE

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ABSTRACT

Dystocia causes adverse effects to dam and calf that results in a significant economic impact to the farmer. Small pelvic area in dams is an important contributor to dystocia causing a physical incompatibility with the foetus or foeto-pelvic disproportion. The relationship between intrapelvic measurements and external morphometric measurements was studied in 50 Friesian cross cattle to determine the usage of external measurements as predictors for intrapelvic parameters. External morphometric parameters taken were thoracic circumference, abdominal circumference, distance between tuber coxae and distance between tuber ischii. A Rice pelvimeter was used to measure the intrapelvic parameters of pelvic height (PH, cm) and pelvic width (PW, cm). The pelvic area (PA, cm²) was calculated using the formula $PA = PH \times PW$. Pearson's correlation showed that all external morphometric measurements were correlated with intrapelvic measurements ($0.43 < r < 0.60$, $P < 0.05$). Regression analyses showed that the internal pelvic parameters can be predicted from body weight and external morphometric measurements. In conclusion, there was an association between the intrapelvic measurements and the external morphometry and by using the models derived, the intrapelvic measurements can be predicted from the external morphometry.

Keywords: dystocia, Friesian cross, cattle, intrapelvic measurements, external morphometry, pelvimeter, pelvic area

**GROSS AND HISTOPATHOLOGIC EVALUATION OF THE
RESPIRATORY ORGANS OF MICE EXPOSED TO AEROSOLISED
PASTEURELLA MULTOCIDA TYPE B:2**

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ABSTRACT

Haemorrhagic septicaemia (HS) is an acute, fatal septicaemic disease in cattle and buffalo caused by *Pasteurella multocida* type B:2 in Malaysia. Haemorrhagic septicaemia is naturally transmitted via aerosol and the respiratory route. This study describes pulmonary lesions in mice inoculated with aerosolised *P. multocida* B:2. Thirty mice were divided equally into 6 groups; 1 control and 5 treatment groups. Treatment groups were treated with to 1×10^9 CFU/mL aerosolised *P. multocida*. Mice from each treatment group were euthanised every 6 h, at 0, 6, 12, 18, and 24 h post-infection (pi), respectively. Control mice were euthanised 6 h prior to inoculation and the clinical signs and gross and histopathologicals lesion recorded. Reduction in mice activities was observed at 12, 18, and 24 h pi. Gross and histopathological lesion that occurred at varying degrees included inflammatory cell infiltration, haemorrhage, and congestion. The severity of inflammatory cell infiltration, haemorrhages, and congestion, beginning 0 h pi, increased significantly in infected mice. PCR analyses of lung tissue samples were positive for *P. multocida* B:2 at 0 and 24 h pi. In conclusion, this study showed that experimental infection with *P. multocida* B:2 via aerosol mimics natural infection.

Keywords: mice, *Pasteurella multocida* B:2, aerosol

EFFECTIVENESS OF EQUINE-ASSISTED THERAPY IN MALAYSIA

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ABSTRACT

Equine-assisted therapy (EAT) is an effective treatment alternative or strategy for a number of psychological disorders in humans. Equine-assisted therapy has the capacity to deliver positive outcomes to children, adolescents, and adults with mental and/or physical health problems. However, the conduct of this therapeutic programme is limited in Malaysia, since the community prefers physiotherapy and medicinal treatments. Thus, the purpose of this study is to determine the effectiveness of EAT in Malaysia, in terms of improving the health condition of patients with various psychological problems. Five trained horses from the PRTC Equestrian Club, Perak, Malaysia were used for riding, socialising, and cognitive effects on patients with different background of physical and mental disabilities, to include Down syndrome (DS), autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD). The behaviour and capability performances of patients (n=5) such as cognitive functions, physical and social were monitored before and after participating in three sessions of the programme. The behaviour data from these sessions were compared using Mann-Whitney U Test analysis. The study showed that the condition of DS, ASD and ADHD patients after participating in the programme improved, indicating that the EAT programme has positive effects. In addition, this study showed that the use of horses with respect to physiological and anatomical movement in the treatment of human conditions, positively impacts patient's physical and mental capabilities. Thus, the EAT programme should be further developed in Malaysia in order to offer a better alternative in the treatment and cure the psychological disorders in humans.

Keywords: equine-assisted therapy (EAT), horses, patients, psychological disorders

SEROPREVALENCE OF ORF VIRUS INFECTION IN SMALL RUMINANTS OF UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

Orf is an infectious disease in sheep and goats that affects skin and general productivity. This study aimed to determine the current status of orf infection among small ruminants in the state of the Selangor, Malaysia. IgM antibody titre, which is employed as an indicator of recent infections were estimated and associated risk factors assessed in this study. Serum samples were collected from 90 goats and 90 sheep of the Universiti Putra Malaysia foster farms. Qualitative enzyme-linked immunosorbent assay (ELISA) was used to estimate IgM antibody titre. The results showed that 33 goats (36.7%) and 7 sheep (7.8%) were positive for orf IgM antibody, indicating that recent infection is more prevalent among goats than sheep. Chi-square test showed that the risk factors that may predispose to orf infections were different for goats and sheep. For goats, the risk factors are species, farm location and history of orf, while for sheep there were species, age, gender, presence of clinical signs, farm location and farm type. In conclusion, this study showed that a significant number of goats of the foster farms already harbour active orf infection in spite of few risk factors. Sheep had fewer recent orf infections in spite of many risk factors.

Keywords: Orf virus, sheep, goat, Ig M antibody, ELISA

SEROPREVALENCE OF BLUETONGUE VIRUS INFECTION AMONG SMALL RUMINANT IN FOSTER FARMS OF UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Bluetongue disease is a notifiable disease listed by the World Organisation for Animal Health (OIE) where it can cause significant problem to the ruminant industry especially sheep. Sheep usually develop clinical signs; however, the same clinical signs can occasionally occur in other ruminants. There is lack of information the status of this disease in goats of the Universiti Putra Malaysia foster farm. In Malaysia, the last known report of this disease was in 1995. Therefore, this experiment was designed to determine the seroprevalence of bluetongue infection among small ruminants in UPM's foster farms and to determine the risk factors associated with the seroprevalence of the disease. Blood samples were collected from 100 goats regardless of their age, breed and gender. The blood serum samples were used to detect the antibody towards Bluetongue virus (BTV) by ELISA. The results were negative for all samples. The zero seroprevalence of bluetongue in this study could be due to the prevention measures implemented by the Department of Veterinary Services (DVS) based on the OIE guidelines. Thus, the goats from UPM's foster farms are free from Bluetongue.

Keywords: Bluetongue, ruminant, goat, outbreak, seroprevalence, blood serum, ELISA.

DETECTION OF LEPTOSPIROSIS IN A DOG SHELTER

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ABSTRACT

Leptospirosis is a zoonotic bacterial disease of worldwide distribution with more than one million human cases reported annually. Limited study has been conducted on canine leptospirosis in Malaysia despite their high risk of transmission to humans. The purpose of this study was to detect the presence canine leptospirosis and possible serovars in a dog shelter in Johore, Malaysia. Blood samples were collected from 73 dogs consisting of 50 vaccinated and 23 non-vaccinated dogs. Microscopic agglutination test (MAT) was used to screen serum samples for anti-leptospiral antibodies. At the cut-off titer of 1:80, two out of 73 dogs (2.7%) were seropositive for *Leptospira borgpetersenii* serovar Javanica. Another two dogs (2.7%) were seropositive for *L. interrogans* serovar Icterohaemorrhagiae and one (1.4%) showed antibody titers against *L. interrogans* sv. Australis. The overall seroprevalence was 6.8% (n=5/73). All seropositive dogs were vaccinated and they consisted of 80% females and 20% males. The seropositive status of these shelter dogs showed that they could be potential disease disseminators to humans and other animals, thus, investigations are warranted on their potential epidemiological roles in leptospirosis.

Keywords: leptospirosis, canine, microscopic agglutination test, seroprevalence, anti-leptospiral antibodies

SEROPREVALENCE OF LEPTOSPIROSIS AMONG MILITARY DOGS FROM JOHORE, MALAYSIA

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ABSTRACT

Canine leptospirosis is a well-known bacteria zoonotic disease with worldwide distribution. A study on seroprevalence of canine leptospirosis was carried out in a dog population (n=40) consisting of 13 searcher, 15 tracker, and 12 guard dogs at the Military Camp, Johore, Malaysia. Forty sera were obtained and examined by Microscopic Agglutination Test (MAT) against 10 *Leptospira* serovar (Canicola, Pomona, Icterohaemorrhagiae, Grippotyphosa, Lai, Australis, Pyrogenes, Javanica, Bataviae, and Celledoni). Titres $\geq 1:80$ were recorded as positive. A seroprevalence rate of 37.5% was observed in this study. *Leptospira interrogans* serovar Icterohaemorrhagiae 15% (n=6/40) was found to be most prevalent serovar followed by Grippotyphosa 12.5% (n=5/40), Pomona 5% (n=2/40), and Canicola 5% (n=2/40). Seropositive dogs were searchers (n=9/40) and trackers (n=6/40) while guard dogs were seronegative. Paired-serum samples were obtained, but there was no 4-fold increase in MAT. In this study, working environment and food contamination were believed to be sources of infection in this population of military dogs. To prevent and control of canine leptospirosis in Malaysia, commercial vaccines against the four major serovars of Canicola, Pomona, Icterohaemorrhagiae, and Grippotyphosa in military dogs are highly recommended.

Keywords: canine leptospirosis, seroprevalence, military dogs, microscopic agglutination test

**EFFECT OF ENROFLOXACIN AND DOXORUBICIN
COMBINATION TREATMENT ON VIABILITY OF CANINE
MAMMARY GLAND TUMOR CELLS *IN VITRO***

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ABSTRACT

Mammary gland tumours are common among intact adult female dogs. Primary tumours can be surgically removed; however, metastasis to lymph nodes and lungs can occur in advanced stages of the disease that ultimately lead to death or decision for euthanasia. In chemotherapy, the role of doxorubicin is to prevent metastasis and local recurrence. However, cardiotoxicity is a common dose-limiting side effect of this drug. Recently, enrofloxacin, a bactericidal antibiotic agent, was shown to demonstrate anti-proliferative activity on human colon cancer and canine osteosarcoma cells *in vitro*. In this study, it was hypothesised that enrofloxacin will reduce cell viability in a dose-dependent manner while enhancing the cytotoxic effects of doxorubicin on canine mammary tumour cells. The objectives of this study are to determine the anti-proliferative effects of enrofloxacin alone and in combination with doxorubicin on the canine mammary tumour (CMT-Stylo) cell line. Doxorubicin at 50, 100, 200, and 400 nM, enrofloxacin at 1, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL, and their combinations were chosen for cell treatment based on previous studies and their IC₅₀s. The proliferation and viability of CMT-Stylo cells treated for 24, 48, and 72 h were determined. There were reductions in proliferation of CMT-Stylo cells treated with enrofloxacin alone and in combination with doxorubicin in a time- and dose-dependent manner. The experiment concluded that the enrofloxacin-doxorubicin combination treatments have synergistic anti-canine mammary tumor cell effects.

Keywords: canine mammary gland tumour, CMT-Stylo cell, enrofloxacin, doxorubicin, enrofloxacin-doxorubicin combination, cell viability, anti-proliferation

ANTIMICROBIAL SUSCEPTIBILITY OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* FROM CAPRINE CASEOUS LYMPHADENITIS CASES

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ABSTRACT

Caseous lymphadenitis (CLA) is a chronic bacterial infectious disease caused by a Gram-positive bacterium, *Corynebacterium pseudotuberculosis*, that infects animals especially small ruminant resulting in economic losses to farms. The use of antibiotics in most cases do not completely eliminate the disease. This is probably due to antibiotic resistance in this bacterium. Using antimicrobial susceptibility test, this study was aimed to identify the susceptibility of *C. pseudotuberculosis* towards selected antibiotics. A laboratory strain of *C. pseudotuberculosis* from caprine CLA cases was tested against 14 antibiotics using the disc diffusion method and zone of inhibition to determine susceptibility. The microorganism was also tested against 4 selected antibiotics using the broth microdilution method, minimal inhibitory concentration, and minimum bactericidal concentration. The microorganism showed susceptibility to amoxicillin with or without clavulanic acid, ampicillin, cephalixin, enrofloxacin, erythromycin, gentamicin, neomycin, oxytetracycline, penicillin G, sulfamethoxazole-trimethoprim and tetracycline but resistant to streptomycin and polymixin B. The minimal bactericidal concentration for neomycin, gentamycin, penicillin g and erythromycin was 1.875, 0.25, 20, and 20 µg, respectively. The minimum inhibitory concentration for neomycin, gentamycin, penicillin g and erythromycin was 0.9375, 0.125, 10 and 10 µg, respectively. From the study, *C. pseudotuberculosis* from caprine CLA cases is susceptible to most of the antibiotic tested including penicillin, cephalosporin, macrolides, tetracycline and aminoglycosides, except streptomycin. The microorganism is resistant to the cyclic peptide antibiotic, polymixin B.

Keywords: caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, AST, antibiotic

PATHOGENICITY AND IMMUNOGENICITY OF LIVE ATTENUATED AND INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS

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ABSTRACT

Fowl adenovirus (FAdV) is a non-enveloped DNA virus that is the primary pathogen of inclusion body hepatitis (IBH) in chickens. Inclusion body hepatitis that is distributed worldwide has been reported in Malaysia. To control IBH outbreak in the poultry industry, vaccination programmes are crucial. However, there is lack of research on the development of local vaccine against FAdV infections. The objective of this study was to determine the pathogenicity and immunogenicity of live attenuated and inactivated FAdV (UPM 1137) in commercial broiler chickens. Fifty-four, 1-day-old Cobb 500 broiler chicks were divided into four groups, namely group A, B, C, and D (control). Feed and water were provided *ad libitum*. The chicks (n=12/group) in groups A, B and C were inoculated with inactivated FAdV (0.2mL) with virus titre of $10^{6.5}$ TCID₅₀/0.2 mL, live attenuated FAdV (0.1 mL) with virus titre of $10^{5.2}$ TCID₅₀/0.1 mL, combination of the inactivated (0.2 mL) and live attenuated (0.1 mL) FAdV, respectively on day 1 (12 chicks of each group) and day 14 of age. Body weight and blood samples were collected prior to necropsy on days 14 and 28 of age, except for the control group, sampling was also conducted on day 1 of age. At necropsy, gross lesions and liver weights were recorded, and samples of liver were collected for histological examination. The study showed that neither clinical sign nor gross lesion were recorded throughout the trial. The body weight of chicken on days 14 and 28 were not significantly ($p>0.05$) different among groups. The liver to body weight ratio of group C was significantly ($p<0.05$) higher than groups A and D on day 28. There was no gross or histopathological lesion in the liver of chicken throughout the trial. The FAdV antibody titre in group D (Control) was 938 ± 1596 on day 1 of age and this was not detected on day 14 and 28 of age. However, the FAdV antibody was induced at high titre in all the inoculated groups at day 14 and 28 of age. The FAdV antibody titre of group C was significantly ($p<0.05$) higher than groups B and D on day 28. In conclusion, live attenuated and inactivated FAdV used in the study have a low pathogenicity but can induce production of antibody in the chickens. Combination of live attenuated and inactivated FAdV showed higher immunogenicity than live attenuated FAdV alone.

Keywords: fowl adenovirus (FAdV), commercial broiler chicken, live attenuated, inactivated, pathogenicity, immunogenicity.

CARRIAGE OF *SALMONELLA* SPP. AND *Escherichia coli* BY HOUSEFLIES AT EATERIES AND POULTRY FARMS IN SELANGOR, MALAYSIA

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ABSTRACT

House flies are of public health concern for their high potential to be carriers of communicable diseases to both humans and animals. There is need to determine the possibility of houseflies carrying disease-causing bacteria in their gastrointestinal tract that may greatly amplify the risk of humans and animal of acquiring the disease. The aim of this study was to determine carriage of *Salmonella* spp. and *Escherichia coli* by houseflies and the occurrence of antibiotic resistance genes in the isolated bacteria. Sixty housefly lies were caught using sticky papers at 3 local eateries frequented by students and staff of Universiti Putra Malaysia and 3 poultry farms in Selangor, Malaysia. The samples were incubated with pre-enrichment media, buffered peptone water (BPW), enrichment media, and Rappaport Vasiliadis (RV) prior to direct plating onto XLD agar. Gram-staining, biochemical and serum agglutination tests showed only 3.33% of the lies sampled were positive for *Salmonella* spp. This bacteria from positive samples were resistant to >2 antibiotics tested. Direct plating using Chromocult agar following pre-enrichment using buffered peptone water (BPW) reveals that 60% of the houseflies caught at the eateries and 53.33% in poultry farms carry *E. coli*. The *E. coli* isolated from two eateries and two poultry farms were mostly resistant to 4 and one antibiotic(s), respectively while from the third eatery and third poultry farm were resistant to two antibiotics. House flies evidently harbour *Salmonella* spp. and *E. coli* that could potentially carry antibiotic resistance genes.

Keywords: house fly, *Salmonella* spp., *Escherichia coli*, antibiotic resistance

TRANSMISSION ELECTRON MICROSCOPIC STRUCTURE OF THE GUTTURAL POUCH

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ABSTRACT

This study on the transmission electron microscopic structure of the guttural pouch (GP) in the horse is complementary to another study on the light and scanning electron microscopic (SEM) structure of the GP concurrently being undertaken. Both studies are aimed at identifying the possible passageway utilised by a fungal infection from the GP into the internal carotid artery (ICA). Referred to as guttural pouch mycosis (GPM), the fungal infection erodes the wall of the said artery resulting in fatal haemorrhage. Under the transmission electron microscope (TEM), the epithelial cells of the GP showed two types of cell surface modifications. The first type is the presence of cilia and microvilli on the surface of the tall columnar cell and mucus secretory cells, respectively. The second type is the presence of tight junction between contiguous epithelial cells. However, more interestingly is the enlarged intercellular space between contiguous epithelial cells; these spaces could be the passage through which mucus droplets from the underlying connective tissue transverse through the epithelium to be deposited on the surface of the mucosa of the GP. Observation on the passage of the mucus through the epithelial lining was demonstrated by SEM in the concurrent study. The present study that demonstrated the existence of distended intercellular spaces between contiguous epithelial cells lend support the passage of mucus droplets from the underlying connective tissue to the mucosal as hypothesised in the concurrent study. Thus, based on the passage of mucous droplets across the epithelial lining of the GP it can be concluded that the possible passage of the mycotic infection is similar to that of the mucous droplets but in the opposite direction. With numerous single layered wall capillaries in the connective tissue below the epithelium, gaining entry into the capillaries would be with relative ease and hence on into the ICA.

Keywords: transmission electron microscopy, guttural pouch, contiguous epithelial cell, intercellular space, passage.

EFFECT OF GINGER AND TURMERIC PEELS ON GROWTH PERFORMANCE AND CRUDE PROTEIN COMPOSITION IN RED TILAPIA

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ABSTRACT

The major objective of aquaculture is to reduce production cost particularly on the cost of feeding and to increase outputs in the shortest period. Turmeric and ginger had been used in feed formulation to improve immunity to infections and contribute in growth performance due to their antimicrobial properties and protein contents. Hence, this study was aimed to determine the effect of turmeric and ginger peels on growth performance and crude protein composition in Red Hybrid Tilapia. Eighty juvenile Red Hybrid Tilapia was divided into 4 groups; Group A: 20% ginger peels + 80% commercial diets; Group B: 20% turmeric peels + 80% commercial diets; Group C: 10% ginger peels + 10% turmeric peels + 80% commercial diets, and Group D: control, without turmeric or ginger peels. Proximate analysis showed that the protein contents were variable with the inclusion of turmeric and ginger peels in the diet. The results showed that, among diets, ginger peels with commercial diet produced the most significant increases in body weight, length, girth, and protein composition of fish. The ginger peel-commercial diet combination had the highest protein and fat contents among groups, thus giving better growth performance and protein composition to Red Hybrid Tilapia.

Keywords: Red Hybrid tilapia, ginger peels, turmeric peels, growth performance, protein composition.

NUTRIENT COMPOSITION OF MILK FROM VARIOUS BUFFALO BREEDS AT PUSAT TERNAKAN KERBAU TELUPID, SABAH, MALAYSIA

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ABSTRACT

Consumption of dairy products among Malaysian has increased steadily over the past few years. Buffalo milk is a potential alternative dairy resource to fulfil the demand for milk. However, the nutrient potential of buffalo milk has not been fully determined and the informations on milk from the various buffalo breeds, in Malaysia, is limited. In this study the nutrient and fatty acid compositions of milk from Murrah crossbred and Swamp buffaloes at the Buffalo Breeding and Research Center, Telupid, Sabah, Malaysia, was determined. Six buffaloes from each breed were used in this study. Milk samples were collected from each buffalo and preserved with 40% formalin, chilled at 4°C before analysis. The milk fat, protein, casein, lactose, total solid, and fatty acid profiles were determined. The results revealed that milk of Murrah cross buffaloes contained 2.21% fat, 5.62% protein, 4.83% lactose, 4.96% casein, and 14.11% total solid. Meanwhile, milk from swamp buffaloes contained 6.25% protein, 1.55% fat, 5.55% casein, 4.65% lactose and 14.04% total solid. There was no significant ($p>0.05$) difference in milk composition parameters between Murrah crossbred and Swamp buffaloes. The fatty acids component of milk samples from Murrah crossbred contained higher monounsaturated (MUFAs) and saturated (SFAs), and low polyunsaturated fatty acids (PUFAs) than samples from Swamp buffaloes. It can be concluded that buffalo milk has great potential as a dairy source for human consumption.

Keywords: milk, milk composition, fatty acid, Murrah, Swamp, buffalo

CONTRIBUTING FACTORS TO THE IMPROVEMENT OF DAIRY AND MEAT GOAT PRODUCTIVITY

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ABSTRACT

Unlike non-ruminant, the ruminant industry in Malaysia still depends on importation to meet local demands for meat and dairy products. Various strategies by the Department of Veterinary Services, Malaysia were implemented to assist smallholder farmers to increase production of animals. Despite all efforts, animal productivity in Malaysia remains low. This study was conducted to investigate the current animal management practices by smallholder goat farmers and to make recommendations to improve meat and milk production consistent with good animal husbandry practice (GAHP). A survey was done on 10 selected including Universiti Putra Malaysia foster farms, comprising of 5 meat and 5 dairy goat farms, to determine their management practices, performance, and health status of their animals. Based on the survey, most farms were lacking in GAHP criteria, especially in the animal management and feeding records. Specific nutrition and feeding management for animals were not practiced in these farms while housing facilities and breeding programme were inadequate. In conclusion, farms in this study only partial practice good animal husbandry.

Keywords: goat, dairy, meat, survey, GAH

**EFFECT OF MALACHITE GREEN TREATMENT ON
HAEMATOLOGICAL PARAMETERS OF
JADE PERCH (*SCORTUM BARCOO*)**

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ABSTRACT

Malachite green (MG) is extensively used as an antiprotozoal and antifungal agent in the aquaculture industry. Since MG is toxic, the present study was conducted to investigate the effect of MG on the hematological parameter of jade perch (*Scortum barcoo*). Sixty jade perch were divided into control and two treatment groups; one group of jade perch was immersed in MG for bath at 0.5mg L⁻¹ daily changed for 5 d and another treated for a single dip at 66.7 mg L⁻¹ for 30 sec. Blood was collected from all groups on days 0, 1, 5, 7, 11, 13, 15, 19, and 21 for determination of erythrocyte (RBC) and leucocyte (WBC) counts, haemoglobin concentration, haematocrit (PCV), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). Analysis of hematological parameter using T-test for dip treatment showed significant (p<0.05) differences in PCV, MCHC and WBC while for bath treatment significant (p<0.05) difference were for PCV, RBC, MCHC, and WBC. Fish given bath treatment showed reddening of pectoral and caudal fins, petechial haemorrhage on the body, erratic swimming, and anorexia during exposure to MG. In conclusion, the study showed that although there were some significant differences in haematological parameters the general trend was not obvious. However, the clinical signs indicate that fish given bath treatment were under stress.

Keywords: Jade Perch, malachite green, dip, bath, hematological parameter

CALVING INTERVAL OF SWAMP AND MURRAH CROSSBRED BUFFALOES UNDER AVERAGE RAINFALL CONDITION

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ABSTRACT

The buffaloes are among animals native to Malaysia. One of the ways to sustain survivability of these buffaloes is through reproduction. Thus, the objectives of this study were to determine the calving interval in Swamp and Murrah cross-breed buffaloes and the relationship between calving interval and average rainfall. This retrospective study was conducted at the Buffalo Breeding and Research Centre, Department of Veterinary Services and Animal Industry, Telupid, Sabah, Malaysia, on 50 female buffaloes comprising of 25 Swamp and 25 Murrah crossbred buffaloes. Buffaloes aged between 3-9 years and with good breeding records were chosen for the study. The age, body weight, and calving interval of the buffaloes was obtained from farm records. Correlation analysis was done to determine the relationship between the calving intervals, average rainfall and breed. The results showed that there was significant ($p < 0.05$) difference between calving intervals and breed. There was also correlation ($p < 0.05$) between breed and rainfall. The calving interval and rainfall also showed correlation, but this was statistically insignificant ($p > 0.05$). The calving interval of Murrah crossbred is shorter (449 days) than that of the Swamp buffaloes (497 days).

Keywords: buffalo, retrospective, calving interval, rainfall.

ANALYSIS OF EPIDEMIOLOGICAL DATA ON URINARY STONES IN DOGS AND CATS AT A REFERRAL CENTRE IN MALAYSIA

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ABSTRACT

Urolithiasis a common urinary problem in dogs and cats. The objective of this study was to evaluate epidemiological data derived from a referral center in Malaysia over a 10 years period, 2007 – 2016. Urolith analytical results of dogs and cats pertaining to their urolith type and composition, were evaluated and compared with breed, age, sex, neuter status, body condition score, urine pH and anatomical location. Out of 1086 urolithiasis cases analysed, 90.4% were from dogs and 9.6% from cats. The majority of uroliths from dogs (89.8%) and cats (92.2%) were from the lower urinary tract. Struvite (61.1%) was most commonly found in dogs and calcium oxalate (CaOx) (51.9%) in cats. The average age of dogs and cats with struvite were 5.8 years and 7.6 years with CaOx. Dogs were 2.7× more likely to develop struvite than cats while cats were 2.4× more likely to develop CaOx than dogs. Bitches were 11.9× more likely to develop struvite than males. Meanwhile, toms were 1.5× more likely to develop CaOx than queens. Most common dog and cat breeds associated with struvite and CaOx were Shih Tzu, mixed-breed dogs, Miniature Schnauzers, Domestic Shorthair, Persians and mixed-breed cats. This is a first epidemiological study on urolithiasis in dogs and cats in Malaysia. This baseline data can be useful for further investigative studies towards species-specific management of urolithiasis.

Keywords: urolithiasis, urolith, struvite, calcium oxalate, breed

THE VALUE OF CONE BEAM COMPUTED TOMOGRAPHY ANGIOGRAPHY IN STUDYING THE ARTERIAL SYSTEM IN VICINITY OF GUTTURAL POUCH IN HORSES

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ABSTRACT

Guttural pouches are the large diverticula of the eustachian tubes that connect the pharynx to the middle ear. It is a unique structure in equine as it is anatomically located very close to other important structures such as internal carotid artery, maxillary artery, external carotid artery, glossopharyngeal nerve, and hypoglossal nerve. These structures are important in the event of guttural pouch mycosis as the damage can cause neurological deficit and severe haemorrhage, which may be fatal to the horse. Hence, a study was conducted to determine the value of cone-beam computed tomography angiography in studying the arterial system in vicinity of the guttural pouch. Two retired horses were humanely euthanised for reasons or problems unrelated to the guttural pouches were used for this study. Iodine based contrast media (Iomeprol) was injected and infused via common carotid arteries to highlight the arteries of the cadaveric heads. Based on the computed tomography angiography images, important arteries in vicinity to the guttural pouch can be recognised and observed in three different planes; transverse, sagittal, and dorsal planes. Although there are several limitations in the use of computed tomography in the study of the guttural pouch, the method provides excellent images of the anatomical pathways of important and associated arteries in relation to the guttural pouch.

Keywords: guttural pouch, cone-beam computed tomography angiography, internal carotid artery, horses

INVESTIGATION OF BOVINE GROWTH HORMONE GENE IN BUFFALO BULLS USING POLYMERASE CHAIN REACTION

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ABSTRACT

The aim of this study was to investigate the ability of specific primers in the detection of the bovine growth hormone (bGH) gene in buffalo bulls (*Bubalus bubalis*) using PCR. The study was carried out in crossbred Murrah and Swamp buffalo bulls maintained at Buffalo Breeding and Research Centre, Department of Veterinary Services and Animal Industry, Telupid, Sabah, Malaysia. Genomic DNA from 12 healthy buffalo bulls was extracted and amplified using primers that were designed from the cattle growth hormone (GH) gene sequences. The DNA quality was checked by running the samples in 2% agarose gel electrophoresis. PCR was used to amplify the growth hormone fragments. *GH1*, 428 bp fragments of the growth hormone gene was amplified with forward (5'-CGG ACC GTG TCT ATG AGA AGC TGA AG-3') and reverse (5'-GTT CTT GAG CAG CGC GTC GTC A-3') primer sequences. *GH2*, 211 bp fragments of the growth hormone gene was amplified with forward (5'-GCT GCT CCT GAG GGC CCT TC-3') and reverse (5'-CAT GAC CCT CAG GTA CGT CTC G-3') primer sequences. The results were negative for both the *GH1* and *GH2* fragments. It is believed that mismatch of the primers could be one of the contributing factors to the failure to detect the bovine growth hormone (bGH) gene.

Keywords: buffalo bulls, bovine growth hormone (bGH), PCR

SEROPREVALENCE OF ORF VIRUS INFECTION AMONG SMALL RUMINANTS IN UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

Contagious ecthyma is an infectious disease caused by Orf virus; characterized by scabby lesions at the nostrils and mouth regions. The disease in huge economic losses due to stunted growth or slaughter of the affected animals. There is inadequate information on the status of long-term Orf infection among small ruminants in Malaysia. This study aimed to detect the IgG antibodies against Orf virus infection in goats and sheep of selected Universiti Putra Malaysia (UPM) foster farms. Associated risk factors of Orf infection were also assessed. Serum samples from 90 sheep and 90 goats and relevant historical information were obtained from 5 randomly selected farms. Serum samples were stored at -20°C and subjected for qualitative Enzyme-Linked Immunosorbent Assay test. It was found that 12.22% of sheep and 14.44% of goats were already infected by Orf. In sheep, the prevalence rate of Orf infection was higher in males than females. Young animals showed higher prevalence than in adults. Poorly managed farm showed the highest disease prevalence. In conclusion, Orf infection is present in sheep and goats from UPM foster farms with prevalence rate in goats higher than in sheep.

Key words: Contagious ecthyma, Orf, risk factor, prevalence rate, IgG, ELISA

EFFECT OF HOLDING TEMPERATURE BEFORE FREEZING ON QUALITY OF BULL SEMEN

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ABSTRACT

A study was conducted to evaluate the effect of holding temperature before freezing on the motility, morphology, and viability of chilled and frozen bull spermatozoa. Semen collection was performed on four mature bulls by electro-ejaculator. After initial evaluation and first dilution using Tris-egg yolk semen extender, four groups of diluted semen samples were kept at holding temperatures of either 4, 15, 25, or 37 °C for 10 min. All semen samples were subsequently chilled at 4°C for at least 3 h. Post-chilling evaluation was done after second dilution with the same extender with glycerol added. The semen samples were packed into 0.25 mL straws before freezing in the liquid nitrogen. Thawing of frozen semen samples were done on day 1 post-cryopreservation in water maintained at 37°C for 30 sec. Semen evaluation was conducted post-thawing. The results showed that there was no significant ($p>0.05$) difference among holding temperatures in post-chilled and frozen-thawed sperm motility, morphology, and viability. It can be concluded that 10 min holding time at holding temperatures of 4, 15, 25, and 37°C before freezing did not affect quality of chilled and frozen bull spermatozoa.

Keywords: bull semen, quality, holding temperature

COMPARATIVE STUDY ON GROWTH PERFORMANCES OF INDIGENOUS SWAMP AND MURRAH CROSSBRED BUFFALOES

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ABSTRACT

Although the demand for beef has increased as a result of increased human population, Southeast Asian countries have reported annual reductions in the buffalo population due to limited land availability for rearing purposes. Therefore, enhancement in buffalo productivity through genetic improvement for the production of dual-purpose crossbred buffaloes (Swamp × River), for milk and beef productions. This retrospective study was conducted to compare the growth performance and determine the benefits of the rearing crossbred versus swamp buffaloes. Records for swamp and crossbred buffaloes born between 2014 and 2016 were acquired from the Buffalo Breeding and Research Centre, Telupid, Sabah, Malaysia. Animal identification and breed, birth weight, weaning weight and the body weights at 3-monthly intervals were recorded and analysed. All calves were weaned at 3 mth of age and released into paddocks to graze. Supplemented feed was provided at 1 kg/animal/d. The average birth weights of crossbred buffaloes were 36.63±5.18 kg, which were significantly ($p<0.05$) higher than that of swamp buffaloes at 34.69±5.28 kg. The average pre-weaning daily weight gain for swamp and crossbred buffaloes was 0.73 and 0.98 kg/d while the average post-weaning daily weight gain was 0.39 and 0.44 kg/d, respectively. Therefore, the 3-monthly body weights of Murrah crossbred buffaloes were significantly ($p<0.05$) higher than that of swamp buffaloes at 24 mth old. The Murrah crossbred and swamp buffaloes achieved the targeted market weight of 250 kg at 15 and 18 mths old, respectively while the target breeding weight was 385 kg at 26 and 30 mth old. In conclusion, the Murrah crossbred buffaloes showed faster growth rate and reached the target market and breeding weights at an earlier age than swamp buffaloes. Thus, farmers can reduce the rearing cost and earn more profit by selling crossbred buffaloes at an earlier age.

Keywords: Swamp, Murrah crossbred, body weight, average daily gain

**HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS
IN CATS SUSPECTED WITH FELINE INFECTIOUS PERITONITIS
AND PRESENTED TO UNIVERSITY VETERINARY HOSPITAL,
UNIVERSITI PUTRA MALAYSIA FROM YEAR 2014 TO 2016**

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ABSTRACT

Feline Infectious Peritonitis (FIP) is a lethal immunopathological disease caused by mutated feline enteric coronavirus found in wild and domesticated cats. This study was conducted to determine the haematological and serum biochemical profile in cats presented to Universiti Veterinary Hospital, Universiti Putra Malaysia, suspected of FIP and to compare between dry and wet form FIP. Medical records of cats suspected of FIP with compatible history and clinical signs admitted to UVH during the period of 2014 to 2016 were reviewed. The sole inclusion criterion was cats tested for FCoV antibody using dot-ELISA, Biogal's Immunocomb® Feline Coronavirus Antibody Test Kit with medium to high positive FCoV antibody titre (S3-S6). The signalment, age, sex, and breed, and the haematology and serum biochemistry results were obtained. Among the 132 FIP-suspected cats, 81.1% had hyperproteinaemia, 88.6% had hyperglobulinemia, 97.7% had A:G ratio \leq 0.8 and 46.2% had hypoalbuminemia while 47.8% had neutrophilia with left shift, 44.7% had lymphopenia, 41.7% had monocytosis, 55.3% had eosinopenia, and 31.8% had nonregenerative anaemia. Parameters such as lymphocyte count (P=0.002), eosinophil count (P=0.009), total protein (P=0.000), albumin (P=0.000), globulin (P=0.041), ALT (P=0.016), ALP (P=0.025) and creatinine (P=0.047) are found to be significantly (p<0.05) different between cats with dry form and wet form FIP.

Keywords: feline infectious peritonitis, cats, hyperglobulinaemia, University Veterinary Hospital, Universiti Putra Malaysia

SEROPREVALENCE OF BOVINE ANAPLASMOSIS AMONG DAIRY CATTLE IN UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

Bovine anaplasmosis is a disease primarily caused by *Anaplasma marginale* that infects erythrocytes, leading to erythrophagocytosis and causing anaemia. According to past prevalence studies, anaplasmosis is a significant disease affecting cattle in Malaysia. However, currently there is no record on the prevalence of this disease among cattle of the foster farms of Universiti Putra Malaysia (UPM). Thus, this study was carried out to determine the seroprevalence of anaplasmosis among dairy cattle in the foster farms. This study also compared the sensitivity and specificity between the serological method using cELISA and the microscopic method in the detection and diagnosis of anaplasmosis. Blood samples were collected via jugular venipuncture from 45 cattle selected based on convenience sampling from 4 farms. Heparinised whole blood was used to prepare Giemsa-stained thin blood smears, for determination of haematocrit, and microscopic detection of anaplasmosis. Serum was extracted from coagulated blood for serological testing using the Anaplasma Antibody Test Kit (VMRD, Inc.). The seroprevalence of bovine anaplasmosis in the study cattle population was 51.11%. There was a significant ($p < 0.05$) association between microscopic detection and serological method for anaplasmosis. The serological method had a higher sensitivity and specificity than the microscopic detection method. In conclusion, the low prevalence rate of anaplasmosis in the dairy cattle of the foster farms is the result of the good herd health programme instituted under the supervision of the Faculty of Veterinary Medicine, UPM.

Keywords: Bovine anaplasmosis, *Anaplasma marginale*, seroprevalence, cELISA, microscopic detection

MOLECULAR DETECTION OF FELINE LEUKEMIA VIRUS IN CLINICALLY ILL MALAYSIAN LOCAL CATS

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ABSTRACT

Feline leukemia virus (FeLV) belongs to the genus of *Gammaretrovirus* and is associated with a wide range of clinical signs. Thus far, the first and only molecular characterisation of local FeLV isolates performed in 2014 revealed that local isolates are closely related to United Kingdom (UK) isolate. Due to limited coverage of the study and the nature of the virus that typically integrates DNA into host genome and undergoes mutation, a study on current status of FeLV infection in Malaysia is necessary. Therefore, this study aimed to detect FeLV antigen in clinically ill cats by RT-PCR and to compare the currently identified variant with previous related virus isolates from Malaysia and other geographical distribution. By using convenience sampling method, plasma and saliva were collected from 15 clinically ill cats and 5 healthy cats at the Gasing Veterinary Hospital, Petaling Jaya, Malaysia. Viral nucleic acid was extracted and subjected to one-step RT-PCR with specific primer targeting the highly conserved U3LTR and partial *gag* regions. Two cats from the clinically ill group tested positive for the antigen. Partial nucleotide sequencing and phylogenetic analysis revealed that the current variant is 93 to 99% homologous to previous Malaysian isolates and is closely related to UK isolate. Interestingly, this FeLV isolate is closely related to an isolate from Japan. In conclusion, this study highlights the possibility of an evolutionary relationship between FeLV from Malaysia and those from UK and Japan.

Keywords: feline leukemia virus, RT-PCR, partial nucleotide sequencing, phylogenetic analysis, variant

EVALUATION OF OPTIMUM SCAN DELAY FOR POST CONTRAST CONE BEAM COMPUTED TOMOGRAPHY OF CANINE ABDOMEN

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ABSTRACT

Cone beam computed tomography (CBCT) is an advanced imaging technology used for various clinical applications including research and diagnostic. The application of CBCT as a veterinary diagnostic tool particularly for soft tissue diseases is under-utilised due to limited information available on the scanning protocol. Therefore, the aim of this study is to determine the optimum scan delay for post-contrast study using CBCT for major blood vessels and various organs in the canine abdomen. Pre-contrast study was performed on 3 dogs and post-contrast images were obtained at different scan delay; 0, 30, and 90 sec. Attenuation for blood vessels; aorta, caudal vena cava and portal vein and also abdominal organs; liver, spleen, and renal pelvis were measured in Hounsfield unit (HU). All blood vessels and abdominal organs were clearly visualised at 30 sec delay. There was no contrast enhancement seen in blood vessels at 0 sec although other abdominal organs were enhanced by contrast. The 30 sec scan delay produced similar contrast enhancement as 90 sec scan delay, however, this protocol is not recommended because of safety issues related to contrast administration. In conclusion, CBCT can be optimised for the evaluation of blood vessels and abdominal organs in dogs. The post-contrast scanning protocol is of diagnostic value to small animal patients.

Keywords: *CBCT*, canine, abdomen, optimum scan delay, Hounsfield unit

EFFECT OF VITRIFICATION ON SPERMATOZOA QUALITY IN BULL SEMEN

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ABSTRACT

Cryopreservation of spermatozoa is part of the assisted reproductive biotechnology to enhance reproductive capacity in livestock. Conventional cryopreservation applies slow-gradual freezing method that permitted ice crystallization and causes both intracellular and extracellular cryodamage resulting in poor post-thawed semen quality. Hence, vitrification is introduced by solidifying the solution into glassy state without causing crystallization in fast and inexpensive manner. This ultra-rapid cooling method requires high concentration of cryoprotectants that is potentially toxic the spermatozoa. Therefore, this study was conducted to determine the effect of vitrification on the quality of bull semen. Eight bull semen samples were collected using electro-ejaculation. Tris-based extender was compared with vitrification using solution of different concentration of cryoprotectants at 10% (Vitrification Solution 1; VS-1) and 20% (VS-2) containing dimethyl sulfoxide (DMSO) and ethylene glycol respectively. The result revealed that high mortality and nearly zero motility in all post-warmed vitrified spermatozoa, but the general and progressive motilities parameters in VS-1 at initial evaluation was 22.45 and 24.87% respectively, which is significantly ($p < 0.05$) better than Tris-based extender. Apart from that, VS-1 showed possibility for cryopreservation as few spermatozoa and some incidental findings of motile microorganisms survived the process. In conclusion, vitrification has potential as an alternative for cryopreservation.

Key words: bull, spermatozoa, cryopreservation, vitrification, electro-ejaculation

CAT OWNERS' PERCEPTION TOWARDS FELINE HEART DISEASE: A BEHAVIOURAL STUDY

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ABSTRACT

Heart disease is a serious medical condition in cats and requires lifelong treatment. Informed decisions to treat cats with heart diseases depend on the cat owners' level of awareness and knowledge on feline heart disease and there is a paucity of such information among the Malaysian cat owners. Therefore, the purposes of this study were to (i) assess owner's awareness, and knowledge on feline heart diseases; (ii) determine owners' barriers to treat cats with heart disease; (iii) examine relationship between attitude, subjective norms, and perceived behavioural control towards intention to treat, and (iv) examine the moderating role of empathic concern towards intention to treat feline that have been diagnosed with heart diseases. One hundred and thirty-nine pet-cats owners participated in the study and data was collected using an interviewer-assisted questionnaires on demographic, cat ownership, awareness of feline heart disease and owners' intention to treat cats with heart diseases. Data were analysed using descriptive analysis and variance-based structural equation modeling. The results revealed that the majority of the respondents (49.6%) were aware that cats can have heart disease, but approximately 89.2% respondents did not understand the disease, and only 11.5% had good ability to identify symptoms related to feline heart disease. The respondents showed that attitude, subjective norms, and perceived behavioural control are positively associated with intention to treat even though cost was the main barrier (45.5%). Findings also indicated that empathic concern only moderates the relationship between perceived behavioural control and intention to treat. In conclusion, educational interventions are warranted for cat owners on feline heart disease. The data collected will assist veterinarians in the counselling and planning of treatment for owners and to improve client compliance.

Keywords: awareness, feline heart disease, intention to treat, attitude, subjective norm, perceived behavioural control

SERUM AND ORAL FLUID ANTIBODY RESPONSES TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is endemic in Malaysia. It is a disease of concern as it can cause great economic loss from reproductive failure in sows, high pre-weaning mortality in piglets, and respiratory disease in growing and finishing pigs. Therefore, it is necessary to have an efficient and effective surveillance method to detect the disease. In this study, antibody responses against PRRS in oral fluid and serum were compared using the IDEXX ELISA test kit. This study was conducted in two (A and B) commercial pig farms. Thirty-two heads of 6-week-old pigs from each farm were used as subjects. These pigs were placed in four pens in equally numbers. Eight serum samples and 3 pen-based oral fluid sample were collected from each pen. Sample collections were repeated on the same individual and same pen 4 weeks later. The serum and oral fluid samples collected were analysed using IDEXX PRRS X3 antibody test kit and IDEXX PRRS oral fluid antibody test kit, respectively. There was significant, very strong and positive correlation between serum and oral fluid samples for Farms A ($r_s=0.976$, $p < 0.01$) and B ($r_s=0.905$, $p < 0.01$). In conclusion, oral fluid is a useful tool for PRRS surveillance at the farm and a good alternative to the traditional serology method.

Keywords: Porcine reproductive and respiratory syndrome (PRRS), serum, oral fluid, IDEXX PRRS X3 antibody test kit, IDEXX PRRS oral fluid antibody test kit

MOLECULAR INVESTIGATION OF FELINE CORONAVIRUS IN MALAYSIAN PET CATS

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ABSTRACT

Feline coronavirus (FCoV) infection is very common in cat populations. The FCoV is classified into two biotypes, namely feline enteric coronavirus (FECV) and mutated feline infectious peritonitis virus (FIPV). The FIPV can cause fatal immune complex disease by altering tropism from enterocytes to monocytes. Previous studies on molecular detection of FCoV in cats were conducted in catteries, however, investigation on the presence of FCoV antigen in local pet cats in Malaysia is limited. Thus, this study aimed to detect FCoV antigen via RT-PCR assay in local pet cats and to compare the identified strain with related virus using phylogenetic analysis. By using convenience sampling, rectal swabs and buffy coat were collected from 16 clinically ill pet cats and 5 healthy pet cats. Viral RNA was extracted and subjected to one-step RT-PCR, targeting polymerase gene. Only 1 of 21 faecal samples was positive for FCoV and none of the buffy coat gave positive result. Phylogenetic analysis revealed that the positive sample was highly homologous, up to 95%, with a FCoV strain from Netherlands on partial sequence of polymerase gene. In conclusion, this study detected FCoV antigen in local pet cats. Negative results could not completely rule out the possibility of FCoV infection because of the complexity of diagnosis that requires a series of analyses.

Keywords: feline coronavirus, feline enteric coronavirus, feline infectious peritonitis virus, RT-PCR, phylogenetic analysis

**PREVALENCE OF ZONOTIC ENTERIC PROTOZOA (*GIARDIA*,
BLASTOCYSTIS AND *CRYPTOSPORIDIUM*) IN SHELTER CATS IN
SELANGOR, MALAYSIA**

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ABSTRACT

Enteric protozoan species like *Giardia*, *Cryptosporidium* and *Blastocystis* in cats are considered to have zoonotic potential. In Malaysia, cats are commonly associated with human living environments. However, there remains a paucity of published information on the epidemiology of these enteric protozoa among owned and stray cats in Malaysia. Thus, this study was carried out to determine the prevalence and risk factors for infection with these protozoa among cats kept in animal shelters in Selangor, Malaysia. Faecal swabs from 105 cats were collected opportunistically at chosen animal shelters. Faecal smears were prepared and stained with Giemsa and Modified Ziehl-Neelsen for microscopy examination. Polymerase chain reaction (PCR) using genus-specific primers was carried out to amplify the small subunit ribosomal RNA (SSU) gene of the protozoa. *Giardia* was detected in 1.9% (2/105) of the faecal samples by conventional microscopy. PCR detection revealed that 37.5% (15/40) and 25% (10/40) of the samples were positive for *Giardia* and *Blastocystis*, respectively. *Cryptosporidium* was not detected in any of the samples examined. The presence of these zoonotic enteric protozoa was neither associated with age nor faecal consistency. The high prevalence of *Giardia* and *Blastocystis* among shelter cats in the country merits further investigation on the epidemiology and risk factors of the infection.

Keywords: *Giardia*, *Cryptosporidium*, *Blastocystis*, zoonotic, cats

EFFICIENCY OF LOCAL FRAGRANCE LEAVES AS RAT REPELLENT

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ABSTRACT

Rodents causing significant damage in terms of physical and dissemination of zoonotic diseases. In Malaysia, house rats are significant as hosts for diseases such as leptospirosis, lymphocytic, choriomeningitis, and rickettsial pox, that can be transmitted to humans. Plants and herbs has been used traditionally as natural rodent repellent. However, usage of local plants and herbs for this purpose is only based on folklore and not scientifically proven. Therefore, this study investigated the potential and effectiveness of local fragrance leaves as natural rodent repellent. Ten, 4-week old, male, Sprague-Dawley rats were exposed to 4 types of local fragrance leaves. Y-maze test used to compare repellent effectiveness between *Annona muricata*, *Pandanous amaryllifolious*, *Cymbopogon nardus*, and *Citrus hystrix*. Approachability, time-spent, and entries of rats towards herbs were recorded and analysed by using one-way (ANOVA). The results showed that all 4 leaves did not show quantitatively or statistically significant natural rodent repellent properties. However, it was observed the rats least approached the arm of *Citrus hystrix* leaves and spent the least time at the arm of *Pandanous amaryllifolious* leaves, and least entered the arm of *Cymbyopogon nardus* leaves. In conclusion, local fragrance leaves may be beneficial as a natural rodent repellent, however, its effectiveness needs further verification.

Keywords: local fragrance leaves, rodent repellent, Y-maze

PATHOGENICITY AND IMMUNOGENICITY OF INFECTIOUS BURSAL DISEASE VIRUS ATTENUATED IN BGM-70 CELL LINES IN SPECIFIC PATHOGEN FREE CHICKENS

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ABSTRACT

Infectious bursal disease (IBD) is a contagious viral disease characterised by high mortality and immunosuppression that can cause heavy economic losses to the poultry industry. Control of IBD is mainly by practicing good biosecurity and vaccination programmes. The objective of this was study to determine the pathogenicity and immunogenicity of very virulent IBD virus (vvIBDV) attenuated in BGM-70 cell lines in specific pathogen free (SPF) chickens for the development of IBD tissue culture-based vaccine against vvIBDV infection. Twenty-five 1-day-old SPF chicks were divided into 5 equal groups, A, B, C, D, and E. Group A and B were inoculated via subcutaneous route with 0.1 mL UPM190 IBDV isolate ($10^{9.7}$ TCID₅₀ /1.0 mL) passage 10 and 15, respectively. Groups C and D were inoculated also subcutaneously with 0.1 mL UPM0081 IBD isolate ($10^{9.5}$ TCID₅₀/1.0 mL) passage 10 and 15, respectively. Group E acted as control group and was not inoculated with the virus. The chicks were provided feed and water *ad libitum* in environment-controlled rooms. Before sacrifice, the body weights of chicks were recorded and serum samples were collected to determine antibody titre using enzyme linked immunosorbent assays (ELISA). Five chicks from each inoculated group were sacrificed each on day 14 and 28 and five from the control group each sacrificed on days 1, 14 or 28. At necropsy, gross lesions and weights of bursa of Fabricius were observed and recorded. This study showed that no mortality, clinical signs or lesions in the bursa of Fabricius occurred in chicks in this study. Bursa to body relative weight was significantly ($p < 0.05$) lower in groups A and D than in in group E at 14 days of age. However, bursa to body relative weight showed did not significantly ($p > 0.05$) differ among groups at 28 days of age. The IBD antibody titre was negative for all chicken in this study. In conclusion, vvIBDV attenuated in BGM-70 cell lines was ineffective in the induction of lesions in the bursa of Fabricius and or raising antibody titre in SPF chickens.

Key words: Infectious bursal disease (IBD), live attenuated, BGM–70 cell lines, day old SPF chicks, IBD antibody titer

STRESS LEVEL OF SEDATED AND NONSEDATED DOGS TO BE EUTHANISED

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ABSTRACT

Euthanasiation of stray dogs is a common practice at Dewan Bandaraya Kuala Lumpur (DBKL), Malaysia. It is expected dogs to undergo euthanasia are under enormous stress. The objective of this study was to evaluate stress indicators, serum cortisol concentration and neutrophil to lymphocyte ratio in sedated and non-sedated dogs before euthanasiation. Forty adult dogs at DBKL comprising of 20 females and 20 males, weighing 8 to 5 kg were used in this study. Blood was collected via jugular venipuncture. Ten male and 10 female dogs were sedated using acepromazine prior to euthanasia, while another 10 male and 10 female dogs were not sedated. Serum cortisol concentration and neutrophil to lymphocyte count were determined by radioimmunoassay and differential count, respectively. The results revealed that there was significant ($p < 0.05$) lower neutrophil to lymphocyte ratio in sedated than non-sedated dogs. However, the serum cortisol concentration did not vary significantly ($p > 0.05$) between the sedated and non-sedated dogs. In conclusion, sedation using acepromazine can reduced stress in dogs prior to euthanasiation.

Keywords: euthanasia, sedation, stress, cortisol, neutrophil to lymphocyte ratio

**ANTIMICROBIAL SUSCEPTIBILITY PROFILING FOR
STAPHYLOCOCCUS AUREUS AND *ESCHERICHIA COLI*
IN SMALL RUMINANTS OF UNIVERSITI PUTRA MALAYSIA
FOSTER FARMS**

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ABSTRACT

Antimicrobial resistance (AMR) has become a major problem worldwide with significance public health impact on animals and humans. *Staphylococcus aureus* is a common pathogen in domestic livestock with *Escherichia coli* being a normal flora in gut and considered as effective indicators in AMR surveillance. This study aimed to evaluate the antimicrobial susceptibility of *S. aureus* and *E. coli* isolated from small ruminants in Universiti Putra Malaysia foster farms. Milk and faecal samples were collected from 36 goats from 3 foster farms and the samples were processed for *S. aureus* and *E. coli* isolation. A total of 92% samples were positive for *E. coli* and 72% of samples were positive for *S. aureus*. Eleven *E. coli* and 3 *S. aureus* isolates on antimicrobial sensitivity test were resistant towards amoxicillin and penicillin. *E. coli* however, was susceptible to trimethoprim-sulfamethazole, neomycin, tetracycline and enrofloxacin. The results showed that both *S. aureus* and *E. coli* isolates have low susceptibility to antimicrobials indicating the presence of high level of antimicrobial resistance in these bacteria from the rampant use of β -lactam antibiotics in the foster farms.

Keywords: Antimicrobial resistance, antimicrobial susceptibility, *E. coli*, *S. aureus*, small ruminants

IDENTIFICATION OF ECTOPARASITES OF RED HYBRID TILAPIA (*OREOCHROMIS SP.*) CULTURED IN EARTHEN PONDS

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ABSTRACT

Red Hybrid Tilapia (*Oreochromis sp.*) in lakes and reservoirs in Malaysia has shown rapid increase in production. Currently the Red Hybrid Tilapia is recorded as the most common fresh water fish being farmed, where up to 46% was cultured in the earthen pond system. Unfortunately, diseases especially parasite infestation has affected the growth and production yield of the farmed fish. The present study was aimed to determine the prevalence and identification of ectoparasites in Red Hybrid Tilapia cultured in earthen pond and the pathological lesions it causes. In this study, 30 Red hybrid Tilapia were collected from three farms in Selangor, Malaysia. Only 17 fishes (56.7%) were infested with ectoparasites. The ectoparasites were counted and morphology of the ectoparasite determined via light microscopy. Only one ectoparasite species viz., *Dactylogyrus sp.* (Monogenea) was found to infest the gills of the fish. Histopathology of the gills tissue showed hyperplastic changes of the lamellar epithelium and erosion of the lamella. These lesions were the result of Opisthaptor of *Dactylogyrus* penetrating deep into the basement of gills membrane, inducing hyperplastic reaction, and subsequently destroying the secondary lamella.

Keywords: Red hybrid tilapia, ectoparasite, *Dactylogyrus*, histopathology

SULPHUR, NAPHTHALENE AND CAMPHOR AS POTENTIAL WILD RETICULATED PYTHON (*MALAYOPYTHON RETICULATUS*) DETERRENT

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ABSTRACT

Reticulated python is the most common species involved in the human-snake conflict in Klang Valley, Malaysia. Various chemicals are being used by people to keep these snakes away from their residential area. Sulphur, naphthalene and camphor are commonly used but the efficacy of these chemicals as a deterrent for reticulated python is not known. In this study, 5 juvenile wild *Malayopython reticulatus* that had gravitated into residential areas were acquired from various sources. The snakes were acclimatised for 2 weeks in individual vivariums bedded with wood shavings in a dark area, while kept off food for a minimum of 1 week before the experiment. A modified Y-maze was used and the snakes were released one at a time at the start arm. The goal arms were each bordered with deterrent material and wood shavings. The path that the snake chose to take was recorded as either positive or negative for deterrents. It was found that all the snakes were able to transcend all chemical borders but naphthalene was the most avoided deterrent, which indicates a positive response, making it a highly efficacy deterrent. Camphor was the least avoided chemical, which indicates a negative response, making it a low efficacy deterrent.

Keywords: wild reticulated python, human-snake conflict, deterrent, Y-maze

DETERMINATION OF PIG IMMUNITY STATUS AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AFTER USING DIFFERENT VACCINATION REGIMES

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is a disease characterised by late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. Malaysia is endemic with both Type I and II viruses. The Type I and II modified live vaccines (MLV) are available in the market while vaccination strategy includes breeders only or whole herd approach. Hence, the objective of this study is, to determine the immunity status of pig herds after different vaccination regimes by using a combination of ELISA and PCR techniques to classify the herd PRRS Virus (PRRSV) status under American Association of Swine Veterinarian (AASV) herd classification. Two hundred and seventy sera samples from animals of different age groups were collected from 3 farms practicing different vaccination regime. The sera were analysed using IDEXX PRRS X3 ELISA antibody test kit for PRRSV antibodies. Pooled sera samples were tested using nested RT-PCR assay that can differentiate Type I from Type II PRRSV. The ELISA showed that the mean S/P ratio of all age group from all farm were positive (>0.4), indicating they had been exposed to the virus. While nested PCR detected both homologous and heterologous virus strain in Farms A and C, indicating presence of field isolates. However, there was no heterologous virus in Farm B; only homologous (Type II) was present in weaners and 8-week-old growers, which is probably due to the vaccination programme in the piglets. Under AASV herd classification, Farms A and C were classified as positive unstable (Stage I) while Farm B classified as positive stable (Stage II-A). In short, the different vaccination regime produced herd with different immunity status and different herd classification. The practice of Type-II MLV combined with whole herd vaccination approach in Farm B appeared to have created immunologically more stable herd and had successfully controlled virus circulation while displacing field virus. The practice of Type-II MLV with breeders in Farm A and Type-I MLV whole herd approach in Farm C had caused heterologous field challenge and immunologically less stable condition than in Farm B.

Keywords: porcine reproductive and respiratory syndrome (PRRS), ELISA, nested-PCR, modified live vaccine, herd classification

**CASES OF CATTLE LAMENESS PRESENTED TO
UNIVERSITY VETERINARY HOSPITAL,
UNIVERSITI PUTRA MALAYSIA FROM 2013 TO 2016**

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ABSTRACT

Lameness is one of the most common cases presented to veterinarians. Lameness in cattle can cause an intense pain and are a major welfare issue. It also can cause serious economic impact which is due to loss of milk yield and meat, loss with premature culling and replacement, and lost associated with cost of treatment. The objective of this study was to retrospectively determine distribution of lameness cases in cattle presented to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). And to analyse these lameness cases for future preventive measures. Data for year 2013 to 2016 were collected from the Ruminant Log Book at the Large Animal Ward, UVH, UPM. These data include date of first visit, animal ID, location of farm, age, gender, weight, legs affected, distribution of lesion, treatment, date of the next visit, and outcome of the lameness, if any. Lameness in cattle usually occurs in April, October and December each year and it is commonly affects adult, female, and dairy cattle. Lesions are usually can be found at hind limbs and foot. Good management practice might help in reducing cases of trauma such as removing sharp objects, maintaining facilities well, and proper handling of the animals.

Keywords: lameness, cattle, management system.

**EFFECT OF HUMAN VISITORS ON CAPTIVE SERVAL CATS
(*LEPTAILURUS SERVAL*) BEHAVIOUR AT THE
MALAYSIAN NATIONAL ZOO**

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ABSTRACT

Serval cat (*Leptailurus serva*) can be found throughout Sub-saharan Africa, near water sources. Nowadays, serval cats are gaining popularity as exotic pets. The objective of the study is to document the behaviour of the serval cats in captivity, and to know whether human visitors affect their activities and behaviour. The study was conducted in Zoo Negara Malaysia, at the serval exhibit area. Two serval cats, one adult female and one young female from the same enclosure were subjects for this study. This study was done by visual observation using behavioural index adapted from previous studies on the cat family. An instantaneous sampling was done during the Zoo opening hours for 6 h in two split sessions; 9.00 a.m. to 12.00 p.m. and 1.00 to 4.00 p.m. 24-h observations were conducted 4 days, from 1st to 4th February 2017. The behaviour and spatial use of servals and number of visitors to the exhibit were recorded. The servals enclosure was divided into 4 areas; area A was for resting, area B was an open area, area C was a wall for the servals to hide, and area D was the feeding and hiding area. Generally, the cats spent most of the day sleeping. Rolling-over was the least behaviour observed. The result showed that the diurnal activity of the captive cats during Zoo visiting hours was negatively correlated with visitor count, and aggression, rolling, walking, alert, resting awake, grooming, and yawning. A positive correlation was with sleeping and being out of sight. However, only aggression showed a strong positive correlation with visitor count. This could be attributed to flight response, which is similar to the behaviour in domestic cats. Both servals spent approximately 85% of their time in area A, 15% each in areas B and D. None of the servals used area C. There was no correlation between the visitors count and the spatial use of the servals. In conclusion, the study showed visitors count did not affect the behaviour of servals.

Keyword: serval cat, captivity, behaviour, human visitors

BEHAVIOURAL ANALYSIS OF CAPTIVE MALAYAN TAPIRS (*TAPIRUS INDICUS*) AT THE MALAYSIAN NATIONAL ZOO

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ABSTRACT

Malayan tapirs (*Tapirus indicus*) in captivity are increasing in numbers, especially with their wild populations facing a declining trend. Good management is imperative to ensure their health and welfare are upheld, along with their public image to improve awareness of these animals. Behavioural observations were carried out with the aim of describing the tapirs' diurnal behavior in captivity. Four Malayan tapirs living as pairs in two exhibits at the Malaysian National Zoo were included in this study. Focal sampling was done with behaviours recorded for six h during the opening hours of the institution. The frequencies of visitors to the exhibits were noted. Diurnal activity budgets, exhibit use, and visitor frequency effects were calculated from the data collected. The study revealed that on average, the tapirs spent a large amount of time resting, swimming, and eating. Neither gender differences nor exhibit differences were observed between the activity levels of the tapirs. A negative correlation between their activity levels and visitor frequency were also identified. These findings may be explained by the tapirs having been bred in captivity, and having adapted to human presence. The results conformed to the tapirs' expected crepuscular behaviour, with no stereotypic behaviour seen.

Keywords: activity budget, behavior, captivity, Malayan tapir (*Tapirus indicus*)

MALACHITE GREEN RESIDUES IN LOCAL AND IMPORTED *PANGASIVS HYPOPTHALMUS* SOLD IN MARKETS IN SELANGOR, MALAYSIA

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ABSTRACT

Malachite green (MG) is an *N*-methylated diaminotriphenylmethane dye widely used for therapeutic purposes in the aquaculture sector across the world. Malachite green can easily oxidize to leuco-malachite green (LMG) and remains in muscles and internal organs for several weeks. Consumption of fish with MG residues may be harmful to humans because it is toxic and carcinogenic. This study was aimed to detect MG residues in local the fresh fish, *Pangasius hypophthalmus*, and its imported frozen fillet from Vietnam. Four fish and 3 fillets were purchased from 7 supermarkets in Selangor, Malaysia. The muscle was analysed for MG residues using enzyme-linked immunosorbent assay (ELISA) and liquid chromatography tandem mass spectrometry (LC-MS). The ELISA kit standard curve was not linear with correlation coefficients of 0.5355. This had caused inaccuracies in the calculation MG concentration. However, the slope of the LC-MS standard curve was at 0.999. Using this standard curve the concentration of MG and LMG residues in the fish and fillet were shown to range from 1.19 to 4.09 µg/kg. The minimum required performance limit (MPRLs) for MG and LMG in fish as set by the European Union is 2 ppb. This means the MG and LMG residues in some fish and fillet samples in this study exceeded the MPRLs.

Keywords: Malachite green, *Pangasius hypophthalmus*, residues, ELISA

FEED FATTY ACID UTILISATION BY THE GIANT PANDA (*AILURUPODA MELANOLEUCA*)

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ABSTRACT

The Giant Panda (*Ailurupoda melanoleuca*) derived most of its nutritional needs from plant materials. Dietary utilisation of these plant materials is complicated by the comparatively lower plant fatty acid contents, and the fact that the digestive system of the Giant Panda has characteristics consistent with carnivores. In the absence of other body fluids and tissues, faecal fatty acid profiles of the Giant Panda provide important hints to the functions of its digestive system. These information are crucial to understanding of how Giant Pandas acquire energy and other nutritional needs from its herbivorous diet. The aims of this research were to determine the fatty acid profiles of bamboo plants in the Giant Panda diet, and faecal fatty acid profile. Four local species of bamboo plants, and faecal samples from two adult Giant Pandas and their cub were obtained for analysis. The study was conducted from 9th January to 20th of February 2017 at the Malaysian National Zoo. Identification and quantification of fatty acid content was done by gas liquid chromatography (GLC) following the procedure of total lipid extraction and fatty acids methyl esters (FAME). The results showed that palmitic acid (16:0) was the dominant fatty acids in the shoots of bamboo plants, while α -linolenic acid (18:3 n-3) is the major fatty acid in the leaves. Total saturated fatty acids (TSFA) were consistently high followed by unsaturated fatty acids comprising of the PUFA n-3, n-6 and MUFA. Faecal fatty acid profiles showed presence of very long chain n-3 and n-6 fatty acids, which are important for cellular functions. In conclusion, the study showed that while bamboo lacked the longer chain essential fatty acids, and the Giant Pandas could satisfy their physiological requirements through *de novo* synthesis. This indicates that the Giant Panda do have the ability, as well as the specific enzymes for the elongation and desaturation of fatty acids, a feature found in most herbivorous and omnivorous animals.

Keywords: Giant Panda (*Ailurupoda melanoleuca*), fatty acid analysis, local bamboos, nutritional preferences, utilization

TURTLE EGGS AS THE ULTIMATE HEALTH DELICACY

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ABSTRACT

A descriptive and comparative study on fatty acids and protein, coupled with *Salmonella* sp. isolation in turtle and chicken eggs were carried out. Thirty turtle and chicken eggs purchased from local wet market raw and homogenised prior to use in this study. Analysis conducted using the Kjeldhal method showed no significant ($p > 0.05$) difference in crude protein content between turtle and chicken eggs at 11.99 and 11.58%, respectively. There was significant ($p < 0.005$) difference in polyunsaturated fatty acid composition between turtle and chicken eggs; Omega-3 was higher in turtle eggs (13.35%), and Omega-6 fatty acids higher in chicken eggs (16.25%). There was also significant ($p < 0.05$) differences in saturated and monounsaturated fatty acids composition where it was higher in turtle eggs at 35.27 and 48.79%, respectively compared to chicken eggs at 1.99 and 45.3%, respectively. Generally, turtle eggs contain high both essential and non-essentials fatty acids. None of the isolates were positive for *Salmonella* sp. and the majority of the isolates were positive for *Proteus mirabilis*. In conclusion, turtle eggs contain significantly ($p < 0.05$) higher fatty acids compared to chicken eggs, but insignificantly lower in protein percentage, and pose risks of bacterial infection upon consumption, especially when raw.

Keywords: fatty acids, protein, bacterial isolation, turtle eggs, chicken eggs

EFFECT OF EFFECTIVE MICROORGANISM ON MEAT QUALITY OF SEMI-COMMERCIAL VILLAGE CHICKEN

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ABSTRACT

One of the biggest challenge faced by the chicken meat industry to increase production efficiency, including to increase feed conversion and meat quality with the addition of antimicrobial agents and natural products in their diets. This study was conducted to investigate the effects of effective microorganism on the breast muscle meat in village chickens. Effective microorganism was added to drinking water of semi-commercial village chicken beginning with one-day-old chicks. At 84 days old, 16 chickens from control and treatment group was randomly chosen and slaughtered to harvest breast muscle for both physical and proximate meat quality analyses. There were significant ($p < 0.05$) differences in colour (lightness and redness), ash, crude protein and crude fat between treatment and control group. However, the pH, colour (yellowness), texture, cooking loss and dry matter did not differ significantly ($p > 0.05$) between groups.

Keywords: village chickens, effective microorganism, ash, crude protein, crude fat

DETECTION OF HAEMOPARASITE IN SEMI-COMMERCIAL AND SCAVENGING VILLAGE CHICKEN

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ABSTRACT

Village chicken (*Gallus gallus domesticus*) usually associated with free-range system are exposed to the environment, seasonal outbreaks of disease, soil bacteria and parasites, wild birds, and other animals. Haemoparasites infections are prevalent and contribute significantly to the health status of village chicken. In this study, the occurrence of haemoparasite infection in semi-commercial and scavenging village chicken was determined. Twenty-four blood samples were collected from chicken in a village located in Simpang Renggam, Kluang, Johore, Malaysia. Twelve blood samples were collected from the semi-commercial village chicken in the Aqil Aqilah Farm and 12 from village chicken found scavenging around the village. Giemsa-stained thin blood smears were prepared and screened for haemoparasites. Antigen detection using conventional reverse transcriptase PCR on blood samples was done and specific prime was designed to target mitochondrial cytochrome b genes of the parasites. In microscopic examination of blood smears, no haemoparasites were detect in any of the samples. RT-PCR analysis was negative for haemoparasite cytochrome b gene in all blood samples. In conclusion, there was no difference in blood parasite infection between semi-commercial and scavenging village chicken.

Keywords: haemoparasite infection, village chicken, RT-PCR, giemsa stain

**ANTIBACTERIAL EFFECT OF *MELASTOMA MALABATHRICUM*,
TETRACERA INDICA, AND *ARDISIA CRISPA* TOWARDS ISOLATES
FROM SUBCLINICAL MASTITIS IN CATTLE**

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ABSTRACT

Subclinical mastitis does not exhibit any obvious clinical signs in animals. However, the infection causes economic losses to the dairy industry. To make matters worse, antimicrobial resistance has become a serious threat to the industry. To treat the infection, herbal alternatives are being sought. In this study, the *Melastoma malabathricum*, *Ardisia crispa*, and *Tetracera indica* leaf extracts were investigated for their antibacterial potential on isolates of *Staphylococcus aureus*, *Staphylococcus hyicus* and *Staphylococcus intermediate* obtained from milk samples of 14 cattle with subclinical mastitis. This study was conducted at the University Agriculture Park, Universiti Putra Malaysia. The Kirby-Bauer or disc diffusion methods showed that all plant extracts possessed antimicrobial activities, with *M. malabathricum* (inhibition zone=22.05±0.83 mm) being the most potent extract against all three strains of *Staphylococcus* spp., followed by *A. crispa* (inhibition zone=13.30±0.83mm), and *T. indica* (inhibition zone=9.73±0.83mm).

Keywords: Subclinical mastitis, *Melastoma malabathricum*, *Ardisia crispa*, *Tetracera indica*

MILK PRODUCTION IN MURRAH BUFFALOES UNDER VARIOUS FARM MANAGEMENT SYSTEMS

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ABSTRACT

Buffalo is an important source of milk, supplying approximately 12% of total world production. The aim of this study was to compare milk yield of buffaloes kept under good (GM) and poor management (PM) practices. Farm management was assessed using the standard management guidelines for efficient dairy buffalo production. Five Universiti Putra Malaysia foster farms were selected: GM farms are located in Mukim Ulu Melaka, Langkawi, and Sungai Tangkas, whereas the PM farms are in Kuah Langkawi, Taman Sri Jelok Kajang and Sungai Batangsi Semenyih, Malaysia. Thirty lactating cows from each farm were selected and the milk yield recorded for 3 to 5 days consecutively. Past records beginning November 2015 for up to 3 months, were also obtained from each farm to determine consistency of milk yield. Farmers were interviewed to obtain information on farm reproductive performance and health status. Clinical mastitis was assessed according to udder and milk abnormalities and lameness by locomotion score. The results revealed that the average daily milk yield in buffaloes was 6.32 ± 1.20 L for GM and 4.42 ± 2.40 L for PM farm. The difference in milk yield among farms was statistically significant (t -value=7.908, d.f.=229, $p < 0.05$). Clinical reproductive problems were similar for both farms. This study suggests that buffalo cows in GM performed better than those in PM farms.

Keywords: dairy buffalo, milk yield, reproductive performance, lameness, mastitis

OCCURRENCE OF PATHOGENIC BACTERIA IN BLOOD COCKLES (*ANADARA GRANOSA*)

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ABSTRACT

Blood cockles (*Anadara granosa*), also locally known as *kerang* is a popular seafood in Malaysia. Cockles are at high risk of being contaminated by pathogenic microorganism because they are filter-feeder organism. In 2015, there was a drastic reduction in harvest of these cockles. One of the main reasons for the poor harvest is deterioration of breeding environment water quality. Microbiological assessment of cockles in Malaysia is lacking; therefore, this study was undertaken to determine type and antibiotic susceptibility pattern of pathogenic bacteria present in blood cockles. Thirty pooled *A. granosa* samples were purchased from 15 wet markets and supermarkets in Klang Valley, Malaysia. All samples were subjected to bacteria isolation and identification using conventional methods. Antibiotic susceptibility test was performed on the bacteria isolated. Eighty-five bacteria isolate from the cockles were Gram-negative. *Aeromonas* spp (23%), *Proteus vulgaris* (20%) and *Vibrio alginolyticus* (15%) were the most dominant genus identified. Other human pathogens also identified were *Vibrio parahaemolyticus* (6%), *Vibrio cholera* (5%) and *Salmonella* spp. (2%). All isolates were most resistant to Ampicilin (10 µg) and most were sensitive to Trimethoprim/sulfamethoxazole (25µg). Among isolates, *Aeromonas* spp., *Klebsiella pneumonia*, and *Vibrio parahaemolyticus* were multidrug resistant. In conclusion, this study showed that cockles are highly contaminated with pathogenic bacteria including some that were multidrug resistant.

Keywords: cockles, microbial assessment, antibiotic susceptibility test, Gram-negative, multidrug resistance

LIGHT AND SCANNING ELECTRON MICROSCOPIC STRUCTURE OF THE GUTTURAL POUCH

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ABSTRACT

Although to date the functional significance of the guttural pouches in the horse is still not clear, this outpouching command considerable veterinary attention from the point of view of fungal infection. The latter referred to as guttural pouch mycosis resulted in erosion of the adjacent internal carotid artery leading to fatal haemorrhage. This study on the light and scanning electron microscopic structure of the guttural pouch is aimed at identifying the possible passageway for the fungus from the guttural pouch into the carotid artery. Two euthanised horses were used in this study and the guttural pouches were approached via the Viborg's triangle. Samples of the membranous guttural pouch from the lateral, media, and the roof were collected and processed for light and scanning electron microscopy. Light microscopy showed that the guttural pouch mucosal epithelium is pseudostratified columnar and ciliated interspersed with goblet cells. In the loose connective tissue below the epithelium are numerous capillaries, venules and mucous glands. Under scanning electron microscopy, mucous droplets were present in glands in the underlying connective tissue of the epithelium, between contiguous epithelial cells and on the mucosal surface of the guttural pouch. There is possibility that the mucous in the connective tissue are carried through intercellular spaces between contiguous epithelial cells and subsequently deposited on the guttural pouch mucosal surface. Based on this observation, fungus from the guttural pouch could follow the same passageway as that of mucous, but in the opposite direction, to reach the underlying connective tissue. The fungus would enter the internal carotid artery via the numerous single layer walled capillaries and venules in the connective tissue below the epithelium.

Keywords: guttural pouch mycosis, passageway, light and scanning electron microscopy, intercellular space, capillaries and venules.

RESPONSE OF THE MALAYAN TIGER (*PANTHERA TIGRIS JACKSONI*) TO COLOURS AS VISUAL ENRICHMENT

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ABSTRACT

The Malayan tiger (*Panthera tigris jacksoni*) is under the category of critically endangered species according to IUCN lists. To conserve this unique species in Malaysia, practicing conservation is vital with the keeping of tigers in captivity. Since the tiger enclosures has a simple environment design compared to the complex environment of the wild, environmental enrichment is commonly used for their welfare and health. Unfortunately, little is known about the effect of the enclosures on this species. Colours are one of the important visual stimulation for tigers in captivity since the enclosure is surrounded by unstimulating walls. We observed the response of the tigers to colours and related that to past research findings. In this study, 4 Malayan tigers (*Panthera t. jacksoni*) and two mix breed tigers (*Panthera t. jacksoni* × *Panthera t. tigris*) were chosen and introduced to the colours red, yellow, green in their enclosure. Three papers, one each of the three colours, were pasted on the wall before releasing the tiger into the enclosure. The association between age, gender, and origin (wild vs born in captivity) of *Panthera tigris* with their responses towards the colours were calculated. There are no significant ($p > 0.05$) difference between response to colours and the age, gender, and origin of the tigers. However, the mean of varies with colour. Five of 6 tigers approached red colour first before other colours. Total bouts and duration the tigers were in contact with the three colours were calculated and the results showed that the tigers made contact more frequently and of longer duration with the red colour. These results suggest that tigers showed more interest towards red colour than yellow or green colour.

Keywords: Malayan tiger (*Panthera tigris jacksoni*), enrichment, colours

SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS ANTIBODIES IN PIGEONS IN SELECTED AREAS IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Newcastle disease (ND) is a disease of domestic poultry and other bird species caused by the virus family *Paramyxoviridae*. Newcastle Disease can be transmitted by direct contact with diseased or carrier birds, fomites, hatching chicks from infected egg and potential zoonotic disease. Pigeons may serve as reservoirs for ND. This study determined the Newcastle disease (NDV) seroprevalence in pigeons in Malaysia. Sixty serum samples were collected from 36 wild and 24 captive pigeons in Klang Valley, Malaysia. The Serum samples were analysed for NDV antibodies using the haemagglutination inhibition test. The study showed that 50% (30/60) of all samples were seropositive for NDV antibodies. Wild pigeons showed 41.7% (15/36) and captive pigeons 62.5% (15/24) seroprevalence. In conclusion, this study showed that pigeons in Malaysia are exposed to NDV and developed NDV antibodies at certain stages of their life.

Keywords: antibodies, haemagglutination inhibition, Newcastle disease virus, pigeon, seroprevalence

PATHOGENICITY AND IMMUNOGENICITY OF FOWL ADENOVIRUS ATTENUATED IN CHICKEN EMBRYO LIVER CELLS IN SPECIFIC PATHOGEN FREE CHICKENS

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ABSTRACT

Fowl adenovirus (FAdV) infection is distributed worldwide and is associated with inclusion body hepatitis (IBH). This disease can cause high mortality in chicken. Thus, it was the objective of this study to determine the pathogenicity and immunogenicity of FAdV isolate (UPM1137) attenuated live in chicken embryo liver (CEL) cells in specific pathogen free (SPF) chickens. Forty-five one-day-old SPF chicks were divided into four equal groups, A, B, C, and D. Groups A, B, and C were inoculated, through the subcutaneous route on days 1 and 14 of age, with 0.1 mL FAdV passage 15 (P15) at a virus titre of $10^{5.2}$ TCID₅₀/0.1 mL, 20 (P20) with virus titre of $10^{5.6}$ TCID₅₀/0.1 mL, and 25 (P25) with virus titre of $10^{5.2}$ TCID₅₀/0.1 mL, respectively. Group D acted as control group and remain non-inoculated. Sampling was done on days 1, 14, and 28 of age. The chicks were observed at least twice daily for abnormal clinical signs. Prior to necropsy, blood was collected, and the body and liver weights were recorded. ELISA was used to determine FAdV antibody titre. Liver tissue samples were fixed in 10% buffered formalin for histological examination. The study revealed no abnormal clinical signs or significant gross or histological lesion in the liver in any of the chickens during the study. FAdV antibody titer was not detected in group D (Control). However, the antibody titre was induced at day 14 of age in all inoculated chicks, which continued to increase by day 28 of age. It was concluded that the live attenuated FAdV in CEL cells is of low pathogenicity and could induce high FAdV antibody titre in SPF chickens.

Keywords: Fowl adenovirus (FAdV), specific pathogen free (SPF) chickens, subcutaneous route, histological examination.

KARYOTYPE OF HORNED AND POLLED SHEEP

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ABSTRACT

A karyotypic analysis was performed on two breeds of sheep, the horned Merino and polled Dorper to compare chromosome features. Lymphocytes were extracted from blood samples and cultured in RPMI 1640 as a culture medium, supplemented with foetal bovine serum and penicillin-streptomycin. The lymphocyte cultures in duplicates, were stimulated to grow with phytohemagglutinin (PHA) or pokeweed (PWM) mitogens. Cultures with PWM produced better quality and quantity of metaphase spreads than those with PHA. Karyotypes were constructed in accordance with the standard procedure of International Cytogenetic Nomenclature of Domestic Animal (ICNDA). The domestic sheep has a diploid number (2n) of 54. The chromosomes morphology of Merino and Dorper are indistinguishable based on conventional karyotypes. Male and female sheep have karyotypes of 3 pairs of submetacentric and 23 pairs of acrocentric autosomes. The X chromosome was identified as the largest acrocentric chromosome while the Y chromosome is the smallest chromosome. The fundamental number for male and female sheep is 60.

Keywords: Sheep, karyotype, Merino, Dorper, horned, polled.

**ULTRASTRUCTURE EVALUATION OF
PASTURELLA MULTOCIDA TYPE B:2 IN RESPIRATORY ORGANS OF
AEROSOLLY-INFECTED MICE**

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ABSTRACT

Haemorrhagic septicaemia (HS) is an acute, fatal, septicaemic disease of buffaloes and cattle caused by *Pasteurella multocida* B:2. Natural cases of HS occur primarily via the aerogenous route of susceptible animals. This study was conducted to determine and evaluate respiratory organ ultrastructures of mice with respect to aerosol inoculation of *P. multocida* that mimics natural HS transmission. Thirty healthy female mice were divided into two groups, control (n=5) and treatment. The treatment groups were further divided equally into 5 groups (n=5/group). All treatment group were inoculated aerosolly with 10 mL of 1×10^9 CFU/mL of *P. multocida* for 20 min. The control group were euthanised at 6 h prior to inoculation while the treatment groups were euthanised at 0, 6, 12, 18, 24 h post-inoculation (pi). Clinical signs were observed for 24 h pi. Lungs tissue were collected for ultrastructural examination. The mice showed reduced activity beginning at 12 until 24 h pi. Ultrastructure evaluation showed the treatment groups to exhibit mild to severe pathological changes in the pneumocytes and endothelial cells. Overall, inoculation of *P. multocida* Type B:2 via aerogenous route caused significant ($p < 0.05$) cellular changes in the lungs of mice.

Keywords: haemorrhagic septicaemia, *Pasteurella multocida* serotypes B:2, aerosol, lungs, ultrastructure, mice

A RETROSPECTIVE STUDY ON PREDILECTION SITES OF FOREIGN BODY IN THE GASTROINTESTINAL TRACT AND THE ASSOCIATED OUTCOMES OF MANAGEMENT IN DOGS AND CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FROM 2012 TO 2016.

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ABSTRACT

Gastrointestinal (GIT) foreign bodies are foreign materials in the gastrointestinal tract (GIT) that could easily passed through the GIT. The objectives of this study were to determine the predilection sites of GIT foreign body in dogs and cats and to investigate the outcome of management of the GIT foreign body cases in dogs and cats presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia from 2012 to 2016. Seventeen foreign body cases, 10 dogs and 7 cats, were identified in this study. The patient medical records were retrieved and reviewed for the signalment, clinical presentation, diagnostic workouts, treatment, surgical approaches, and post-treatment complications. The most common location for GIT foreign body in dogs based on the number of cases is the stomach (41.7%), small intestines (41.7%), and large intestines (16.7%). In cats, the most common location is the large intestines (44.4%) followed by small intestines (33.3%), stomach (11.1%), and omentum and abdomen (11.1%). Minor post-treatment complications in dogs were mild haemorrhage from endoscopic removal (10%), swelling at suture site (10%) and bruises at suture site (10%). Cats did not show post-treatment complication. This study showed that the overall response to treatment options in GIT foreign cases is good.

Keywords: foreign body, cats, dogs, University Veterinary Hospital, Universiti Putra Malaysia

SEROPREVALENCE OF NEWCASTLE DISEASE VIRUS ANTIBODIES IN PSITTACINE BIRDS IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Newcastle Disease caused by Newcastle Disease virus (NDV) from the family *Paramyxoviridae* is a devastating disease in poultry and other avian species. It is also a potential zoonotic disease that can cause conjunctivitis and flu-like symptoms in humans. Psittacine birds may serve as reservoirs or carriers for NDV to other galliformes. This study was conducted to determine NDV antibody titre in pet birds of psittacine species in Klang Valley, Selangor, Malaysia. Forty-two birds of psittacine species consisting macaws, parrots, and cockatoos were obtained from clients, zoo, and pet shops and blood was sampled. Sera from these birds were subjected to haemagglutinin inhibition (HI) assay. Out of 42 samples, 54.76% (23/42) were positive for ND antibodies. The seroprevalence of client-owned, zoo, and pet shop birds was 21.43% (9/42), 4.76% (2/42) and 28.57% (12/42), respectively. Parrots showed the highest seroprevalence at 33.33% (14/42) followed by cockatoos at 21.43% (5/42), and macaws at 9.52% (4/42). Chi-square analysis revealed an association between the risk factor of psittacine birds and NDV seroprevalence. In conclusion, psittacine species of birds are susceptible to NDV infection.

Keywords: cockatoo, hemagglutination inhibition test, macaw, Newcastle Disease virus, parrot, Psittacine birds, seroprevalence

HISTOLOGICAL CHANGES IN THE LIVER OF GOATS WITH KETOSIS

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ABSTRACT

The demand for goat milk and meat in Malaysia has increased due to rapid population growth, urbanisation, and changes in consumer preferences. However, small ruminant dairy production in Malaysia is small and cannot meet demand. One of the major factors that restrict the production is the metabolic disorder ketosis. This case-control study was done to describe the histopathological lesions in liver of goats clinically diagnosed with ketosis and to associate the histopathological changes with the clinical features. The study was carried out without knowing the clinical background of the cases. Nine liver samples inclusive of control cases were fixed in 10% buffered formalin. Histological examinations were carried out and severity of lesions determined using the % lesion distribution and graded into mild, moderate, and severe. Clinical signs including dullness, depression, teeth grinding, and recumbency and the blood glucose and serum β -hydroxybutyric acid (BHBA) determined. In this study, the clinical signs were only observed in the ketosis group. Blood glucose level of ketosis group was significantly ($p < 0.05$) lower while BHBA of ketosis group significantly ($p < 0.05$) higher than controls. The lesions observed in the liver included fat deposition, congestion, and thrombosis. In goats with ketosis, fatty liver lesion was significantly ($p < 0.05$) more severe than congestion or thrombosis. Correlation analyse reveals that the BHBA have negative correlation with blood glucose but positive correlation with histological score. Similarly, histological scoring had negative correlation with blood glucose level. In conclusion, fatty liver is strongly associated with ketosis, in severe ketosis with clinical signs. Therefore, there is high likely that a goat exhibiting fatty liver lesion also suffering from severe clinical ketosis with clinical signs.

Keywords: ketosis, fatty liver, congestion, thrombosis, β -hydroxybutyric acid

INTESTINAL MORPHOLOGY CHANGES IN SEMI-COMMERCIAL VILLAGE CHICKEN TREATED WITH EFFECTIVE MICROBES

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ABSTRACT

Effective microorganisms were proven to have beneficial effects in preventing infections, maintain intestinal microbial balance, improving digestion and absorption rate, thus improving production. However, studies on morphological changes of intestine caused by effective microorganisms is lacking. An experiment was conducted to determine the effect of diet supplemented with effective microorganisms on the morphology and gross anatomy of the intestines of village chickens. Sixteen male semi-commercial village chickens were assigned into two groups; control and treatment. The treatment group of chicken was supplemented with effective microorganisms (EM-1) in drinking water for the period of the experiment. Effective microorganism supplementation caused significant ($p < 0.05$) increase in the width of villi of the ileum. However, the height of villi of ileum in the chicken given effective microorganism-supplemented diet did not differ ($p > 0.05$) from those on non-supplemented diet. Effective microorganism supplementation also did not significantly ($p > 0.05$) affect the height or width of the villi of the duodenum and jejunum. The small intestine of semi-commercial village chickens supplemented with effective microorganisms was significantly ($p < 0.05$) longer than that of control chicken. It is concluded that effective microorganism supplementation to the drinking water increases the width of villi in the ileum and causes the small intestine to increase in length in village chicken.

Keywords: effective microorganisms, small intestine, villi length, villi width.

DETERMINANTS OF OBESITY IN DOGS

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ABSTRACT

Obesity is characterised by the accumulation of excessive amount of body fat, resulting in grossly fat or overweight individuals. Dogs that are overfed and had minimal amounts of exercise are most at risk for becoming obese. This study was carried out because of the potentially deleterious health effects and negative financial consequences associated with obesity in dogs. It was hypothesized that the occurrence of obesity in dogs is associated with the occurrence of obesity among their owners. Therefore, the objectives of this study were to determine the owner's perception of their dogs' body weight, determinants of obesity in dogs, and correlation between obese owners and obese dogs. Seventy dog owners were interviewed via a questionnaire adapted from the University of Glasgow Nutrition Questionnaire. The results showed that 35.71% of dogs were overweight, 21.43% were obese, and 40% were of ideal weight. The results also showed that dogs perceived to be greedy were 3 times more likely to be obese (OR 3.041, 95% CI 1.074 – 8.460) while those perceived by their owners to be hungry all the time had significantly high ($p < 0.05$) frequency of obesity at 61.43%. This study showed that owner's perception of hunger "signs" in dogs is a major determinant in the feeding of their pets, putting the dogs at higher risk of being obese. Dogs that overeat tend to have BCS of 4 ($n = 25$) and 5 ($n = 15$). The freedom given by owners for dogs to scavenge for food was also a determinant of obesity. Dogs that scavenged had higher BCS scores corresponding to being overweight and obese. However, there were no significant ($p > 0.05$) correlation between the dog owners' body mass index (BMI) and the BCS scores of their dogs. Owners that were interviewed were overweight and obese, with 6% morbidly obese and they own many dogs with BCS of 3 to 5. The current study also showed that owners tend to underestimate the body condition scores of their dogs. Over 77.14% of these owners believed their dogs were underweight or of ideal weight, although clinical evaluation showed that only 41.43% were in the underweight and ideal weight categories. These clearly shows that owners tend to underestimate their dog's BCS, and will continue to excessively feed their overweight and obese dogs. The study showed that owners misconception of their dog's weight is a major determinant in the effectiveness of weight management programmes for dogs. Dogs perceived by their owners to be greedy, hungry all the time and allowed to scavenge are most at risk of being obese. There were no correlations between owners' weight and obesity in dogs.

Keywords: dogs, obesity, owners

CLINICOPATHOLOGY OF CATS WITH LOWER URINARY TRACT DISEASES PRESENTED TO THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Feline lower urinary tract disease (FLUTD) is among the most common diseases in cats. A retrospective cohort study was conducted on FLUTD cases recorded in 2016 at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) with support of data from the case profiles at the University Veterinary Hospital, UPM. Information collected from 173 FLUTD cases in the study included patient signalments, haematological, serum biochemical, and urinalysis results. The specific risk parameters were age, breed, sex, lifestyle, diet, and body weight. The results showed that domestic short hair male cat, weighing 3.0 to 4.0 kg with average body condition score (BCS) of 3, living indoor in multicat households and consuming non-prescription dry feed were most affected with FLUTD. The common clinical signs were stranguria (42.2%), haematuria (38.7%), turgid urinary bladder (37%), non-compressible urinary bladder (28.9%), and vomiting (28.3%). Among the 173 cases, 32.4% showed normal leukogram while 15% had mild hyperglobulinaemia. Urinalysis showed severe haematuria with mild pyuria, moderate proteinuria, and mild bacteriuria. The most common diagnosis in these cats was bacterial cystitis.

Keywords: feline lower urinary tract disease, clinicopathological parameters, stranguria, haematuria, bacterial cystitis

**ANTIMICROBIAL USAGE AND AWARENESS ON
ANTIMICROBIAL RESISTANCE AMONG SMALL RUMINANT
FARMERS OF UNIVERSITI PUTRA MALAYSIA
FOSTER FARMS**

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ABSTRACT

Recent trends on the use of antimicrobials have become an alarming risk factor for antimicrobial resistance (AMR) in ruminant farming. The use of antimicrobials in livestock production can be affected by farmer's knowledge, attitude, and practices. A cross-sectional study involving small ruminant farmers of Universiti Putra Malaysia Foster Farm Programme was conducted to assess their practices on antimicrobial usage and understanding and awareness on AMR. Thirteen respondents, from 9 small ruminant farms in Selangor, Malaysia were enrolled in the study. Questionnaires comprised of five sections: demographic characteristics, general management of the farm, assessment on knowledge and practices on antimicrobial usage, and AMR. The results showed that the farms used 3 to 12 types of antimicrobials with penicillin (41%) and tetracyclines (36%) being the most common antibiotics. Generally, respondents were found to have sufficient knowledge on certain aspects on antimicrobials usage but not in the practices. Approximately 54% of respondents did not understand the actual AMR scenario and its impact on livestock farming. The results also suggested an association between the level of education and work position of the respondents and their awareness on AMR ($p < 0.05$). This study showed that there is lack of awareness on AMR among farmers as the majority were ignorant of the need for prudent use of antimicrobials. Thus, increasing awareness on AMR through guidance and education to the farmers are greatly warranted.

Keywords: antimicrobials, antimicrobial resistance, farmer awareness, small ruminants

MICROSCOPIC AND MOLECULAR DETECTION OF *GIARDIA* SPP. IN FAECAL SAMPLES OF SHELTER DOG IN SELANGOR, MALAYSIA

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ABSTRACT

Giardia spp. is a protozoan found in faeces of humans and animals and is considered to be of zoonotic potential. In vertebrates, including mammals, birds, reptiles and fishes, infection with *Giardia* spp. frequently produces clinical signs like diarrhoea, vomiting, weight loss and lethargy while in humans, various clinical manifestations due to the infection range from asymptomatic to acute, intermittent or chronic diarrhoea. This study determined the occurrence of *Giardia* spp. in faecal samples of shelter dogs in Selangor, Malaysia by using microscopic and polymerase chain reaction (PCR) detection methods. The association between occurrence of *Giardia* spp. and diarrhoea was investigated. One hundred and thirty dogs were random selected based on convenience sampling. Faecal swabs were obtained, rolled onto glass slides, air-dried, and stained with both Giemsa and Ziehl-Neelson for microscopic examination. Seventy faecal samples were subjected to nested-PCR assay using primers specific for *Giardia*. Gel electrophoresis was used to verify the PCR product sizes corresponding to the expected band size for *Giardia* spp. The *Giardia* spp. was detected in 2 of 130 dogs (3.1%) on microscopic evaluation which was confirmed on PCR detection where all the four dogs was also positive. Other parasites detected by microscopic examination include *Cryptosporidium* and coccidia. A total of 17.1% (n=12/70) of the samples were positive for *Giardia* spp. by nested-PCR detection method. There was four times higher positive detection of *Giardia* spp. in dogs with diarrhea and it was statistically significant through Pearson's chi-squared analysis (P=0.044). In conclusion, this study reports for the first time molecular detection of *Giardia* spp. in 17.1% of shelter dogs in Malaysia using molecular detection method. It is recommended that all shelter dogs should be periodically dewormed to prevent transmission of *Giardia* spp. among the shelter animals within the same enclosures and avoid potential zoonotic transmission to care takers of the shelter. Phylogenetic characterization of *Giardia* spp. in dogs in Malaysia merit further studies.

Keywords: *Giardia* spp., zoonotic, dog, microscopy, PCR

SEROPREVALENCE OF MELIOIDOSIS AMONG CATTLE OF UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

In the endeavour to make Malaysia as the major producer of *halal*, the livestock animals should be free from infectious disease. Melioidosis is endemic to Southeast Asia and northern Australia. The disease can reduce productivity of animals and cause loss of valuable animal protein. A study on the seroprevalence of melioidosis in livestock animals in 2000-2009 was conducted by the Department of Veterinary Services, Malaysia showed that of 100,262 animals tested, 5,729 (5.7%) were positive for melioidosis. Since then there was no follow-up study on this disease in Malaysia. This study focuses on determination of seroprevalence of melioidosis in the foster farms of Universiti Putra Malaysia. Fifty cattle mix-age and gender were selected randomly for blood sample collection. Complement Fixation Test (CFT) was used to detect antibody against melioidosis in serum. The result from CFT were negative for all samples. The lack of positive case is probably due to small sample number and/or related to the management practices. In this management practice cattle are mostly kept under a semi-intensive system and had little contact with soil and therefore at a low risk of contracting the organism. The zero seroprevalence in this study shows that the foster farms were free of melioidosis.

Keywords: melioidosis, seroprevalence, complement fixation test, foster farm

PREVALENCE OF ZONOTIC ENTERIC PROTOZOA AMONG CAPTIVE PRIMATES IN PENINSULAR MALAYSIA

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ABSTRACT

The transmission of zoonotic wildlife diseases has increased exponentially, especially in non-human primates mainly due to social, demographic and environmental alterations. At present, there remains a dearth of published data on the occurrence and prevalence of zoonotic enteric protozoa among non-human primates in Malaysia. This study was therefore conducted to determine the prevalence of zoonotic enteric protozoa among captive primates housed in four zoological facilities in Peninsular Malaysia. Forty pooled faecal samples were collected from Sumatra Orangutans, Borneo Orangutans, Chimpanzees, White-hand Gibbons, Siamangs, Lion-tailed Macaques, Stump-tailed Macaques, Bonnet Macaques, Mandrills, Brown Capuchin monkeys, Common Marmosets and Brown Lemurs. Faecal smears were prepared and stained with Giemsa and Modified Ziehl-Neelsen for microscopic examination. Polymerase chain reaction (PCR) using genus-specific primers was carried out to amplify the small subunit ribosomal RNA (SSU) gene of *Giardia*, *Cryptosporidium*, *Blastocystis*, *Balantidium* and *Entamoeba*. Microscopic examination revealed the presence of *Blastocystis* (30%), *Entamoeba* (12.5%), *Cryptosporidium* (10%), and *Balantidium* (2.5%). *Giardia* was not detected in the faecal smears. PCR amplification demonstrated that *Entamoeba* (65%) was the most dominant enteric protozoa in primates, followed by *Blastocystis* (50%), *Balantidium* (20%), *Cryptosporidium* (20%) and *Giardia* (5%). There was no significant ($p>0.05$) association between prevalence of protozoa and primate hosts or captive facility. The high prevalence of zoonotic enteric protozoa among captive primates in the country merits further investigation on the epidemiology, risk factors for infection, and their potential for zoonotic transmission to the staff and visitors.

Keywords: non-human primates, zoonotic enteric protozoa, *Giardia*, *Cryptosporidium*, *Blastocystis*, *Balantidium*, *Entamoeba*

EFFECT OF EXTENDERS STORED AT VARIOUS TEMPERATURES ON THE QUALITY OF BOAR SEMEN

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ABSTRACT

In artificial insemination, semen extenders are important to maintain the quality and survivability of sperm. This study aimed to determine the effect of storage temperatures of KRUUSE Betsville Thawing Solution (BTS) powder on motion characteristics, live dead percentage and morphology of extended boar semen. Semen samples were collected from 7 boars for the study. Each sample was mixed with 3 different BTS extenders stored at 4 and 16°C and room temperature prior to preparation. All the extended semen samples were kept at 16°C and evaluated at 24-h intervals for 3 days. General motility and live dead percentage of spermatozoa decreased gradually over time at all temperatures used in this study. However, there was no significant ($p>0.05$) difference in the general motility, live dead percentage, and abnormal morphology of semen with extenders stored at all temperatures used in this study. There was positive correlation ($p<0.05$) between general motility and live dead percentage of the sperm. Negative correlation ($p<0.05$) was observed between general motility and live dead percentage and abnormal morphology percentage of sperm. In conclusion, extenders stored at room temperature prior to preparation can be of highest quality for boar semen. However, statistically there was no difference ($p>0.05$) in survivability of sperm in extended boar semen at 4, 16°C and room temperature.

Keywords: Betsville thawing solution (BTS), extender, boar semen, general motility, live dead percentage, abnormal morphology

**EX VIVO CORRELATION OF ULTRASONOGRAPHIC
APPEARANCE OF GASTRIC SUBMUCOSAL FAT WITH
HISTOLOGY IN CATS**

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ABSTRACT

Ultrasonographic assessment of the stomach and its layers are performed routinely in companion animals. Varying submucosal thicknesses are seen in cats ultrasonographically, however their attribution to fatty infiltration, anatomical variation or disease processes remain unknown. This study was conducted to correlate appearance of gastric submucosa on ultrasound with its histology in cats. Twenty-five cats from animal shelters and pounds were used in this study. Following humane euthanasia, the stomach was resected and *ex vivo* ultrasonography was performed at the fundus, body, and pylorus. Ultrasound measurements include thickness of gastric wall (GWUS) and submucosa (GSMUS). The presence of gastric rugae fold (RF) was also recorded. Histology of stomach sections measured at ultrasound was performed. Histological measurements include thickness of gastric wall (GWH) and submucosa (GSMH) and submucosa fat score (GSMFAT). Fat was present in the gastric submucosa in 96% of cats. However, no association was found between GSMUS and GSMFAT in all regions. GSMUS was correlated to GSMH ($r=0.459$; $P=0.021$) in the body. RF was associated with GSMUS in the fundus and body. Gastric submucosal thickness is not associated to fatty infiltration on histology. Nevertheless, ultrasonographic thickness in gastric submucosa corresponds to histology and was also associated to presence of rugae fold.

Keywords: cat, histology, stomach, submucosa, ultrasound

SEROPREVALENCE OF MELIOIDOSIS IN SMALL RUMINANTS IN UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

Melioidosis is a zoonosis, tropical disease caused by *Burkholderia pseudomallei* which is a ubiquitous soil saprophyte, facultative anaerobic bacteria, non-spore forming, and motile Gram-negative oxidase-positive bacteria with bipolar staining. This disease is commonly found in domesticated animals such as sheep, goats, and pigs. The disease also affects other species including cattle, buffaloes, horses, deer, dogs, cats, primates, birds, tropical fish, reptiles, and humans. Melioidosis is of public health concern because it is zoonotic and can cause significant animal health problems leading to chronic debility and reduction of productivity in animals. Animals from the Universiti Putra Malaysia (UPM) foster farms was recruited in this study. In this study, 100 goats and 100 sheep were sampled. Blood samples were taken and processed for complement fixation test (CFT) to qualitatively determine IgG antibody against *B. pseudomallei* titre. The analysis was done at the Veterinary Research Institute Ipoh, Perak, Malaysia. A survey with a set of questionnaires was given to each farm to identify risk factors for melioidosis. Out of 100 goat samples only 1 (1%) was positive for *B. pseudomallei* antibodies. All sheep samples were seronegative. The positive sample from the goat had 0% endpoint whereby it has a score of +4 of complete button formation. All the negative results showed 100% endpoint with full lysis, thus no button formation. The questionnaire revealed that all the farms supply treated water to their animals although their management system varies between semi-intensive and intensive farming. In conclusion, the seroprevalence melioidosis is very low among small ruminants of UPM Foster Farms.

Keywords: *Burkholderia pseudomallei*, melioidosis, complement fixation test, seroprevalence, goat, sheep

LENTIVIRUS SEROPREVALANCE IN SMALL RUMINANTS OF UNIVERSITI PUTRA MALAYSIA FOSTER FARMS

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ABSTRACT

Caprine arthritis-encephalitis virus (CAEV) is an incurable disease that has both social and economic impacts. Clinical disease in goats includes encephalitis in kids, and chronic arthritis, inflammatory mastitis, and progressive respiratory disease in adults. The first reported case of caprine arthritis-encephalitis (CAE) in Malaysia was in year 2010, and another suspected case was reported in 2013. Since then no screening for the disease was done in Malaysia. Therefore, the study was undertaken to determine CAEV seroprevalance among the goat population of Universiti Putra Malaysia foster farms and risk factors associated with this disease. Blood samples were collected from 91 goats using the convenient sampling method. Sera were used in the competitive ELISA to detect antibody towards CAEV. Among the 91 goat samples, 8 (8.8%) were positive for CAEV. Biosecurity management, source of origin, and gender of the animal were important risk factors for the prevalence of CAEV in the foster farms. Therefore, it can be concluded that CAEV seroprevalance among goats in the foster farms is low. It should be noted that control measures such as testing and culling positive animals or segregation of infected animals away from test negative animals are important to control horizontal spread of the disease among goats

Keywords: goats, caprine arthritis encephalitis, foster farm, seroprevalance

DIROFILARIASIS IN MALAYSIAN SHELTER DOGS

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ABSTRACT

As a result of globalisation and climate change, *Dirofilaria immitis* and *Dirofilaria repens* has become the most common species of filarial nematodes in dogs. Dirofilariasis is a potential zoonotic disease that may be transmitted to humans. There is limited study on canine dirofilariasis in the dog population in Malaysia. Hence, this study is undertaken to detect filarial parasites, i.e. *Dirofilaria immitis*, *Dirofilaria repens* and *Brugia malayi* in shelter dogs in Pekan Nanas, Johore, Malaysia. A single-step multiplex polymerase chain reaction (PCR) was performed to amplify the internal transcribed spacer-2 (ITS-2) region of extracted DNA for the detection and differentiation of three species of filarial parasites. Blood sample from 40 dogs were collected from the shelter. Twenty dogs were given monthly oral Ivermectin and doxycycline and another 20 dogs did not receive any heartworm prevention treatment. The prevalence of dirofilariasis in the dogs was 17.5% (7/40). All positive samples in 4 female and 3 male dogs were suggestive of *D. immitis*. All the affected dogs were those that did receive heartworm prevention treatment. Thus, implementation of heartworm prevention is important in dog shelter management.

Keywords: dirofilariasis, zoonotic, polymerase chain reaction, prevalence

DETERMINATION OF POST-MORTEM INTERVAL VIA IMMUNOHISTOCHEMICAL LOCALISATION AND EXPRESSION OF CADAVERINE

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ABSTRACT

There is a dearth of knowledge in spite of extensive research on the reliable post-mortem interval (PMI) indicator. Biogenic amine has never been used as an indicator of PMI and its potential is evaluated in this study through the immunologically expressed cadaverine. Brain, liver, muscle, and testis tissue samples obtained from 3 dogs at 0, 12, 18, and 24 h post-euthanasia was immediately fixed in 10% formalin and routinely processed for histology analysis and cadaverine immunohistochemistry (IHC). The H&E-stained samples were also subjected to morphometric analysis. Cadaverine expressions at 18 and 24 h were higher ($p<0.01$) than that at 0 and 12 h post-mortem. Hepatic cadaverine expression was higher than in other tissues, except the brain ($p<0.01$). The cadaverine expression was time-dependent in all organs tested. The morphometry of hepatocytes, neurons, and seminiferous tubules differed significantly ($p<0.01$). In conclusion, the study showed that cadaverine expression can be used a PMI indicator.

Keywords: cadaverine, immunohistochemistry, post-mortem interval

SEROPREVALENCE OF LEPTOSPIROSIS AND BRUCELLOSIS IN LONG-TAILED MACAQUES (*MACACA FASCICULARIS*) OF PENINSULAR MALAYSIA

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ABSTRACT

Leptospirosis and brucellosis are important zoonotic diseases worldwide with high incidence in tropical countries affecting biodiversity, human and livestock health, animal welfare and the economy. The expanding human population along with rapid urbanisation have increased the likelihood of wildlife and human interaction. In Malaysia, the increased human-macaque conflicts have resulted in the concern about zoonotic disease transmission. This study was conducted to determine the seroprevalence of leptospirosis and brucellosis in wild long-tailed macaques of Peninsular Malaysia. One hundred serum samples were screened for antibodies against *Leptospira* and *Brucella* using microscopic agglutination test (MAT) and Rose Bengal Plate test (RBPT), respectively. Fourteen percent of macaques were seropositive for leptospirosis with serovar Cellodoni (4%), and Pyrogenes (4%) as the most common serovar identified, followed by Icterohaemorrhagiae (3%), Bataviae (2%) and Lai (1%). The prevalence in males were significantly ($p < 0.05$) higher than females. Males were 4.5 times more likely to be seropositive for leptospirosis than females. This suggests that gender differences in behaviour influences exposure of macaques to leptospirosis. There was no significant ($p < 0.05$) difference in seroprevalence among age, habitat, and region. All samples were seronegative for brucellosis. This study concludes that leptospirosis is prevalent in long-tailed macaques and poses a public health risk of cross-species transmission.

Keywords: leptospirosis, brucellosis, microscopic agglutination test, Rose Bengal Plate test (RBPT), zoonosis, long-tailed macaques (*Macaca fascicularis*), non-human primate, Peninsular Malaysia

DOG OWNERS' PERCEPTION TOWARDS CANINE HEART DISEASE: A BEHAVIOURAL STUDY

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ABSTRACT

Factors that may affect owners' decision on treatment of heart disease are their level of awareness and knowledge of canine heart disease. This study intends to determine the level of awareness and knowledge among dog owners in Malaysia and to examine the relationship between attitude, subjective norms, perceived behavioural control and empathic concern towards intention to treat if their dog was diagnosed with heart diseases. A behavioural study was conducted using an interviewer-assisted questionnaire and data was collected from 131 dog owners who visited to University Veterinary Hospital, Universiti Putra Malaysia. Information on demographic, dog ownership, level of awareness of canine heart disease and intention to treat was collected. The majority of the dog owners (79.4%) were aware that dogs can have heart disease but 77.1% rated that they do not understand the disease and only 5.3% have good level of knowledge and understanding of canine heart disease. The main barrier for treatment was cost-related (31.1%). In this behavioural study, the intention of treatment was found to positively associated with attitude and perceived behavioural control. In addition, empathic concern of owners towards their dogs played a significant role in influencing their attitude and intention to treat their heart diseased dogs. Owners with lower empathic concerns were found to have a stronger association between the relationship of their attitude and intention to treat their heart diseased dog. Hence, the study concludes that owner awareness and knowledge of canine heart disease can be improved by veterinarians educating and advising them to seek reliable information from social media so that their attitude will change for the better with respect to treating heart diseases in their dogs.

Key words: canine heart disease, attitude, subjective norm, perceived behavioural control, empathic, intention

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