

7th Proceedings
of the Seminar
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Faculty of Veterinary Medicine UPM
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EDITORS

RASEDEE ABDULLAH
MOHAMED ARIFF OMAR
ABDUL RAHIM MUTALIB
ABDUL RANI BAHAMAN



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PREFACE

The process of research starts by turning an idea into a research question and does not end until a report of the study has been completed. The 7th Proceedings have endeavoured to accomplish this task as a repository of the reports of final year students' projects conducted in 2012. In a single publication of the 7th Proceedings one has the opportunity to discern the vast array of research topics covered by these projects. Among the 65 projects listed in the Proceedings there were 10 projects on disease investigation, 8 projects on aquaculture and 7 projects each on cattle and buffalo and wildlife species. The quality of research conducted by a majority of these students is at par with the best students' projects in many veterinary schools in developed countries. This understatement is attested by one of the external examiners of the Doctor of Veterinary Medicine programme of Universiti Putra Malaysia. As usual, many of the research conducted by the final year students were based on the current research of their supervisors and thus the information gathered are often publishable in journals. Although we understand the sentiments of the supervisors, it is rather unfortunate because the proceedings to a certain extent fail to fully serve as a complete reference to staff and students alike on the research conducted at the Faculty. However, on the upside, the proceedings is still a memento and record of their successes in conducting organised research during their tenure as veterinary students at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. For that we are thankful.

The editors would like to express their gratitude to students and staff for their full cooperation and contributions toward the publication of the 7th proceedings.

The Editors

Rasedee Abdullah
Mohamed Ariff Omar
Abdul Rahim Mutalib
Abdul Rani Bahaman
Saleha Abdul Aziz
Mohamed Ali Rajion

RISK FACTORS AND CLINICOPATHOLOGICAL FINDING OF EFFUSIVE-FORM FELINE INFECTIOUS PERITONITIS AT UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

**Chan Jia Xin,¹Rasedee Abdullah,²Malaika Watanabe
& ³Mohamed Ariff Omar**

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

³*Department of Veterinary Preclinical Sciences*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

Abstract

A retrospective study on the prevalence of effusive feline infectious peritonitis (FIP) cases at the University Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM) from 2009 to 2011 was conducted. Data regarding patient signalment, history of risk factors, clinical and laboratory profiles were recorded. Eighty-three cases of suspected effusive FIP were diagnosed in UVH-UPM during that period. The study showed that 87.7% cases of effusive FIP occurred in cat less than 3 years old, 60.2% in intact male, 86.4% in Domestic Short Hair, 79.2% in cats from multi-cat household, 40.3% were in cats kept indoor and 46.8% were in those kept outdoor. Multi-cat household was a risk factor for effusive FIP. The most commonly reported clinical findings were abdominal distension, dyspnea, weight loss, jaundice, ascites and pleural effusion. The clinicopathological findings associated with effusive FIP were hyperglobulinaemia, decreased A:G ratio (<0.4), hypoalbuminaemia, hyperproteinaemia, hyperbilirubinaemia and non-regenerative anaemia. The abdominal or pleural fluid was typically exudate and modified transudate.

Keywords: effusive FIP, risk factors, clinicopathological findings, UVH-UPM

INTRODUCTION

Feline infectious peritonitis (FIP) is a lethal immune-mediated disease triggered by feline coronavirus (FCoV) infection. The first FIP case reported in Malaysia was in 1981 (Wong *et al.*, 1983). The virus is present ubiquitously in multi-cat environment. The disease is usually transmitted via faecal-oral route because of use of communal litter tray. Feline infectious peritonitis may be effusive and non-

effusive. Generally, cats with effusive FIP have high protein peritoneal and/or pleural effusion, fever, weight loss, anaemia and elevated serum globulin levels. The signs of non-effusive FIP are often unclear and nonspecific to include fever, weight loss, malaise and inappetance, which are usually associated with the affected tissues. Presumptive diagnosis of FIP is frequently made based on its affinity to younger cats, strong inclination to involve multi-cat household, typical historical and physical findings, and some characteristic laboratory abnormalities. Definitive diagnosis can be made based on the detection of FIP virus in macrophage using immunohistochemistry and with histopathology findings. However, definitive diagnosis of FIP in very ill young cats with laboratory findings suggestive of FIP is difficult because of absence of non-invasive diagnostic method. In this study the objectives were to determine the potential risk factors associated with effusive FIP and clinical signs, disease manifestation and clinicopathological findings that may aid in the diagnosis of effusive FIP.

MATERIAL AND METHODS

Patient medical records from Universiti Veterinary Hospital (UVH) and Hematology and Clinical Biochemistry Laboratory, Universiti Putra Malaysia were reviewed to identify cats diagnosed with effusive FIP during the period 2009 to 2011. Cases of FIP were classified as 'confirmed' if histopathology confirmed the diagnosis and 'suspected' if the attending clinician concluded that FIP was the most likely diagnosis based on history of risk factors, patient signalment, clinical abnormalities, and laboratory findings but without histopathology. Data concerning the patient signalment, physical and clinical findings as well as laboratory profiles, results of diagnostic tests performed were recorded. Descriptive statistical analysis was done to obtain the sample profile of the cats studied and non-parametric chi square was conducted to compare frequencies of specific group of risk factors of FIP. All statistical analyses were performed using SPSS 19.0 statistical software.

RESULTS AND DISCUSSION

Signalment

Of the cats sampled 87.7% diagnosed as suspected effusive FIP were less than 3 years old and 12.3% more than 3 years old. Among these cats, 60.2% were intact male, 25.3% intact female, 8.4% castrated male and 6% spayed female. These cats were composed of 86.4% Domestic Short Hair and 13.6% pedigree cats. However, there was no risk association between sex, age and breed with seroprevalence of FCoV infection. Although age cannot be proven as a risk factor in this study, a survey done elsewhere found 50% of cats with FIP were below 2 years old (Pederson, 2008; Addie and Jarret, 1998). On the other hand, Domestic Short Hair cats appeared to be over-represented because of limited variation of cat breeds presented to UVH. Intact male had the highest occurrence of FIP which could be attributed to their roaming behavior.

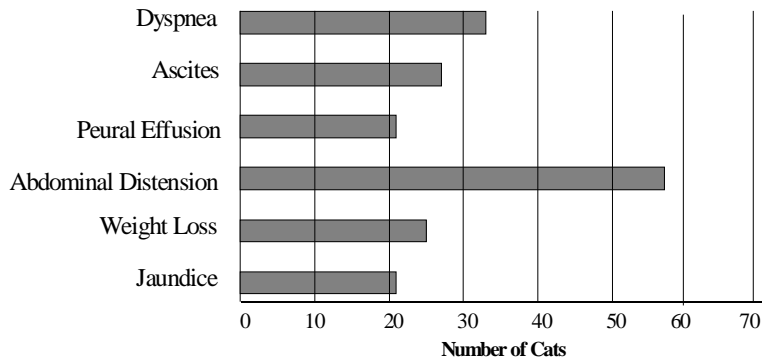


Figure 1. Frequency of clinical findings in cats diagnosed with suspected effusive FIP

Table 1. Haematological findings of cats diagnosed with suspected effusive FIP

Haematology	Unit	Mean	Observation range	Reference range	Frequency and percentage
Anaemia					21/51 (41.0%)
RBC	$\times 10^{12}/L$	4.10	2.58-5.12	5-10	-
Hb	g/L	64.93	42.00-78.00	80-150	-
PCV	L/L	0.21	0.12-0.25	0.24-0.45	-
Leukocytosis	$\times 10^9/L$	27.91	20.40-55.3	5.5-19.5	29/36 (80.5%)
Leukopenia	$\times 10^9/L$	-	-	5.5-19.5	0/36 (0%)
Neutrophilia	$\times 10^9/L$	21.73	13.58-50.10	2.5-12.5	29/36 (80.5%)
Lymphocytosis	$\times 10^9/L$	21.80	21.80	1.5-7.0	1/36 (2.7%)
Lymphopenia	$\times 10^9/L$	0.84	0.27-1.53	1.5-7.0	10/36 (27.7%)

Table 2. Biochemical findings of cats diagnosed with suspected effusive FIP

Biochemistry	Unit	Mean	Observation range	Reference range	Frequency and percentage
Elevated ALT	U/L	133.21	103.9-182.6	10-90	7/49 (14.3%)
Elevated AP	U/L	226.00	226.0	<80	1/19 (5.3%)
Elevated bilirubin	$\mu\text{mol}/L$	83.38	50.4-109.7	1.7-17	5/9 (55.5%)
Elevated urea	mmol/L	13.22	10.4-18.3	3-10	14/41 (34.1%)
Elevated creatinine	mmol/L	-	-	60-193	0/41 (0%)
Hyperproteinemia	g/L	87.34	61.4-129.6	55-75	38/53 (71.7%)
Hyperglobulinemia	g/L	60.11	32.2-104.4	25-45	45/53 (84.9%)
Hypoalbuminemia	g/L	21.98	16.9-25.4	25-40	38/53 (71.7%)
Decreased A:G	Unit	0.38	0.2-0.5	0.5-1.4	46/55 (83.6%)

Historical Findings

The FIP cases comprised 79.2% multi-cat and 20.8% single-cat of household cats. There was significant relationship between numbers of cats in the multi-cat household and the seroprevalence of FCoV infection. Cats in multi-cat household were seven times ($p=0.005$) more likely to be seropositive for FCoV infection than cats in single cat household, which may be the result of sharing of litter-sharing, feeding or drinking bowls. 40.3% of cats with suspected effusive FIP were kept indoors, while 46.8% were outdoor and 12.9% were semi-roamer cats.

Clinical Findings

The most common clinical findings were abdominal distension (69.9%), dyspnea (39.8%), weight loss (30.1%), jaundice (25.3%), ascites (32.5%) and pleural effusion (25.3%) (Figure 1).

Clinicopathological Findings

Of the 83 cats with suspected effusive FIP, 36 had complete haematological data; 15 had only PCV values. The results showed 41% of cats had anaemia, 80.5% had neutrophilic leukocytosis and 27.7% had lymphopenia. In total, 6% of cats with suspected effusive FIP had non-regenerative anaemia, neutrophilic leukocytosis and lymphopenia (Table 1). On the other hand, serum biochemistry analyses were done on 55 cats to analyse the following parameters; CK, Urea, ALT, AP, bilirubin, total protein, globulin, albumin and A:G ratio. From these analyses 71.69% showed hyperproteinaemia, 84.9% showed hyperglobulinaemia, 71.7% showed hypoalbuminemia and 83.6% showed decreased A:G ratio. In addition, 14.28% had elevated ALT; 5.3% had elevated AP; 55.5% had elevated bilirubin; 34.1% had elevated urea. In total, 51% of cats had hyperproteinemia, hypoalbuminemia, hyperglobulinemia and decreased A:G ratio. The abdominal or pleural fluid was typically modified transudate or exudate (Table 2).

CONCLUSION

In conclusion, this study shows that multi-cat household is a risk factor for effusive FIP. Commonly observed clinical findings associated with effusive FIP were abdominal distension, dyspnea, jaundice, ascites and pleural effusion. The clinicopathological findings associated with effusive FIP in order of frequency were hyperglobulinemia, decreased A:G ratio (<0.4), hypoalbuminaemia, hyperproteinaemia, hyperbilirubinaemia and non-regenerative anaemia. Last but not least, abdominal or pleural fluid was typically exudate or modified transudate.

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DETECTION OF NEWCASTLE DISEASE VIRUS IN EDIBLE-NEST SWIFTLET (*AERODRAMUS FUCIPHAGUS*) RANCHED UNDER AN OIL PALM PLANTATION IN SUNGKAI, PERAK, MALAYSIA

Chow Guo Hao,^{1,3} Jalila Abu,² Siti Suri Arshad,⁴ Tan Sheau Wei,² Siti Khatijah Muhamad, & ¹Ong Kang Woei

¹*Department of Veterinary Clinical Studies*

²*Department of Pathology & Microbiology*

³*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

⁴*Institute of Bioscience*

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Newcastle Disease is regarded as one of the most important disease of avian species and was listed as ‘A’ disease by the Office International des Epizooties (OIE). Studies have shown this disease infects more than 250 species of birds. The edible-nest swiftlet industry in Malaysia is on the rise for the last 10 years and swiftlet houses can be seen everywhere. This is due to the high price of edible bird nests at international market and high income generated by the farming of edible bird nest. Nevertheless, edible-nest swiftlet (*Aerodramus fuciphagus*) is can either be infected with or carrying the deadly Newcastle disease virus (NDV) which is a great threat to the poultry industry in this country. In Peninsular Malaysia, no study on this subject was reported so far in this swiftlet species. In this study, 60 swiftlet carcasses were sampled. Post-mortem was conducted and lung and trachea tissues were collected. Tissue samples were tested for NDV using the reverse-transcriptase polymerase chain reaction (RT-PCR). Newcastle Disease Virus was not detected in any of the samples. Thus, it can be concluded that edible nest swiftlets (*Aerodramus fuciphagus*) ranched under oil palm plantation in this study are not infected with or carry NDV.

Keywords: swiftlet, Newcastle disease, Newcastle disease virus, PCR

INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease of avian species (Alexander, 2006). Newcastle disease was first reported in Malaysia in 1934 and today the clinical diseases are still present in Malaysia as well as in several countries in the region (Aini, 2006; OIE Wahis, 2009).

Edible-nest swiftlet (*Aerodramus fuciphagus*) is classified under the kingdom *Animalia*, phylum *Chordata* and class *Aves*. Swiftlet ranching in Malaysia began in the 1980s. However, this industry only started to mushroom after 1997 (Lim and Cranbrook,

2002). In December 2001 it was estimated that there were approximately 1,000 swiftlet ranches in Malaysia and the number has grown to about 10,000 by 2005 (Burhanuddin, 2005). The high price of edible-bird nest is the main reason for the tremendous increase in the number of swiftlet ranches (Lim and Cranbrook, 2002). With the high price of edible-bird nest, it fetches a lucrative income and hence there is an increase in the number of people involved in swiftlet ranching. This study was conducted to determine the prevalence of ND in edible –nest Swiftlet ranches under an oil palm plantation in Sungkai, Perak, Malaysia.

MATERIALS AND METHODS

Degenerative primers 5'-ATGGGC (C/T)CCAGA(C/T)CTTCTAC-3'(forward) and 5'-CTGCCACTGCTAGTTGTGATA ATCC-3'(reverse) previously described by Berhanu *et al.* (2010) used to amplify the F-gene fragment were used in this study. The primer pair is universal primer which is able to detect all strains of Newcastle disease virus (NDV) and is expected to generate an amplicon size of 535 bp (nt 47 – 535 of nucleotides of F protein).

Sixty dead swiftlet carcasses were collected at one swiftlet ranch in Sungkai, Perak, Malaysia. Post-mortem was conducted and trachea and lungs were sampled. Organ samples collected from each bird were pooled and treated as one sample. Samples from each bird were minced in 1000 µL 1X phosphate buffered saline (PBS) (Merck, Germany). The pooled sample was then transferred into a 1.5 mL microcentrifuge tube. 1X PBS was added to each tube until the total volume of each tube reached 1.5 mL. The sample was then freeze-thawed three times followed by centrifugation at 3000 rpm for 15 min at 4°C. 300 µL of the supernatant was transferred into a new 1.5 mL tube and RNA extraction was conducted using TRIzol® reagent solution (Invitrogen, USA) and a series of steps as manufacturer recommendation.

Standard reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using Access® One-step RT-PCR kit (Promega, USA) with 2 µL of extracted RNA from sample resulting in a total reaction volume of 25 µL. NDV strain AF2240 was used as positive control while sterilised deionised water was used as negative control. RT-PCR was carried out in MyCycler™ (BIO-RAD, USA). The cycling parameters were set at 48°C for 45 min (reverse transcription), and 35 cycles of 94°C for 2 min (denaturation), 56°C for 2 min (annealing) and 72°C for 1 min (extension), followed by 72°C for 10 min (final extension).

Two microliters of loading dye (Vivantis, USA) was added to RT-PCR product of each sample. 10 µL of sample mixed with loading dye was loaded into agarose gel (Promega, USA). 100bp DNA marker (Vivantis, USA) was used as the indicator. Electrophoresis was performed by running the gel in 1X TAE buffer (Merck, Germany) under 80V for 40 min. The gel was then stained with 1% ethidium bromide and viewed under UV light (ProteinSimple, USA). The resulting band for each sample was observed. Sample(s) with band observed between 500 to 600 bp was considered as positive while sample with absence of such band was considered negative.

RESULT

None of 60 samples tested showed any band in 500 to 600 bp region, which was expected in NDV-positive cases. It is suggested that edible-nest swiftlet (*Aerodramus fuciphagus*) is not naturally infected with or act as a carrier or reservoir for NDV.

DISCUSSION

There are various reasons which could the absence of NDV in the samples. This includes low prevalence of NDV infection in wild birds population, the flying height and habit or this bird itself may be naturally resistant to NDV infection.

According to a study done by Tan (2005), the prevalence of NDV infection in wild birds' population is in fact low. We showed that only 20.2% of the samples were seropositive for NDV antibody. This study showed that the prevalence of NDV infection in edible next swiftlet population is low and the risk of these birds contracting this disease is very low.

Edible-nest swiftlet fly at a different level from that of other species of birds, which is at a specific height between 30 to 60 meters above the forest canopy. Edible-nest swiftlet also has the habit of not mixing and interacting with other species of birds even with their close relative, the black-nest swiftlet, *Aerodramus maximus*. Hence, there is minimal contact between edible-nest swiftlet with other wild avian species (Lim and Cranbrook, 2002; Aini, 2005).

Edible-nest swiftlet may be naturally resistant to Newcastle Disease Virus infection. According to Howe *et al.* (1961) and Biddle and Belyavin (1963), the edible-bird nest extract has inhibitory properties on the viral haemagglutinin and neuraminidase activity of Myxovirus and influenza virus respectively. Newcastle Disease Virus possesses haemagglutinin-neuraminidase surface glycoprotein which is important in the process of attachment to the host cells (Nagai, 1993). The substances in the nest produced by edible-nest swiftlet also have inhibitory properties on viral haemagglutination and neuraminidase activities and this might indirectly protect the bird from infection from viruses such as NDV.

CONCLUSION

From this preliminary study, it is suggestive that edible-nest swiftlet, *Aerodramus fuciphagus* is not naturally infected with or act as a carrier or reservoir for NDV. This is good news to the swiftlet industry and the conservation of the species because edible-nest swiftlet do not suffer from ND. Subsequently the expanding swiftlet industry would also not be a threat to the poultry industry in term of disease transmission.

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A COMPARATIVE STUDY OF CONDITIONING PROGRAMS IN RELATION TO PERFORMANCE OF MALAYSIAN ENDURANCE HORSES

**Hazira Filza Mohd Zulkaffli,¹Noraniza Mohd Adzahan
& ²Mohamed Ariff Omar**

¹Department of Veterinary Clinical Studies

²Department of Veterinary Preclinical Sciences

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Conditioning provides physiological adaptation, resulting increase in fitness of athletic horses, which eventually increase their performance. The primary aim of a conditioning program is to produce the best performing athletic horse with minimal injuries. A survey was conducted using a questionnaire to identify various conditioning programs practiced by the local endurance horse establishments with regards to techniques and duration of the program. Fifteen athletic endurance horses with three horses each representing five equine establishments were used in this study. The duration of every conditioning program were recorded. Performance parameters that were monitored were the average recovery time, the average speed, the completion rate and the level of performance. Kruskal-Wallis test was used to compare conditioning programs practiced by different establishments in relation to their performances. The mean of the average speed of horses from different establishments ranged from 9.30 to 15.20 km hr⁻¹. Stable A that practices all basic endurance conditioning techniques with a duration period of conditioning of >3 months showed the highest speed (15.20 ± 0.05 km hr⁻¹) and the shortest mean of the average recovery period (12.28± 1.18 min) with a 100 % completion rate in 120 km endurance race category. Therefore, the conditioning program practiced by Stable A is suggested to be an optimal conditioning program suited for horses under Malaysian conditions in producing good and competitive endurance horses locally.

Keywords: endurance horse, conditioning program, performance level

INTRODUCTION

In an endurance race, the horse needs to maintain its speed over a long ride without encountering any health problem that commonly occurs due to metabolic ailments and lameness (Robert *et. al*, 2011). As such, conditioning program needs to be planned to ensure horses are fit and well-prepared to compete in an endurance race. The ultimate aim of a conditioning program is to produce the best performing endurance horses with minimum risk of injuries. Formulating conditioning program is very subjective and

produces different performance levels of endurance horse. Effect of conditioning depends on different techniques – long-slow distance (LSD), flatwork, strength training and speed play; and the exercise volume – intensity, duration and frequency. Establishment that practices the most optimal conditioning program produces the best performing endurance horse. Therefore, it is important to identify such establishment in order to obtain important information for the formulating conditioning regime for endurance horses particularly under Malaysian condition. The objective of this study was to identify and compare the conditioning programs practiced by different establishments in relation to their endurance horses' performance under Malaysian conditions.

MATERIALS AND METHODS

Various conditioning methods practiced by local endurance practitioners were observed and recorded. After gaining the information, the performance of endurance horses from that particular establishment were monitored and assessed in actual endurance races. At the end of the study, a conditioning program that produced the most performing endurance horses at races was identified and was justified as to why it was considered as an optimal conditioning program formulated and suited for endurance. Equine establishment with poor conditioning program and did not produce good performing endurance horses were also identified and advice was given accordingly to improve and benefit the local horse endurance society.

Animals

Fifteen horses from different establishments practicing diverse types of conditioning programs were used in this study. Competing horses were first being inspected by the event veterinarian to ensure that these horses were fit to participate in an endurance race. Three horses from five different establishments were included in this study in a 1* endurance ride event under FEI rules.

Conditioning Programs

Information and details of conditioning program were obtained for all horses from different establishment participated in the 1* Endurance Ride in January 2012 at the Terengganu International Endurance Park (TIEP), Lembah Bidong, Setiu, Terengganu. Conditioning programs practiced by different establishments consisted of LSD, flat work, strength training and speed play. Various ranges of frequency, duration and intensity of the conditioning programs were noted and recorded.

Average Recovery Time and Average Speed

Average recovery time and average speed of each horses participated in the race were recorded as to evaluate the performance during actual event. Average recovery time is the average time taken of an individual horse to have a heart rate of 64 beats min⁻¹ within 20 min of maximum recovery period while the average riding speed is the average riding velocities of each individual horse to finish the race.

Completion Rate

Completion rate was based on the numbers of horses from a particular establishment that have completed the race successfully. The highest completion rate was reflected by a

high percentage of horses from similar establishment successfully completed the race in good condition within required time. Data was presented as mean \pm standard deviation. The effect of conditioning techniques and duration of the conditioning program on performance – the completion rate, placing, the average speed and the recovery time were evaluated using Kruskal-Wallis. Significance was inferred at $p < 0.05$.

RESULTS AND DISCUSSION

Only 40% of the local endurance horse establishments practiced proper conditioning programs, while the rest practiced incomplete conditioning programs. This shows that only a few local horse owners are aware of the importance of having an appropriate conditioning program to prepare horses for and to prevent the occurrence of health problem during competitions. An optimal conditioning program is the most important factor in stimulating the physiological adaptations within the animal's body to improve performance of equine athletes (Rogers *et al.*, 2007). Forty percent of establishments, represented by Stables A and C had proper conditioning program using all basic conditioning techniques for duration of 3 months or more. Other establishments practiced either one, two or three basic technique(s). Duration of conditioning program practiced by the local endurance establishments ranged from less than a month to more than 3 months. Optimal conditioning program duration is very subjective although adaptation changes as explained by Hiney *et al.*, (2004) occurred within as least 8 weeks. Figure 1 shows the comparison among establishments based on performance in races. Stable A had the best average speed ($15.20 \pm 0.05 \text{ km hr}^{-1}$) and recovery time ($12.28 \pm 1.18 \text{ min}$) among establishments. The average recovery time among establishments were insignificantly different ($p > 0.05$). The performance of establishment based on the completion rate and level of category are illustrated in Table 1. From 15 horses participating in the study, 13 (87%) completed the race while 2 (13%) were unable to complete the race due to metabolic problem and lameness.

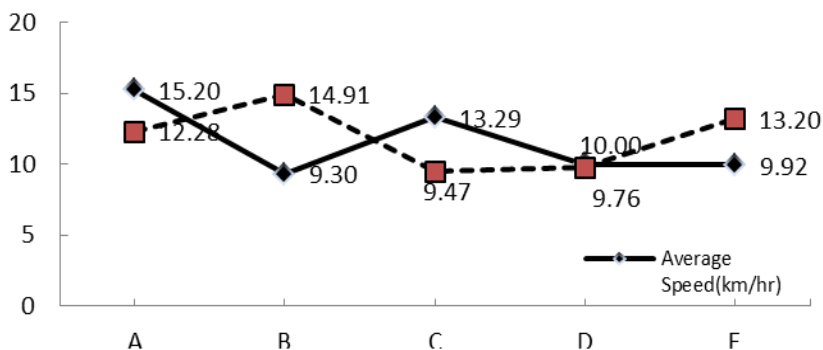


Figure 1. The average speed and the average recovery time for different equine establishments practicing different conditioning program

Table 1. Completion rate and racing category in endurance races for horses from different establishments practicing different conditioning program

Equine Establishment	Completion Rate (%)	Racing Category (km)
A	100	120
B	67	40
C	100	40
D	100	80
E	67	40

CONCLUSION

Conditioning program practiced by Stable A is suggested to be optimal based on a few factors – it consisted of all basic conditioning techniques, horses were participating in higher racing category and 100% completion rate in endurance race.

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NUTRITIONAL EVALUATION OF HOUSE CRICKET (*BRACHYTRUPES PORTENTOSUS*) MEAL FOR POULTRY

**Ismasyahir Abdul Razak, ¹Yusof Hamali Ahmad
& ¹Engku Azahan Engku Ahmed**

*¹Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

This study was conducted to evaluate the nutritional value of house cricket meal (HCM) as an alternative feedstuff for poultry. The proximate chemical composition, amino acid (AA) content and total metabolisable energy (TME) of HCM were determined. The protein quality of HCM was evaluated using protein efficiency ratio (PER) and net protein ration (NPR) methods. Treatment diets consisted of basal diet (N-free), basal + HCM, basal + soy bean meal (SBM) and basal + fish meal (FM) and were arranged in a completely randomized design. The crude protein (CP) content of HCM was 60.4% and the value was higher than that of either SBM or FM. Total metabolisable energy value for HCM was similar to that of corn but was much lower than that of SBM. Total amount of tryptophan, tyrosine and valine in the HCM were 2.8, 2.4 and 3.2%, respectively. These values were higher than those in SBM and FM. The percentages of lysine, methionine and cysteine in HCM were 2.4, 0.5 and 0.8%, respectively. These values were similar to those in SBM (2.9, 0.6 and 0.7%) but lower than FM (4.5, 1.7 and 0.8%). Chicks fed HCM diet recorded higher ($p < 0.05$) weight gain than chicks fed SBM but slightly lower than chicks fed FM. The PER values for HCM, SBM and FM were 3.42, 3.11 and 3.71, respectively. NPR values for HCM, SBM and FM were 3.66, 3.29 and 3.96, respectively. The PER and NPR values of HCM were higher ($p < 0.05$) than that of SBM but slightly lower than that of FM. The results suggest that the HCM has a substantial amount of protein and energy which could to be included in poultry diets.

Keywords: house cricket meal, crude protein, true metabolisable energy, protein quality, poultry

INTRODUCTION

The poultry production in Malaysia is too dependent on imported feedstuffs especially protein feed ingredients. Presently, local production of soybean and other beans to be used as protein feed ingredients are not economically feasible. The current supply of local fish meal is small and future production is expected to decrease due to limited availability of trash fish. Therefore, alternative protein sources for livestock in the country should be explored and studied so that they could replace some of the imported protein feed ingredients. Among potential protein sources that could replace soybean meal and fish meal is insect protein. Insects can be used to produce cheaper proteins

from non-food animals. Insects are rich in protein, with reported protein contents ranging from 44-70 percent (Ramos–Elorduy, 1987). One of the local insects that have potential to be used as protein source for poultry diets is house cricket. House crickets are being sold in most pet shops and tropical fish shops as fishing bait and food for birds, reptiles and aquarium fish. The house cricket is easily adapted to domestic rearing and has not been seriously studied as a potential source of nutrients for non-ruminants in the country. Comprehensive studies on the potential of cricket meal as protein source for poultry or other livestock species have not been conducted. The objective of the study was to determine the chemical composition, true metabolisable energy and protein quality of house cricket meal.

MATERIALS AND METHODS

Eight weeks old house crickets were bought from an insect breeding farm in Kuala Selangor, Malaysia. The crickets were sacrificed by placing them overnight in a freezer at -20°C for 24 hours. All crickets were washed with tap water and then dried in the oven at 60°C for 48 hours. The crickets were freeze-dried in liquid nitrogen then grounded to 60 mesh size.

The crude protein (CP), ether extracts (EE), crude fiber (CF), ash, gross energy (GE), calcium (Ca) and phosphorous (P) for HCM and other feed ingredients were analyzed according to the procedures of AOAC (1990). Amino acids (AA) were determined with Biochrom 30 Amino Acid Analyzer from United Kingdom after hydrolysing with 6 N hydrochloric acid for 22 hour at 110°C.

TME was conducted by adapting the method of Parsons et al. (1982). Seven caecectomized roosters were used in this experiment and placed in individual cages. All roosters were fasted for 24 hours and during the fasting period, all the endogenous excreta produced were collected. After 24 h fasting, all seven roosters were force-fed using crop-intubation and each cockerel received 15 g of HCM. During 48 hours after forced feeding, excreta from all roosters were collected. Feathers and debris were removed from all excreta collected and then dried in the oven for 48 h at 60°C. GE for endogenous excreta and excreta were determined by using a bomb calorimeter. Calculation of TME was done using the following formula: $ME = GE \text{ intake} - (GE \text{ excreta} + GE \text{ endogenous droppings})$.

One hundred and twenty one-day-old crossbred broilers were used in this study. Feed and water were given *ad libitum*. The chicks were fed a 24% CP corn-SBM pretest diet during the first 7 days post-hatching. Following an overnight period without feed, the chicks were weighed and allotted to dietary treatments as described by Sasse and Baker (1973). Treatment diets consisted of basal diet (N-free), basal + HCM, basal + SBM and basal + FM. All treatment diets contained 10% CP except the basal diet. The chemical compositions of the experimental diets are shown in Table 1. Three groups of ten chicks per cage were assigned to each treatment. The protein quality was determined using PER and NPR. The formulae used for net protein ratio (PER) and net protein ratio (NPR) calculations are shown below:

$$\text{Protein Efficiency Ratio} = \frac{\text{Body weight gain (g)}}{\text{CP intake (g)}}$$

$$\text{Net Protein Ratio} = \frac{\text{BW gain (g)} - \text{BW gain (g) of chicks fed N-free basal diet}}{\text{CP intake}}$$

All data from the experiments were analysed using ANOVA procedure of Statistical Analysis System (SAS) (SAS Institute, 1990) for completely randomised designs. Statistical significances of differences among treatments were assessed using the Duncan's multiple-range test.

RESULTS AND DISCUSSION

The CP percentage of HCM was 60.4% (Table 2) and was higher than that of SBM but slightly lower than FM (McDonald et al., 1995). The HC contained higher percentages of protein as compared to other insects, such as in Mormon cricket (*Anabrus simplex*) (DeFoliart et al., 1982), Field cricket (*Gryllus testaceus*) (Wang et al., 2005) and Lepidoptera larvae (Landry et al., 1986), but was somewhat lower than the protein contents of silkworm pupae (68%) as reported by Wei and Liu (2001).

TME value for HCM was 13.03 MJ/kg (Table 2) and this value was similar to that of corn (13.40 MJ/kg) but much higher than that of SBM (9.60 MJ/kg). The high value of TME in HCM in this study was probably attributed to high level of fat content (22.7%). TME value for HC was also higher than that of Field cricket, 12.39 MJ/kg (Wang et al., 2005). The high TME value in HCM is comparable to conventional energy supplements of most grains.

The amino acid composition of HCM is shown in Table 3. Percentages for tryptophan, tyrosine and valine in HC were 2.8, 2.4 and 3.2%, respectively. These values were higher than those of SBM (0.6, 1.4 and 2.4%) and FM (0.7, 1.8 and 3.0%). The values for histidine (0.9%), leucine(2.9%), methionone (0.5%), threonine (1.5%), tryptophan (2.8%) and cysteine (0.8%) for HCM were almost similar to the corresponding values of SBM and FM. The percentages of lysine, methionine and cysteine in house cricket were 2.4, 0.5 and 0.8%, respectively. These values were quite similar to those in SBM (2.9, 0.6and 0.7%) and in FM (4.5, 1.7 and 0.8%) except for lysine, indicating that the essential amino acids in HCM were adequate for poultry. However, these values were lower than the values in Field cricket (4.79, 1.93 and 1.01%) as reported by Wang et al. (2005). Thus, HC has an advantage on amino acid composition compared with other insects reported because it contains high quantities of AA.

Chicks fed the basal diet (N-free) lost weight during the assay period, whereas chicks fed HCM, SBM and FM had a positive weight gain response (Table 4). The chick's growths were significantly affected by protein source in their diets. These results suggested that the protein of HCM could support normal growth in chicks. Chicks fed HCM diet recorded higher ($p < 0.05$) weight gain than those chicks fed SBM but slightly lower than those chicks fed FM diet and the differences were not significant. The PER and NPR values of HCM were significantly higher ($p < 0.05$) than that of SBM but slightly lower than FM. The lower PER and NPR values of HCM compared to FM were probably due to higher crude protein content and slightly lower amino acid digestibility.

Besides that, it was ascertained that the protein of HCM had no adverse effect as a feedstuff.

Table 1. Chemical composition of treatment diets

Components	Treatment diets			
	Basal	Basal + 16.56% HCM	Basal + 22.47% SBM	Basal + 16.95 % FM
ME (MJ/kg)	11.70	11.79	11.45	11.87
Crude protein	0	10	10	10
Calcium (%)	1.0	0.8	0.8	0.9
Phosphorus (%)	0.7	0.7	0.5	0.6

ME=metabolisable energy; HCM=house cricket meal; SBM=soy bean meal; FM=fish meal

Table 2. Chemical composition of HCM, SBM and FM (% as fed DM basis)

Proximate analysis	Content %							
	Dry Matter	Crude protein	Crude fibre	Fat	Ash	Ca	P	TME (MJ/kg)
HCM*	89.55	60.4	8.3	22.7	5.4	1.4	1.0	13.03
FM**	89.50	59.95	0.5	4.8	20.5	5.1	2.9	15.00
SBM**	89.45	44.0	5.8	1.0	6.5	0.3	0.7	9.06
Corn**	-	-	-	-	-	-	-	13.40

*Results from laboratory analysis, ** McDonald *et al.*, 1995

HCM=house cricket meal; FM=fish meal; SBM=soy bean meal; TME=total metabolisable energy

Table 3. Amino acid profile of HCM, SBM and FM

Amino acid	(% dry matter basis)		
	HCM1*	SBM**	FM**
Arginine (Arg)	2.2	3.2	4.0
Histidine (His)	0.9	1.1	1.3
Isoleucine (Ile)	1.6	2.5	2.7
Leucine (Leu)	2.9	3.4	4.4
Lysine (Lys)	2.4	2.9	4.5
Methionine (Met)	0.5	0.6	1.7
Phenylalanine (Phe)	1.6	2.2	2.3
Threonine (Thr)	1.5	1.7	2.6
Tryptophan (Try)	2.8	0.6	0.7
Valine (Val)	3.2	2.4	3.0
Cysteine (Cys)	0.8	0.7	0.8
Tyrosine (Tyr)	2.4	1.4	1.8

* Results from UPM laboratory analysis, ** McDonald *et al.*, 1995

HMC=house cricket meal; SBM=soy bean meal; FM=fish meal

Table 4. Mean values of weight gain, gain to feed ratio, protein efficiency ratio and net protein ratio

Dietary treatments	Weight gain (g)	Gain:feed (g:g)	PER	NPR
Basal diet	- 6.2	-	-	-
Basal + HCM	88.5 ^b	3.14 ^a	3.42 ^a	3.66 ^a
Basal + SBM	81.7 ^a	3.42 ^b	3.11 ^b	3.29 ^b
Basal + FM	92.1 ^b	2.70 ^a	3.71 ^a	3.96 ^a

^{a-c}Means within a column with no common superscript differ significantly
PER=protein efficiency ratio; NPR=net protein ratio

CONCLUSION

House cricket meal has substantial nutritional value for poultry. The HCM contained not only high amount of protein but had the advantage on the composition of amino acids for poultry, especially the percentage of tryptophan, tyrosine, valine and cysteine. House cricket meal also had a higher TME value which was equivalent to that of corn. The PER and NPR values were higher than SBM suggesting that it could replace SBM as a protein source in poultry diets. House cricket meal has the potential to be a new protein source for poultry in this country, or at least would be extremely beneficial as a complement to a domestic animal diet and could be fitted into meal patterns in a variety of ways.

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EFFECT OF A COMBINED NAPIER GRASS-OIL PALM FROND FEED ON THE *IN VIVO* AND *IN SACCO* RUMEN FERMENTATION AND DIGESTIBILITY IN GOATS

Leong Zi Ping, ¹Mohamed Ali Rajion, Wong Meng Joo, Mahdi Ebrahimi
& ^{1,2}Goh Yong Meng

¹Department of Veterinary Preclinical Sciences

²Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

In Malaysia, the lack of high-quality pasture remains the main factor slowing down the development of the ruminant livestock industry. The oil palm fronds (OPF), abundant and readily available agricultural by-products, appear as a promising solution, though they are unsuitable to be used as a sole feed. Therefore, this study evaluated a combined Napier grass (NP) and OPF (NP+OPF) feed by monitoring the digestibility, *in vivo* and *in sacco*, as well as the changes of rumen fermentation parameters (volatile fatty acids, rumen fluid pH and total protozoal counts). Fifteen two-year-old male rumen-fistulated Kacang crossed goats were used and divided into three groups, where Treatment 1 group was fed 50% NP + 50% concentrate, Treatment 2 group 25% NP + 25% OPF + 50% concentrate, and Treatment 3 group 50% OPF + 50% concentrate. Following dietary adaptation of 10 days, the *in vivo* and *in sacco* digestibility and rumen fermentation parameters were determined. Compared to the 50% NP diet, the combined NP-OPF feed showed a significantly lower *in sacco* digestibility and total volatile fatty acid production ($p < 0.05$). However, it produced good *in vivo* digestibility, rumen pH and total protozoal counts which were comparable to the 50% NP diet and significantly better than the 50% oil palm frond feed. Thus, it is concluded that the combined NP-OPF diet is suitable as a ruminant feed.

Keywords: oil palm fronds, rumen fermentation kinetics, *in vivo* digestibility, *in sacco* degradability, rumen protozoa

INTRODUCTION

Malaysia is one of the biggest producers and exporters of palm oil and palm oil products, which currently accounts for 39% of the world palm oil production and 44% of the world exports (MPOC, 2009). However, the ruminant livestock sector is growing at a slow rate due to the lack of a continuous supply of quality feed and land for the majority of the livestock producers. While the oil palm frond (OPF) has been identified as a potential feed for ruminants and other herbivores (Ebrahimi *et al.*, 2011), its low digestibility, high lignin content and low nutritive energy render it unsuitable to be used as a sole feed. Thus, this study evaluated the *in vivo* and *in sacco* digestibility, as well as assessing the

in vivo rumen fermentation characteristics resulting from the OPF inclusion, in order to determine its suitability to be incorporated into ruminant feed.

MATERIALS AND METHODS

Animals

Fifteen rumen-fistulated, two year old Kacang crossbred male goats were used. They were housed individually in metabolism crates and divided into three treatment groups. Treatment 1 animals received 50% Napier grass (NP) and 50% concentrate, Treatment 2 animals received 25% NP, 25% OPF and 50% concentrate while Treatment 3 animals received 50% OPF and 50% concentrate. All the animals were fed twice daily (at 09:00 and 18:00) at 2.5% of body weight on DM basis. The feeds were added with liquid molasses, with water provision *ad libitum* and free access to mineral licks. After dietary adaptation of 10 days, measurements of *in vivo* and *in sacco* digestibility and rumen fermentation parameters (pH, total protozoa and volatile fatty acids) were carried out.

Determination of In Vivo Digestibility

Ten percent of the refusals and feces were sampled daily for one week and oven-dried at 60°C. The digestibility coefficient (DC) of dry matter (DM) was calculated as:

$$\text{DC of DM (g/day)} = \frac{\text{DM intake} - \text{DM voided in faeces}}{\text{DM intake}} \times 100\%$$

Determination of In Sacco Digestibility

Two grams of NP, 1.0 g of OPF and 1.0 g of NP, and 2.0 g of OPF were weighed into nylon bags (Ankom Technology, Part # R510) and incubated in the rumen for 0, 4, 8, 24, 48, 72, and 96 hours. After removal, the bags were washed in a washing machine (Panasonic, NA-F70GS) together with the zero time bags not rumen-incubated, for 40 min and dried at 60°C. Disappearance of DM was measured as the loss in weight of the bag contents.

Determination of Rumen Fermentation Parameters

Ten milliliters of rumen contents were collected before the morning feeding designated as zero hour, and at 3, 6, and 9 hours post-feeding. Rumen pH, total protozoal counts and total volatile fatty acids were measured.

RESULTS AND DISCUSSION

All treatment groups did not exhibit significant differences in the pH of the rumen fluids (Table 1). Nevertheless, the feeding of NP-OPF combination yielded the highest mean rumen pH values compared to the other two groups. Furthermore, the combined NP-OPF feed produced rumen pH values ranging from 6.17 - 6.73 which indicated that effective fiber digestion had taken place (Orskov and Ryle, 1990).

Table 1. Rumen fluid pH from fermentation of different diets

Time (h)	Napier 50%	Napier 25% + OPF 25%	OPF 50%
0	6.58 ± 0.12	6.67 ± 0.15	6.91 ^x ± 0.13
3	6.24 ± 0.13	6.28 ± 0.08	6.07 ^y ± 0.15
6	6.26 ± 0.09	6.17 ± 0.24	6.29 ^y ± 0.08
9	6.41 ± 0.17	6.73 ± 0.11	6.30 ^y ± 0.13

All values are expressed as Mean ± SE (n = 5)

^{x, y}Means columns differed significantly at $p < 0.05$ due to time effect

OPF=oil palm frond

Table 2. Total volatile fatty acid production (mMolar) from fermentation of different diets at different hours

Time (h)	Fatty acid (mM)		
	Napier 50%	Napier 25% + OPF 25%	OPF 50%
0	35.93 ^a ± 3.01	30.02 ^{b, x} ± 2.49	30.36 ^{a, b, x} ± 2.21
3	54.23 ^a ± 4.58	52.33 ^{b, y} ± 3.34	60.43 ^{a, b, y} ± 4.10
6	52.26 ^a ± 4.49	39.98 ^{b, x} ± 2.10	42.73 ^{a, b, z} ± 2.75
9	46.00 ^a ± 7.57	31.10 ^{b, x} ± 1.57	36.04 ^{a, b, x, z} ± 2.48

All values are expressed as Mean ± SE (n = 5)

^{a, b}Means within row differ significantly at $p < 0.05$ due to treatment effect

^{x, y, z}Means within column differ significantly at $p < 0.05$ due to time effect

OPF=oil palm frond

The *in vivo* fermentation of the NP 50% produced the highest total volatile fatty acids (VFA) at the zero, sixth and ninth hour post-prandially (Table 2), in line with its high digestibility. The OPF with higher lignin content (13-25%) resulted in a lower microbial production of VFA in the OPF 50% group as lignin restricted the degradability of structural polysaccharides by the microbial hydrolytic enzymes, thereby limiting the bioconversion of OPF fiber into VFA (Brown, 1985; Jung and Deetz, 1993). This OPF-NP combination produced the least amount of VFAs, in contrast with its highest *in sacco* degradability in the first 12 hours of rumen incubation (Table 4). Since the sampling for VFA measurement was carried out within the time frame where the *in sacco* digestibility was at the highest, a rapid rate of VFA absorption from the rumen might have occurred to explain the above finding. This study confirmed the inverse relationship between increasing pH value and decreasing total VFA concentration (Pamungkas *et. al.*, 2006).

The combined NP-OPF feed had a lower but statistically comparable *in vivo* digestibility with the NP 50% diet (Table 3).

To evaluate the OPF inclusion with NP, not only was the *in sacco* degradability (Table 4) of the NP-OPF combined diet close to 40% at 48 hours, but significantly higher than that of the OPF 50% diet at all times where it approached the NP 50% diet at 96 hours. As expected, the NP 50% showed the greatest *in vivo* dry matter digestibility, significantly different from the OPF, again confirming the higher cell-wall lignification

of the OPF since the digestibility of forage in the rumen is related to the proportion and extent of lignification (Van-Soest, 1994).

Table 3. *In vivo* dry matter digestibility of different diets

	Napier 50%	Napier 25% + OPF 25%	OPF 50%
DM Digestibility (%)	89.06 ^a ± 1.18	78.80 ^{a,b} ± 2.99	56.39 ^b ± 4.32

All values are expressed as Mean ± SE (n = 5)

*^{a, b} Means within row differ significantly at $p < 0.05$ due to treatment effect

Table 4. *In sacco* dry matter degradability (%) of different diets

Time (h)	Dry matter (%)							
	0	4	8	12	24	48	72	96
Napier	20.5±0.3	21.3 ^{a,w} ±0.4	22.6 ^{a,w} ±0.4	27.3 ^{a,w,x} ±1.2	38.9 ^{a,w,y} ±2.0	51.9 ^{a,y,z} ±4.2	55.6 ^{a,z} ±4.4	58.3 ^{a,z} ±4.4
Napier ±OPF	20.4±0.4	22.0 ^{b,v} ±0.3	23.8 ^{b,v} ±0.8	28.1 ^{b,v,w} ±0.8	34.4 ^{b,w,x} ±0.6	38.5 ^{b,w,y} ±1.5	46.5 ^{b,y,z} ±2.3	54.5 ^{b,z} ±5.3
OPF	15.3±0.7	17.0 ^{c,w} ±1.7	17.5 ^{c,w} ±1.6	18.5 ^{c,w} ±1.6	20.5 ^{c,w} ±1.1	22.2 ^{c,w,x} ±1.7	24.8 ^{c,w,x} ±1.6	28.5 ^{c,x} ±1.5

All values are expressed as Mean ± SE (n = 5)

*^{a, b, c} Means within row differ significantly at $p < 0.05$ due to treatment effect

^{w, x, y, z} Means within column differ significantly at $p < 0.05$ due to time effect

Table 5. Total protozoal counts in goats fed different diets

Time (h)	Protozoal count (10 ⁶ cells/mL)		
	Napier 50%	Napier 25% + OPF 25%	OPF 50%
0	0.68 ^a ± 0.22	0.46 ^a ± 0.11	0.36 ^b ± 0.20
6	0.57 ^a ± 0.23	0.64 ^a ± 0.13	0.29 ^b ± 0.09
9	0.65 ^a ± 0.21	0.62 ^a ± 0.08	0.32 ^b ± 0.17

All values are expressed as Mean ± SE (n = 5)

^{a, b} Means within row differ significantly at $p < 0.05$ due to treatment effect

OPF=oil palm frond

In conclusion, the 25% OPF-25% NP combination was shown to be suitable for goats as reflected by its good *in vivo* digestibility and satisfactory *in sacco* degradability, as well as acceptable rumen pH values and total protozoal counts (Table 5).

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DISEASE PREVALENCE AND CLINICOPATHOLOGICAL CHANGES IN SENIOR AND GERIATRIC CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

**Lim May Lyn,¹Rasedee Abdullah,²Gurmeet Kaur Dhaliwal
& ³Mohamed Ariff Omar**

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

³*Department of Veterinary Preclinical Sciences*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

This retrospective study reports on the prevalence of disease, clinicopathological changes and diagnosis in senior and geriatric cats presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia between 2009 and 2011. The age distribution of cats presented to UVH, level of preventive health care and cost of treatment per cat per annum were also determined. The majority of the feline patients were less than 3 years old. Senior and geriatric cats made up 1.4% of all cats in the study. Kidney disease, dental disease, neoplasia and feline upper respiratory tract disease were the most commonly reported disorders in senior and geriatric cats. Furthermore, blood lymphocytes count and albumin level were significantly lower in older cats compared to adult as a result of immuno-senescence and age-related physiological changes. Preventive care was poor for senior and geriatric cats, with only 9% presented for wellness examination. In general, cost of veterinary care was significantly lower for those that received preventive health care than those that did not. The results from this study can be used by veterinary practitioners to better understand, anticipate health problems of senior and geriatric cats and to encourage clients to subscribe to a semi-annual wellness programme for their older cats.

Keywords: senior, geriatric, cats, preventive care, kidney disease

INTRODUCTION

Just like humans, cats are now living longer; the greying population continues to grow. For example, in Canada more than 35% of cats are greater than 8 years old and in United States 30-40% of the cat population are senior cats (Little, 2011). Cat life expectancy has also risen reaching an average age of 14 to 16 years. The term ‘senior’ has been used to describe the aging group of companion animals and those in their last 25% of their expected life-span (Epstein *et al.*, 2005). The Association of American Feline Practitioners defines senior cats as 11 to 14 years old and geriatric cats as 15 years or older. The most common causes of death in senior cats are renal failure, cancer, and infectious disease (Hoskins, 1997). There are marked increase in incidence of some

illnesses related to age in senior cats compared to adult cats, which consist of metabolic disturbances related to the urinary tract (chronic kidney disease, lower urinary tract disorders), endocrine system (hyperthyroidism, diabetes mellitus), osteoarthritis, dental diseases and neoplasia (Scheck, 2009). Even though the impact of aging is irreversible, with the intervention of nutrition and regular wellness examination, many of these conditions could be controlled and indirectly improve the quality of life of these cats. Furthermore, regular senior wellness examination can help in early detection of diseases and early interventions could be taken. However, there is a scarcity of information on the population of senior cats, general health and disease conditions in adult and senior cats in Malaysia. This study was conducted to provide data on the disease status of geriatric cats which will form the database for clinicians and diagnosticians to use in the management of these cats.

MATERIALS AND METHODS

The medical records of the University Veterinary Hospital (UVH), Universiti Putra Malaysia from January 2009 to December 2011 were searched for cases of adult (3-6 years old), mature (7-10 years old), senior (11-14 years old) and geriatric (15 years old and above) cats. These medical records were reviewed and information collected were signalment of patient (case number, patient identification, sex, breed, age, vaccination and deworming status and body condition score), number of visits throughout the year, diagnosis and cost of treatment. In addition, the haematology and serum biochemistry results were also obtained for 17 clinically healthy cats aged 3-6 years and 10 years and above to determine age-related changes.

Statistical Analysis

The statistical analyses were made using SPSS version 19.0. Descriptive statistics, including means and standard errors of means for continuous data and frequency for categorical data, were produced. Pearson Chi-square Test was used to assess associations between categorical variables and odd ratio to determine the likelihood of diseases in different age groups. Independent T-test was used to test the statistical significance of differences between continuous variables. Significance for all analyses was set at 0.05.

RESULTS AND DISCUSSION

The majority (79.4%) of feline patients were found to be less than 3 years old. Senior and geriatric cats were 1.4% of all cats. This proportion was low compared to counterparts in the United States where 19.1% of cats presented to private veterinary practices were aged 10 years and above (Lund *et al.*, 1999). The difference in senior population in Malaysia from that in developed countries could be due to environmental factors, since most of cats in Malaysia are outdoor cats and roam freely, which make them more prone to infection and fatal motor vehicle accidents.

Clinicopathological findings of healthy adult and senior cats showed that there was no significant difference ($p>0.05$) in erythrocyte parameters. However, the total leucocyte, segmented neutrophils, and monocytes counts were significantly higher

($p < 0.05$), and lymphocytes were significant lower ($p < 0.05$) in older than young cats. This is contrasted with a previous study by Campbell *et al.* (2006), where it was shown that older cats were found to have lower total leucocyte and neutrophil counts. It is probably attributable to the free roamer status of most cats in Malaysia, which constantly expose them to various infectious agents. Being senior cats with poor immune response, response to infections takes, thus causing increase in circulating leucocyte counts. However, consistent with other studies, the circulating lymphocytes in senior cats was low (Campbell *et al.*, 2004; Heaton *et al.*, 2001). These decreases were in CD4+ T cells, B cells and natural killer cells due to age-related immunological changes in cats (Day, 2010). This is the reason why senior cats are more susceptible to infection and neoplasia. Albumin was significant lower in older than young cats because of decreased nutrient digestibility and hepatic function in older cats (Harper, 1998) (Table 1).

Table 1. Haematology and biochemical parameters of cats presented to Universiti Veterinary Hospital, Universiti Putra Malaysia

Parameter	Age (years)	
	3-6 (n=17)	>10 (n=17)
RBC ($\times 10^{12}/L$)	7.49 \pm 0.34	7.221 \pm 0.375
Hg (g/L)	117.12 \pm 5.45	113.818 \pm 4.639
PCV (L/L)	0.33 \pm 0.01	0.315 \pm 0.011
WBC ($\times 10^9/L$)	11.19 \pm 0.76	14.406 \pm 1.280
Band neutrophils ($\times 10^9/L$)	0.36 \pm 0.06	0.35 \pm 0.11
Seg neutrophils ($\times 10^9/L$)	7.30* \pm 0.64	10.10 \pm 1.00
Lymphocytes ($\times 10^9/L$)	2.24* \pm 0.23	1.38 \pm 0.11
Monocytes ($\times 10^9/L$)	0.35* \pm 0.03	0.57 \pm 0.07
Eosinophils ($\times 10^9/L$)	0.88 \pm 0.13	1.19 \pm 0.27
Urea (mmol/L)	8.24 \pm 0.46	9.78 \pm 0.67
Creatinine (μ mol/L)	129.82 \pm 5.20	138.71 \pm 8.00
ALT (U/L)	54.43 \pm 6.60	49.49 \pm 5.63
Albumin (g/L)	32.73* \pm 0.69	27.63 \pm 1.47
Globulin (g/L)	47.25 \pm 2.96	52.28 \pm 3.43

* For each row, means are significantly different ($p < 0.05$)

The most common diseases reported in geriatric cats presented to UVH were kidney disease (33.3%), feline upper respiratory tract disease (33.3%), mammary gland tumours (11%) and wound (11%), while in senior cats there were dental disease (24%), kidney disease (17%), feline upper respiratory tract disease (17%) and skin tumour (10%). However, in middle-aged cats, feline upper respiratory tract disease (11%), wound (10%), dental disease (9%) and kidney disease (8%) were among the top 4 conditions. In contrast, ectoparasitism (12%) was the most common disease reported in adult cats and followed by Feline upper respiratory tract disease (11%), wound (10%) and sporotrichosis (8%) (Table 2).

The frequency of kidney disease increased with age; geriatric cats had the highest frequency of the disease (33.3%) followed by senior cats (17.2%). Odds ratio showed that senior and geriatric cats were 3 times ($\chi^2 = 10.233$, $p = 0.001$) more likely to have kidney disease compared to middle-aged cats and 7 times ($\chi^2 = 37.132$, $p = 0.000$)

compared to adult cats. Age is indeed an important risk factor for kidney disease. Dibartola *et al.* (1987) and Watson (2001) reported that chronic kidney disease affected 7.7% of cats over 10 years old and 15.3% of those over 15 years old. The increase in frequency of kidney disease in older cats could be due to age-related physiological changes. Kidneys of senior cats are smaller in size; there is decrease in tubular size and increased fibrosis. These changes cause a decreased glomerular filtration rate and blood flow and thus a decline in kidney function.

Table 2. Frequency of diseases in cats presented to Universiti Veterinary Hospital, Universiti Putra Malaysia for the period of 2009-2011

Diseases	Age (Years)							
	3-6		7-10		11-14		≥15	
	n	%	n	%	n	%	n	%
Urinary system								
Kidney disease	23	3.8	16	7.6	10	17.2	6	33.3
Non-obstructive FLUTD	28	4.6	10	4.7	4	6.9	0	0
Obstructive FLUTD	15	2.5	6	2.8	0	0	0	0
Dental disease								
FURD	64	10.6	23	10.9	9	15.5	3	16.7
Integument system								
Wound/abscess	63	10.4	21	10.0	2	3.4	2	11.1
Ectoparasites	71	11.8	13	6.2	6	10.3	0	0
Sporotrichosis/fungal infection	50	8.3	7	3.3	3	5	1	5.6
Diabetes mellitus								
	2	0.3	3	1.4	2	3.4	1	5.6
Tumours								
Mammary gland tumour	1	0.2	8	3.8	1	1.7	2	11.1
Skin tumours	2	0.3	3	1.4	6	10.3	1	5.6

In this study, most of the cats were presented only when they were sick. Only 3% of senior cats were presented for wellness examination, as compared to the United States where more than 14 % of senior cats received regular wellness examination. The level of preventive care was reflected in the cost of veterinary care. The veterinary care expenditure in adult and senior cats was quite similar, ranging from RM321 to RM473 per year. However, the veterinary care costs for cats that received preventive health care were lower than in those that did not. The reason was that when cats were presented for preventive health care, early detection of disease could be done, resulting in easier disease management, which was less costly and more successful than crisis management (Epstein *et al.*, 2005).

In summary, senior and geriatric cats made up only a small proportion of feline patients at UVH. Kidney disease, dental disease and neoplasia were among the top three conditions in senior and geriatric cats. Blood lymphocyte count was significantly lower in older cats. Preventive care for feline patients can be improved. Cost of veterinary care is cheaper in the long run if cats received preventive health care.

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DETECTION OF ORGANOCHLORINE COMPOUND IN PUYU, SEPAT AND HARUAN FISH CAUGHT FROM IRRIGATION CANALS IN PADDY FIELDS IN SELANGOR AND PERAK, MALAYSIA

Nur Azhani Abdul Rahman & ¹Mohamed Ariff Omar

¹*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

A case study was conducted to investigate the concentration of organochlorine pesticide residues in fish samples collected from irrigation canals in paddy fields in Tanjong Karang and Sekinchan, Selangor and Seberang Perak, Perak, Malaysia. The samples comprised of three fish species, namely puyu (*Anabas testudineus*), haruan (*Channa striatus*) and sepat (*Tricogaster pectoralis*). Samples were chopped and homogenized and later digested and extracted using QuEChERS method and characterised for organochlorine compounds using gas chromatography. Seven organochlorine compounds, namely aldrin, dieldrin, endrin, endosulfan, o,p-DDT, o,p-DDD and p,p-DDT were identified and quantified. The water samples taken from the irrigation canals were analysed only for endosulfan at 0.04 mg/kg, which exceeded the maximum residue level for endosulfan pesticide residue for water sewage by Environment Protection Administration of the Republic of China on Taiwan (2000) at 0.03 mg/kg. However, the concentrations of endosulfan in all fish samples were below the Codex Alimentary Maximum Residue Limit of 0.2 mg/kg for meat.

Keywords: organochlorine pesticide residues, gas chromatography, paddy-field fish, maximum residue limit

INTRODUCTION

Paddy cultivation is one of the major agricultural activities in Malaysia, mainly concentrated in the major granary areas in Kedah, Kelantan, Perak and Selangor which are served with a system of water ways including irrigation canals, drains, rivers and dams. A variety of fish can be found to inhabit these water ways and these fish contribute a common protein source, apart from marine fish, chicken, meat and eggs, to the general populace (Chen *et al.*, 1984). Among the common fish caught from irrigation canals in paddy fields are puyu (*Anabas testudineus*), haruan (*Channa striatus*) and sepat (*Tricogaster pectoralis*).

Pesticides have been used extensively in paddy planting activities in Malaysia and many parts of the world to control the infestation of major pests such as brown plant hoppers (*Nilaparvata lugens*) and golden apple snails (*Pomacea canaliculata*). Among the common pesticides used are those belonging to the two major pesticide categories: organochlorine and organophosphate - extremely hazardous Class I and II chemicals -

which are either banned or severely restricted for use in some countries even when used with high levels of protection. The use of organochlorines in farming activities has caused concern due to their effect on human health and aquatic ecosystem, particularly so in view of their persistent and bioaccumulative properties (George and Frear, 1966). These chemicals have also been implicated in a broad range of adverse human health effects including reproductive failures, birth defects, immune system malfunction, endocrine disruptions, and cancers (Garabrant *et al.*, 1992)

The residue levels of pesticides found in paddy field fish were moderately high, considering the short exposure period during the fish growing process. These levels generally do not cause toxic effects to the fish. However the residue can accumulate to alarming levels in human subjects, particularly when contaminated fish are consumed frequently and in great quantities (Chen *et al.*, 1984). There is little scientific research been carried out to address the safety of use of these pesticides by paddy farmers. This study was conducted to investigate the concentrations of organochlorine compound residues in sepat (*Tricogaster pectoralis*), haruan (*Anabas testudineus*) and puyu (*Channa striatus*) fish from paddy fields in Sekinchan, Tanjong Karang and Seberang Perak.

MATERIALS AND METHODS

Fish sampling

Fish samples used in the present study were caught live from irrigation canals in paddy fields in three rice growing areas, namely Tanjong Karang and Sekinchan in Selangor and Seberang Perak in Perak, Malaysia during the month of December 2011. The fish were of three common species: sepat (*Tricogaster pectoralis*), puyu (*Anabas testudineus*) and haruan (*Channa striatus*). The samples comprised of 5 fish of each species (*Tricogaster pectoralis*, *Anabas testudineus* and *Channa striatus*) caught in Tanjong Karang, 3 fish each of *Channa striatus*, *Tricogaster pectoralis* and *Anabas testudineus* obtained from Seberang Perak and Sekinchan. The fish samples were kept in thermal insulator boxes and transported to the MARDI Central Laboratory at Serdang, Selangor, Malaysia the following day after collection.

Sample storage

Upon arrival at the laboratory the samples were washed several times with deionized water to remove sediments and other entangled materials. Each fish sample was given an individual identification code. Fish samples were wrapped in aluminum foil and stored at 4°C prior to sample preparation.

Sample preparation

Haruan samples from Tanjong Karang were thawed at room temperature (25°C) for an hour, cut and blended as whole samples to include skin, internal organs and head. Other puyu, sepat and haruan samples from Sekinchan and Seberang Perak were blended and homogenised separately according to internal organs and fillet.

Detection of pesticide residue

The extraction method used in this study was QuEChERS extraction which involved single-step buffered acetonitrile (MeCN) and salting out liquid-liquid partitioning from the water in the sample with MgSO₄. Dispersive solid phase extraction clean-up was done to remove organic acids and excess water from the samples. Then the extracts were analyzed by mass spectrometry after a chromatographic analytical separation. Organochlorine compound residue analysis was done for endosulfan, aldrin, o,p-DDT, p,p-DDT, o,p-DDD, dieldrin and endrin. A well-chopped 15-g fish sample along with 1 mL 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous MgSO₄ per g sample were centrifuged for 5 minutes followed by the addition of a portion of the MeCN extract to anhydrous MgSO₄ sorbent, and then mixed and recentrifuged. This final extract was transferred to auto-sampler vials for analysis by gas chromatography (Agilent 7890N) and mass spectrometry to determine the concentration of the specific organochlorine residues.

RESULTS AND DISCUSSION

Pesticide residues in water of irrigation canals in paddy fields

Table 1 shows the organochlorine compound residue concentration in water samples taken from irrigation canals in Tanjong Karang and Sekinchan paddy fields. These samples were taken to ascertain the pesticide residue status in water from the sampling areas. The water samples were analyzed for organochlorine compounds, mainly endosulfan, aldrin, dieldrin, o,p-DDT, o,p-DDT, and p,p-DDT. The residue levels of organochlorine compound in the water samples taken from Tanjong Karang and Sekinchan indicated endosulfan was present at a concentration of 0.04 mg/kg. According to Environment Protection Administration of the Republic of China on Taiwan (2000), endosulfan pesticide residue levels in sewage water should not exceed 0.03 mg/kg. The present study showed that the endosulfan pesticide residue exceed the minimum level, which is something of concern. This result showed that endosulfan was still being used in the paddy fields in the two sampled areas. Although organochlorine endosulfan pesticide has been banned since 2008 in many countries including Malaysia for use in crop cultivation, it is still being used because of availability in the Malaysia market.

Pesticide residues in fish samples

Tables 2 and 3 show the levels of organochlorine compounds in puyu, haruan and sepat fish samples from Tanjong Karang, Sekinchan and Seberang Perak. Fish sampled from these three areas had pesticide residue concentration of 0.02 mg/kg or less. This concentration is below the Codex Alimentary minimum residue level for meat. The organochlorine residual analysis used in the present study has been programmed to detect residue concentration of 0.02 mg/kg or less. Thus concentration of less than 0.02 mg/kg was indicated as non-traceable. The concentration of organochlorine compound residue of less than 0.02 mg/kg was considered to be very low and the fish samples were not free of organochlorine compound residue since the concentration of organochlorine compound residue in the water of irrigation canals from the same areas was 0.04 mg/kg. The residue detection method adopted by the MARDI Laboratory was meant to

differentiate samples exceeding 0.02 mg/kg in organochlorine compound residue from those with less than 0.02 mg/kg for gross classification of samples.

Table 1. Organochlorine (OC) pesticide residue in water samples from irrigation canals in Tanjung Karang and Sekinchan paddy fields

Area	OC residue (mg/kg)
Tanjung Karang	0.04
Sekinchan	0.04

Table 2. Organochlorine compound residues in whole samples of three fish species caught in irrigation canals in paddy fields in Tanjung Karang

Fish species	No. of samples detected above BDL ¹	BDL ¹ (mg/kg)
Sepat	Nil	<0.02
Puyu	Nil	<0.02
Haruan	Nil	<0.02

¹Below detection limit

Table 3. Organochlorine compound residues of different parts of fish sampled from irrigation canals in paddy fields in Seberang Perak (S.Perak) and Sekinchan areas

Fish species	Samples Type	Sampling area	No. of samples detected above BDL ¹	BDL ¹ (mg/kg)
	Organ	S. Perak	Nil	<0.02
		Sekinchan	Nil	<0.02
Haruan	Meat	S. Perak	Nil	<0.02
		Sekinchan	Nil	<0.02
Sepat	Meat	Sekinchan	Nil	<0.02
Puyu	Meat	Sekinchan	Nil	<0.02

¹Below detection limit

Pesticide application was carried out at several stages in the paddy cultivation process and many paddy farmers applied “cocktail pesticide”, a combination of pesticides made up of endosulfan, organophosphate chemicals and cypermethrine. In this “cocktail pesticide”, the endosulfan concentration was low. This explained the low endosulfan compound residue level in the water samples. However, because endosulfan is a hydrophobic compound, it might be higher in the soil than in water. The low endosulfan concentration in water samples from irrigation canals would lead to low exposure of this organochlorine pesticide to the fish. Thus, this situation has led to low

bioaccumulation of pesticide residues in the fish. This study shows that there is a need to determine biometabolism of endosulfan in future researches.

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ECOLOGY OF *HAEMAPHYSALIS WELLINGTONI* IN RED JUNGLEFOWL

Nurul Aizzati Nadri,^{1,2} Shaik Mohamed Amin Babjee & ¹Jasni Sabri

¹Department of Veterinary Pathology & Microbiology

²Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The ecology of the parasitic stages of *Haemaphysalis wellingtoni* was studied in 30 Red Junglefowls in a farm in Jenderam Hulu, Sepang, Selangor, Malaysia. The free ranging Red Junglefowls are trapped using leg traps and individual bird was carefully examined for tick infestations. The ticks were collected from the neck, comb, and outer ear canal, counted and preserved in 70% ethanol. The identification of the tick species and their stages at each site was done through examination using a stereomicroscope and keys to tick identification. Tissue biopsies with ticks attached were also obtained to determine the pathology of tick-feeding sites. The comb tissues were processed with the standard histological technique and stained with hematoxylin and eosin to observe the cellular changes initiated by the ticks. Only one species of the tick was identified, which was *Haemaphysalis wellingtoni*. The mean of tick numbers in the outer ear canal was the highest (1.77 ± 0.193), followed by the dorsal aspect of the neck (1.32 ± 0.329) and the comb (0.72 ± 0.190). In this study, there are significant differences in stage of the ticks at different infestation sites suggesting that they have preferential site for different stages of their life-cycle. Generally, the mean count for tick stages (adult, nymph and larval) were almost similar on the dorsal part of neck and the outer ear canal. The mean numbers of nymph and larval stages in the neck and outer ear canal were similar, except for the adult stage which was 3.63 on neck and 3.33 in the outer ear canal. However, adult stage was absent on the comb while the mean of nymph stage was 0.16. The larval stage was lower in number in the comb (0.87) compared to the neck and outer ear canal (both 2.2). The cellular changes observed at tick feeding sites consisted of eosinophil and very high lymphocyte infiltrations which indicated chronic inflammation. The congestion, hyperkeratosis and detachment of the keratin layer were also observed at tick feeding sites.

Keywords: *Haemaphysalis wellingtoni*, Red Junglefowl, eosinophils, lymphocyte, Inflammation

INTRODUCTION

The common ectoparasites infesting poultry includes ticks, mites and also lice. The ectoparasites such as ticks and mites can cause heavy morbidity due to their blood sucking habits and cause irritation to the birds causing economic loss (Phulan *et al.*, 1984). Heavy infestation of ticks cause severe blood loss and leading to anaemia and

eventually death (Bergstrom *et al.*, 1999). Ticks and other ectoparasites are able to transmit viral, bacterial and parasitic diseases such as Pasteurellosis, Avian encephalomyelitis, Aegyptiniallosis, fowl Cholera and Borreliosis (Permin and Hansen, 1998).

The ticks can be divided into two families: Ixodidae and Argasidae. Basically the Ixodidae has a chitinous covering, known as the scutum which extends over the whole dorsal surface of the male ticks. However, the scutum only covers a small area behind the head in the larvae, nymph and female ticks. The tick species such as *Haemaphysalis cinabarina*, *H. leporispalustris*, *H. chordeilis*, *Amblyomma haebraeum* and others feed on the poultry and wild ground birds (Johannes, 1996). The species of ticks infesting birds in Malaysia has been reported in Malaysian Parasites XLIX, (Audy *et al.*, 1960), and they consist of the genera *Amblyomma*, *Haemaphysalis*, and *Dermacentor*.

Haemaphysalis species are inornate ticks and have festoons but the eyes are absent. The sensory palps for this genus are short and broad with the second segment extending beyond the basis capituli. In Malaysia, the species *Haemaphysalis wellingtoni* has been identified, infesting the poultry. The genus *Haemaphysalis* is the second largest in the family of Ixodidae. The ixodidae family undergoes four stages: eggs, larvae, nymphs and finally adult stage. This family only has one nymphal stage. Most of the Ixodidae family requires three hosts to complete their life cycle (Oliver, 1989).

The saliva of the blood sucking arthropod has been identified as the sources of toxins or allergens. There is a vast literature on both the toxic and antigenic properties of this secretion (Ribeiro, 1987). The type of cells infiltrating tick feeding sites can be predominantly mononuclear cells or neutrophils. The type of cells infiltrating tick feeding sites can change after multiple infestations (Heinz, 2001). This study was conducted to cover the species of ticks infesting the Red Junglefowl, the predilection sites of infestation, the stages of ticks found at each site and the description of microscopic lesions produced by the host-ticks reaction.

MATERIALS AND METHOD

Animals

This study was conducted on a one-acre Red Junglefowl farm situated in Jenderam Hulu, Sepang, Selangor, Malaysia. These Red Junglefowls were kept under a free-range system and was mixed with village chickens and geese. They were fed once daily in the morning with commercial poultry feed.

Sampling

Eight to 10 birds were randomly trapped daily using the leg trap. Thirty Red Junglefowls were used in this study and these birds were more than 6-month old. In this study the Red Junglefowls were randomly sampled. The individual bird was examined for the ticks on the head region especially the comb, neck and the ears. The ticks found on individual birds at different sites were collected and kept in separate bottles containing 70% ethanol. The numbers collected at each site were recorded. Each individual bird had 3 bottles to collect ticks from the 3 sites.

Identification and Counting the Number of Ticks

The counting and determination of the species of tick were done using stereomicroscope. For each of the 3 sites, the stages of the ticks were also recorded. For identification of the tick species, the specimen was first mounted and then identified based on the mouth parts, presence of eyes, anal groove, presence of festoons and also the basis capituli. *Haemaphysalis* sp. can easily be identified based on the following: the second segment palpi is laterally projected, the eyes are absent, the anal groove is situated below the anus and the basis capituli is rectangular and also the presence of the festoons.

The larval stages are identified based on the presence of 3 pairs of small legs. The nymphal stage is small, have 4 pairs of legs and do not have the genital aperture at the ventral site of the body. In the adult stage, the male and female ticks are identified based on the presence of the scutum. In the male, the scutum covers almost the entire body, while in the female ticks the scutum covers part of the anterior of the body.

Tissue Biopsy and Histopathology

The tissue biopsy of the comb containing the attached ticks was taken from one bird. The birds were anaesthetised locally with 0.1 mL of 2% Xylocaine. The site of combs attached with ticks and an uninfested site was cut and the samples preserved in the 10% Formalin before staining with hematoxylin and eosin.

RESULT AND DISCUSSION

Identification of Tick Species

In previous studies, the most common ectoparasite found was the tick *Haemaphysalis wellingtoni* in 60% of the village chickens (Amin-Babjee *et al.*, 1997). In this study, the species of the ticks identified to affect the Malayan Red Junglefowls was confirmed to be *Haemaphysalis wellingtoni*. The genus of *Haemaphysalis* was identified based on some characteristic morphological parts of these ticks. These ticks had broad palpal segment and the second palpal segment laterally projected. The anal groove of these ticks was seen contouring posteriorly of the anus and there was presence of the festoon.

Predilection Sites of Tick Infestation

Based on the descriptive analysis of variance, ANOVA, $p < 0.05$, there was a significant difference in the number of ticks at the different sites of infestation. The results showed that the ears were the site which has the highest percentage (46%) of ticks, followed by the dorsal aspect of the neck (34%) and the comb (19%). The results also showed the outer ear canal was the site with the highest mean tick number 1.77, followed by dorsum of the neck (1.32) and the comb (0.72). The results indicated the ticks preferred sites which are less exposed to grooming, such as the outer ear canal and the dorsal aspect of the neck.

Stages of Ticks at Specific Sites

This study shows that the stages of ticks found in different sites varied significantly ($p < 0.05$). The different stages of tick's life cycle have their own predilection feeding site on the host. The mean numbers of nymphs and larval stages in the neck and outer ear canal were similar, except for the adult stage which was 3.63 on dorsum of the neck and

3.33 in the outer ear canal. However, there was no adult stage on the comb, but the mean numbers of the nymphal stages was 0.16 was lower than in the neck and outer ear canal (both 0.3). The larval stage is lower in number in the comb (0.87) than in the neck and outer ear canal (both 2.2). This behavior could reflect the feeding stage in the tick life-cycle. The lower mean number of nymphal stages in the host could also be the result of them dropping off after feeding.

Histopathology of Tick Feeding Site.

There were evidence of separation and detachment of keratin layer as the result of tick attachment. The thickening of the keratin layer, hyperkeratosis was observed at the feeding site with monocytes being the predominant cells and presence of several eosinophils. Lima e Silva *et al.*, (2004) described the microscopic features of tick-bite lesions in anteaters and armadillos to include dermal changes with infiltration by variable numbers of inflammatory cells, oedema, haemorrhage and vascular dilatation. In our study, we observed similar cellular eosinophilic and massive lymphocytic infiltrations. High numbers of red blood cells were seen occupying the sinus of capillary in this tissue. In conclusion, there was only one species of tick identified which was *Haemaphysalis wellingtoni*. Based on the results, the outer ear canal is the most common predilection site of these ticks followed by the neck and the comb. Adult ticks were predominantly found on the neck and in the ear canal while nymph and larval stages of the tick appeared to be present at all the three sites. Tick infestations induced pathological changes to the skin structure especially at bite sites, which showed heavy cellular reactions.

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IDENTIFICATION OF EQUINE ESTABLISHMENTS WITH DIFFERENT PERFORMANCE STATUS OF ENDURANCE HORSES

Nurul Hafizah Faisal, ¹Noraniza Mohd Adzahan & ^{2,3}Goh Yong Meng

¹*Department of Veterinary Clinical Studies*

²*Department of Veterinary Preclinical Sciences*

³*Ruminant Diseases Research Center*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

A population survey was conducted on seven equine establishments in Peninsular Malaysia, which actively involved in equine endurance sports. This study presents data obtained through questionnaires and interviews of horse personnel. Total number of horses involved in the study was 115 endurance horses of different breed, gender, age and performance level from various equine establishments. Data collected were analyzed using SPSS version 19 (Kruskal Wallis). More than 50 % of horse population from the equine establishments involved was represented by pure Arabians. The pure Arabians were also observed in all performance categories – 40, 80, 120 and 160 km of racing distances. Majority of the endurance horses were geldings and females with the total of 51 % and 36 % respectively. About half of the population was between the age of 6 to 8 years and most of them were found in all performance categories. There were significant relations between breed, gender, age and establishments based on the performance categories in this study. Majority of the equine establishments were involved in the 40 and 80 km racing categories, both representing approximately 30 % of the total population, followed by the 80 km. Both 120 and 160 km racing categories were represented equally by 18 % of the total endurance horse population surveyed.

Keywords: endurance horse, racing categories, breed, age, gender

INTRODUCTION

Endurance race is an equestrian sport based on controlled long-distance races. Since 1950's, endurance race has been a competitive sport and it has been approved as one of the international competition recognized by FEI (Fédération Équestre Internationale) discipline since 1982. Most breed have been tested and used for endurance races. The most competitive are Arabian or Arabian-Crosses (Anglo-Arabian) due to their muscle fibre composition. Other breeds including Thoroughbreds, Criollos, Ponies, and Appaloosas also have been used successfully for endurance races. Arabian horses are better adapted to endurance race to work for longer distance because of their superior oxidative capacity compared to Thoroughbreds. Arabian has the highest composition of Type I muscle fibers and derived their energy by oxidative phosphorylation via electron transport system, allowing them to go for a longer distance as compared Thoroughbred

which only has Type II muscle fibers that derived their energy by anaerobic glycolysis, thus allowing to go for racing in a short period of time (Rivero *et al.*, 1989).

There are quite numbers of equine establishments with various purposes of horse registered with the National Federation (NF) – the Equestrian Association of Malaysia (EAM). The endurance horse's populations were selected from the individual owners, private clubs as well as government horse establishments. There were seven equine establishments, which were actively involved in endurance races were used in this study. Performance status of endurance horses can be classified into 4 categories based on various distances – 40 km (amateur), 80 km (intermediate), 120 km (advanced), and 160 km (professionals).

The objectives of this study were to determine the relationships between breed, gender, age and equine establishments based on performance categories of endurance horses; and to determine the performance categories among local equine establishments in Peninsular Malaysia.

MATERIALS AND METHODS

A population survey was conducted on 7 different equine establishments within Peninsular Malaysia. Only endurance horses that are actively involved with endurance races registered under the EAM, the country's NF were chosen for this study. All the data were collected using questionnaires and interviews of horse personnel (stable manager, horse owners and riders) to obtain information on the total population of endurance horses according to their breed, gender, age, establishments and performance categories. The performance categories of endurance horses can be classified into different levels, which were the amateurs (40 km), the intermediate group (>40 km to 80 km), the advanced group (>80 to 120 km) and the professional group (>120 to 160 km). Data obtained were recorded in Excel 2007 and were statistically analyzed using SPSS 19. The total population of local endurance horses in Peninsular Malaysia was compared based on various variables (breed, gender, age, and establishment) in relation to the performance categories using a non-parametric ANOVA (Kruskal Wallis). Significance was set at $p < 0.05$ for all comparisons.

RESULTS AND DISCUSSION

The most common breed that present in almost all equine establishments in Peninsular Malaysia was pure Arabian. This breed is the most successful breed used as equine endurance athlete and better suited for racing a longer distance (Nielsen *et al.*, 2006). Majority of the endurance horses were geldings followed by mares and the least was stallions. Stallions often have unpredictable temperament in the presence of mares resulting eliminations. Fully matured horses for endurance is at the ages of 6 to 10 years and the peak performance of endurance horses is at the age of 9 to 11 years. Some of the establishments may not have the knowledge in planning to have horses for variety of categories. Professional establishment may have horses for each of the categories for

long term planning. More than 50 % of endurance horses were from Australia and these proved that the equine establishments have the knowledge in endurance horses.

CONCLUSION

It was concluded that there were significant relationships between breed, gender, age and establishments with performance categories of endurance horses.

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BEHAVIOURAL PROFILE OF NEWLY-CAPTURED STRAY DOGS AND THE EFFICACY OF DOG APPEASING PHEROMONE IN REDUCING POST-CAPTURE STRESS AND FEAR-RELATED BEHAVIOURS

Purshyla Manikam,¹Gurmeet Kaur Dhaliwal & ^{2,3}Sumita Sugnaseelan

¹*Department of Clinical Studies*

²*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

³*Department of Animal Science, Faculty of Agriculture*

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

This study aimed to outline the behavioural profile of stray dogs post capture, as well as to determine the efficacy of Dog Appeasing Pheromone (DAP) in reducing post-capture stress and fear-related behaviours. The behaviour of 17 stray dogs was observed using instantaneous scan sampling for 3 days after being caught from the streets with loop snares, as is the current practice for stray dog control in Malaysia. Following capture, 7 dogs were kept in kennels fitted with DAP diffusers, while 10 were untreated. The untreated adult stray dogs exhibited persistently high coping behaviour for 3 hours post-capture, followed by decreased panting and increased ‘sternal recumbency with head down’ by Day 2. Coping behaviour peaked during kennel cleaning. Significantly higher frequency of maintenance behaviour, whimpering, and ‘exit squeeze’ was observed in puppies compared to adult dogs. DAP-treated dogs had significantly decreased panting and ‘sternal recumbency with head low’, as well as increased standing and ‘sitting with head low’, as compared to the untreated group. Dogs treated with DAP also had an earlier decline in coping behaviour post-capture. This suggests that DAP is a useful tool in reducing some behavioural indicators of stress in dogs post-capture.

Keywords: stray dogs, behaviour, capture, Dog Appeasing Pheromone (DAP), stress

INTRODUCTION

An average of 6600 stray dogs is caught every year by the Kuala Lumpur Municipal Council, in an effort to reduce the risk posed to the community in terms of safety and disease transmission. These dogs are caught using loop snares and kept in a pound for a minimum of 2 weeks before being euthanized. High stocking densities in the pound as well as workers attitudes to the dogs further worsen the welfare implication to these stray dogs.

Dog Appeasing Pheromone (DAP, Ceva Santé Animale) is a synthetic pheromone developed based on the natural pheromones secreted by post-parturient female dogs to provide comfort and reassurance to their newborn puppies, who absorb it *via* inhalation (CEVA Santé Animale-DAP product instruction leaflet). It has been found to be helpful in a variety of situations including separation anxiety (Kim *et al.*, 2010), dogs in public

shelters (Tod *et al.*, 2005), and fireworks anxiety (Sheppard and Mills, 2003), among others. However, to date, there have been no studies of the efficacy of DAP in pounds following capture.

The objectives of this study were to describe the behavioural profile of stray dogs post-capture, in terms of the behavioural manifestations of fear and distress, time taken to recover from the capture, as well as adaptation to the kennel conditions. Apart from that, this study also aimed to determine the efficacy of Dog Appeasing Pheromone (DAP) in reducing stress and fear-related behaviours in newly-captured stray dogs.

MATERIALS AND METHODS

A total of 17 stray dogs were used in this study, recruited upon capture and entry to a municipal council pound. The behaviour of these dogs was recorded beginning from the moment of entry to the pound, and subsequently at 10-minute intervals using instantaneous scan sampling for 3 consecutive days from 08.30 to 17.30 hours. This was done based on an ethogram of canine behaviours. The untreated group, which consisted of 10 dogs, was observed in an unaltered environment in the pound. The treatment group, made up of 7 dogs, was placed in specific kennels fitted with Dog Appeasing Pheromone (DAP) diffusers. A total of 3 diffusers were used to cover an area of 48 m². Data was analysed using SPSS version 19.0, with $p < 0.05$ statistically significant, using the Mann-Whitney U Test and Friedman's Test.

RESULTS

Behavioural profile of stray dogs post-capture

There was a wide range of behaviours exhibited during the 3-day post-capture period, including maintenance, locomotion and stance, vocalisation, investigative behaviour, conspecific interaction, aggression, avoidance, coping behaviour and other miscellaneous behaviours.

There was a 50 % reduction in panting on Day 2 and a subsequent 75 % reduction from Day 2 to Day 3. Also, a significant ($p=0.008$) increase in the frequency of 'sternal recumbency with head down' was seen by Day 2 post-capture. It was also observed that the frequency of coping behaviour peaked during daily kennel cleaning, as seen in Figure 1.

Significantly higher frequency of maintenance behaviour ($p=0.044$), whimpering ($p=0.044$), and 'exit squeeze' ($p=0.044$) as an avoidance behaviour was observed in puppies as compared to adult dogs.

Efficacy of DAP in reducing post-capture stress and fear-related behaviour

The DAP-treated group had a significantly ($p=0.024$) lower frequency of coping behaviour as compared to the untreated group. As shown in Figure 1, coping behaviour in the DAP-treated group declined immediately post-capture, whereas the untreated group experienced a persistently high frequency of coping behaviour for 3 hours post-capture before declining.

There was also a significant increase in standing ($p = 0.029$), and ‘sitting with head low’ ($p = 0.04$), as well as a significant ($p = 0.029$) decrease in ‘sternal recumbency with head low’ in the DAP-treated group.

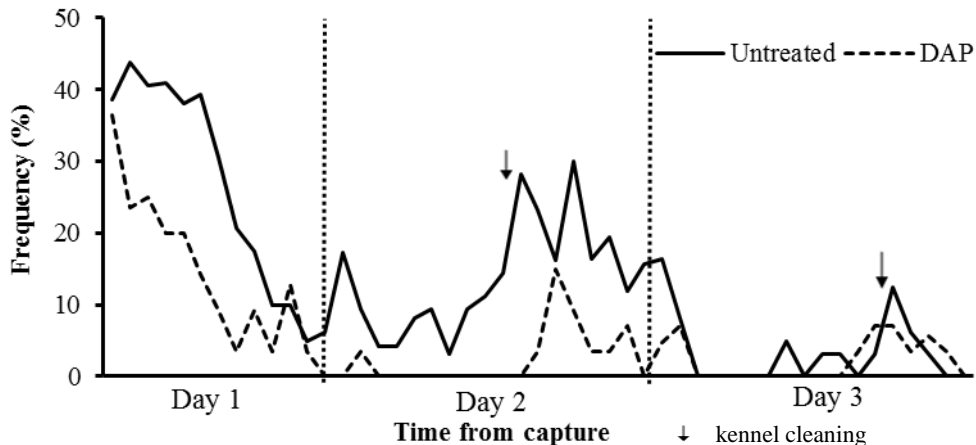


Figure 1. Coping behaviour in untreated ($n = 8$) and DAP-treated ($n = 7$) adult stray dogs post-capture

DISCUSSION

Behavioural profile of stray dogs post-capture

The behaviour that showed the most significant change over the 3 days post-capture period was coping behaviour, which comprised of panting, trembling and hypersalivation. Apart from its thermoregulatory function, these behaviours are also proven to be fear-associated (Schull-Selcer and Stagg, 1991). It can be deduced from this that the reduction in coping behaviour by Day 2 is indicative of a reduction in fear-related behaviour. Concurrently, the dogs spent more time resting by Day 2, as shown by increased ‘sternal recumbency with head down’.

The increased frequency of coping behaviour during kennel-cleaning may be attributed to the use of high-powered water jets while the dogs were still in the kennel.

The higher frequency of whimpering and avoidance behaviour in puppies as compared to adults may be interpreted as increased stress and fear-related behaviour.

Efficacy of DAP in reducing post-capture stress and fear-related behaviour

A similar finding of reduced panting was reported as an effect of DAP in dogs with fireworks anxiety (Sheppard and Mills, 2003). Based on the finding of Schull-Selcer and Stagg, (1991) as noted above, it can be postulated that DAP reduced fear-related behaviour such as panting in stray dogs post-capture. Also, the more rapid decline in coping behaviour in the DAP-treated group indicates that DAP hastens the adaptation process to a stressful experience such as capture.

Positive changes were seen in the locomotion and stance of the DAP-treated group. The lower frequency of ‘sternal recumbency with head low’ in the DAP-treated group is

indicative of lower stress levels as low postures such as this was described by Beerda *et al.* (1997) as being one of the most consistent indicators of stress.

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CLINICAL RESPONSE AND PATHOLOGICAL CHANGES ASSOCIATED WITH *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* INFECTION IN MICE

Randolf Pazatsen Sam Shun,^{1,3}Faez Firdaus Jesse Abdullah,
^{2,3}Mohd Zamri Saad & ^{1,4}Abd. Wahid Haron

¹Department of Veterinary Clinical Studies

²Department of Veterinary Pathology & Microbiology

³Ruminant Diseases Research Centre

⁴Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Corynebacterium pseudotuberculosis is a causative agent of caseous lymphadenitis (CLA). Caseous lymphadenitis is a chronic granulomatous infectious disease characterised by the formation of abscesses, typically located in superficial lymph nodes and lungs. Difficulties in early clinical identification of CLA-infected animals have limited the effectiveness of controlling and eradicating this disease. This study was conducted to acquire a better understanding the pathogenesis of CLA through the mice model. Sixteen healthy male mice were divided equally into 2 groups, where the first group of mice were orally inoculated with 1.0 mL of sterile phosphate buffer solution (PBS), pH 7 and the second group of mice were orally inoculated with 1.0 mL of 10⁹ colony forming unit (CFU) of *C. pseudotuberculosis*. Clinical signs and histopathological changes in visceral organs were compared between the diseased and non-diseased group within the period of 120 hours of post-inoculation. Data was analyzed using the SPSS version 19. This study showed that there were significant (p<0.05) differences in histopathological changes in the lungs, liver and kidneys between diseased and non-diseased mice. Clinical signs were not observed.

Keywords: *Corynebacterium pseudotuberculosis*, caseous lymphadenitis (CLA), mice, clinical response, cellular changes

INTRODUCTION

Caseous lymphadenitis (CLA) or cheesy gland is a disease that can potentially threaten the livestock industry of Malaysia. This disease is found in the major sheep and goat production areas worldwide, causing significant economic losses (Paton *et al.*, 2003; Williamson, 2001; Stoops *et al.*, 1984). The causal pathogen of CLA is *Corynebacterium pseudotuberculosis*, a rod shape, gram-positive, non-spore forming bacteria. It is a facultative intracellular parasite that is found on fomites, in soil and manure contaminated with prudent exudate (Cahn and Line, 2005). Caseous lymphadenitis is a chronic granulomatous infectious disease of sheep and goats characterised by the

formation of abscesses, typically located in superficial lymph nodes and lungs (Batey, 1986; Paton *et al.*, 1994; Arsenaut *et al.*, 2003).

Transmission of CLA among sheep or goats occurs mainly through contamination of superficial wounds that commonly appear following routine procedures, such as shearing, castration and ear-tagging, or body injuries caused by traumatic events (Dorella *et al.*, 2006). Controlling CLA with antimicrobial or antibiotics is challenging because the bacteria stay protected inside abscesses due to the thick capsule that surrounds them (Piontkowski and Shivvers, 1998; Williamson, 2001). In general, the best strategy to control this disease is by vaccination of healthy animals and identification as well as removal of the infected animals (Menzies *et al.*, 2004; Paton *et al.*, 1995; Williamson, 2001). However, the difficulties in early clinical identification of infected animals have caused a limitation to the effectiveness of this strategy. A number of serodiagnostic tests have been developed to overcome the problem of clinical identification of CLA in infected animals. Most tests have been reported to lack either sensitivity or specificity (Burrell, 1980; Menzies and Muckle, 1989; Menzies *et al.*, 2004; Williamson, 2001). Therefore, this study was conducted to determine a better method of detecting and diagnosing CLA in subclinical cases through the mice model. Furthermore, there is a lack of information on the pathogenesis and clinical signs of CLA in mice infected experimentally via oral route infection. Thus the objective of the present study was also to determine the development of clinical signs and pathological changes in mice following the oral route inoculation with *C. pseudotuberculosis*.

MATERIALS AND METHOD

Experimental animals

Four-week old healthy mice (16 ICR, Institute of Cancer Research) were used in this study. The mice were kept at a stocking density 8 per cage in an air-conditioned room. They were fed commercial mice pellets and drinking water *ad libitum* throughout the study. The mice were acclimatised for a period of 1 week before the commencement of study.

Bacteria

Blood bacterial agar culture made from a lymph node that was naturally infected with caseous lymphadenitis from a previous CLA outbreak at the Taman Pertanian Universiti, Universiti Putra Malaysia and was culturally and biochemically identified as *C. pseudotuberculosis*. The bacteria was then subcultured in Brain Heart Infusion broth for 24 hours and concentration estimated to the standard dose of 1×10^9 CFU/mL using the MacFarland technique.

Experimental design

The mice were separated into Group 1 (non-diseased) and Group 2 (diseased) with 8 mice per group. Each mouse in Group 1 was orally inoculated with 1.0 mL of sterile phosphate buffer saline (PBS), pH 7. The mice in Group 2 were orally inoculated with 1.0 mL of 10^9 CFU of *C. pseudotuberculosis*. Development of clinical signs and mortality were observed in the mice within 120 hours after inoculation. Immediate post-mortem examination was performed on mice that died within and after 120 hours of

post-inoculation. Heart, lungs, lymph node, kidney, intestine and brain tissue samples were obtained for histopathology. All changes and abnormalities were recorded.

Clinical signs and scoring

The clinical signs recorded were ruffled hair, eye discharge, movement and responsiveness. Clinical signs scoring for ruffled fur in mice was evaluated and scored according to the criteria in Table 1.

Table 1. Clinical signs and lesion scoring in mice treated with *C. pseudotuberculosis*

Criteria	Score			
	0	1 (Mild)	2 (Moderate)	3 (Severe)
Clinical Sign				
Ruffled fur	Normal fur	30% of body	60% of body	>60% of body
Movement	Normal movement & appetite	30% reduction	60% reduction	>60% reduction
Eye discharge	Normal, no discharge	30% discharge	60% discharge	>60% discharge
Responsiveness	Normal	30% reduction	60% reduction	>60% reduction
Lesion	Normal	<30% of field	30-60% of field	>60% of field

Lesion scoring done on heart, lung, lymph node, liver, kidney and intestine tissues fixed in 10% formalin and brain tissue fixed in 40% formalin and stained with haematoxylin and eosin. Examination and scoring under 200 × magnification.

Histopathology method and lesion scoring

Post-mortem examination was conducted on all mice that died within 120 hours after inoculation. Mice that survived after 120 hours were euthanised by cervical dislocation and post-mortem performed. Heart, lung, lymph nodes, liver, kidney, intestine and brain tissues were obtained for histopathology evaluation. These tissue samples were fixed with 10% formalin except for the brain tissue which was fixed with 40% formalin and processed in an automatic tissue processor. The tissues were processed in paraffin blocks and stained with haematoxylin and eosin. Cellular changes were scored following observation of 5 slides per organ. Six microscopic fields for each slide were observed under 200× magnification. Lesion scoring is according to Table 1.

RESULTS AND DISCUSSION

Clinical signs

Within 120 hours after inoculation of *C. pseudotuberculosis*, the mice did not show clinical signs. These findings are not similar to that of previous studies where significant clinical signs were observed in mice induced to develop the disease (Jesse *et al.* 2011). However in some cases, as in sheep and goats, CLA infections do produce clinical signs (Brown *et al.*, 1987). It is possible that the short observation period (5 days) of the study

and the effectiveness of different inoculation routes may have contributed to the variation in observations. It is possible that had the mice had been observed longer, the disease might have developed to show clinical signs and progress to other organs (Gorman *et al.*, 2010). However, post-mortem examination is still necessary to confirm diagnosis.

Histopathological findings

There was no significant histopathological change in the non-diseased group of mice but the diseased mice did show significant ($p < 0.05$) changes in lungs, liver and kidneys. The lungs showed infiltration of neutrophils and macrophages, congestion, increased vascularization, thrombosis, hemorrhages in the alveolar lumen bronchiole and formation of microabscesses. In the liver of diseased mice there were neutrophils and macrophages infiltrations, degeneration, vacuolation (necrosis), haemorrhage and formation of microabscesses, while the kidneys showed hemorrhages, degeneration, congestion and tubular necrosis.

The lesions observed in this study were similar to those found in sheep and goats infected with CLA via other routes of inoculations (Brown *et al.*, 1986) and in mice (Jesse *et al.*, 2011). The lesions in the lungs, liver and kidneys may be due to two main virulence factors of *C. pseudotuberculosis*, namely phospholipase D (PLD) and mycolic acid (Hodgson *et al.*, 1992). Phospholipase D is a potent exotoxin of *C. pseudotuberculosis* that promotes hydrolysis of ester bonds in mammalian cell membrane, resulting in the damage or destruction of host cell membranes leading to dysfunction or disruption (Salyers and Witt, 1994), which is lethal at high doses to different laboratory and domestic animals (Songer, 1997). Mycolic acid on the other hand causes pyogranulomatous lesions, localized swelling, congestion and necrosis in the lymph nodes, liver, kidneys and mammary glands (Jolly, 1966; Valli and Parry, 1993). The spread of infection in the host may occur via the blood or lymphatic system (Williamson, 2011).

CONCLUSION AND RECOMMENDATION

This study shows that oral route inoculation of *C. pseudotuberculosis* can produce CLA in mice. Future researches that include ultrastructural studies need to be conducted on larger scale and for longer periods to determine obtain more conclusive results. The knowledge and understanding gained from these further studies will be able to contribute to the development of an effective treatment, control and eradication programs for the disease.

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SEROLOGICAL PREVALENCE OF LEPTOSPIRA INFECTION IN MALAYAN PORCUPINES (*HYSTRIX BRACHYURA*) IN CAPTIVITY

Siti Nurdiyana Hj. Abdul Kadir,^{1,3} Abdul Rani Bahaman,^{1,3} Reuben Sunil Kumar Sharma,^{3,4} Sumita Sugnaseelan,^{2,3} Azlan Che' Amat & Mohd Firdaus Ariff Abdul Razak

¹Department of Veterinary Pathology & Microbiology

²Department of Veterinary Clinical Studies

³Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

⁴Department of Animal Science, Faculty of Agriculture

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

⁵Department of Wildlife and National Parks Peninsular Malaysia

Km 10 Jalan Cheras, 56100 Kuala Lumpur, Malaysia

Abstract

Leptospirosis is recognised as one of the important zoonotic diseases in the world including Malaysia and rodents are the major natural reservoir of this disease. A study was conducted to determine the serological prevalence of leptospiral infection among a rodent species, the Malayan Porcupine (*Hystrix brachyura*). A total of 50 serum samples were obtained from the porcupines kept in captivity at the Wildlife Conservation Centre, Sungai Dusun, Selangor. The microscopic agglutination test (MAT) was performed to detect the presence of agglutinating antibodies to a panel of 16 *Leptospira* serovars (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Djasiman, Hardjobovis, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Sejroe). From the serological test, 18% (9/50) of the porcupines had leptospiral antibodies to serovars Javanica (8%), Hurstbridge (4%), Ballum (2%), Celledoni (2%) and Hardjoprajitno (2%). Hence, this study disclosed that leptospiral infection was prevalent among the Malayan Porcupines and could be a potential source of infection to humans.

Keywords: leptospirosis, leptospira, Malayan Porcupines, MAT

INTRODUCTION

Leptospirosis is an acute septicaemic disease of domestic and wild animals as well as humans (zoonosis) that is caused by different serovars within the species *Leptospira interrogans*. Rats are believed to be the major natural reservoir for this bacterium (Bahaman and Ibrahim, 1988). Leptospire can survive for long periods of time in the environment under favourable conditions. Moisture, pH values of the soil and surrounding temperature will influence the survivability of the pathogenic leptospire which can lead to a high incidence of leptospirosis (Gordon, 1977). There are two modes

of transmission for leptospirosis namely by direct or indirect contact. Interactions between the maintenance host, contaminated environment and susceptible animals play a role in the epidemiology of leptospirosis.

Rodents such as rats and mice are known to be one of the most important reservoir hosts for *Leptospira* in the wild. Porcupines are also of rodents and ranked third largest. These mammals are commonly herbivores, nocturnal and terrestrial. The Malayan Porcupine (*Hystrix brachyura*) is one of the wildlife species proposed by the Department of Wildlife and National Parks (DWNP) of Malaysia to be commercialised as a meat product due to its high reproductive performance, large body size and easy management (Zainal, 1988).

Leptospirosis as mentioned earlier has zoonotic potential and a concern towards public health safety issues with this particular type of farming. The status of leptospirosis in the Malayan Porcupines is still not known. A previous study done by Mitchell *et al.* (1966) reported that *Leptospira pomona* was isolated from a porcupine's kidney among 15 species of wildlife trapped from a farm following an outbreak of leptospirosis in the farm studied. This certainly showed that porcupines are no different from rats or any other rodent species which are able to harbour leptospiral organisms and spread the infection.

Thus, this study was conducted to determine the serological prevalence of *Leptospira* infection in the Malayan Porcupines and to identify the leptospiral serovars infecting them. These would provide background information on leptospiral infection in porcupines and would facilitate the understanding of the epidemiology of leptospirosis in Malaysia.

MATERIALS AND METHODS

Animals

Fifty Malayan Porcupines (*Hystrix brachyura*) kept in metal wire pens at the Wildlife Conservation Centre, Sungai Dusun, Selangor, Malaysia were selected for this study. They were made up of 43 adults and 7 juveniles. All animals were judged to be in good health based on their general behaviour and physical conditions. They were fed twice a day with fruits, vegetables, tubers and concentrates.

Blood sampling

The Malayan Porcupines were restrained, anaesthetised, and 3 mL of blood were extracted from the femoral artery into a plain tube using a 23-gauge needle and a 5 mL syringe. Serum samples were kept at -20°C until analysis.

Serological Examination

A microscopic agglutination test (MAT) was performed according to Cole *et al.* (1973) for the detection of agglutinating antibodies against a panel of 16 leptospiral serovars (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Djasiman, Hardjobovis, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Sejroe). The leptospiral cultures (antigens) were adjusted to a cell density of 1.5×10^8 leptospores/mL based on MacFarland standard. Serum positive at titre 1:20 was further titrated until 1:5120. A serum sample was considered positive when more than

50% agglutination was observed compared to the negative control and at a titre of 1:40 or higher. A negative MAT would demonstrate live and active leptospiral antigen with few or without any agglutination.

RESULTS

From the serological test (MAT), out of 50 serum samples, 18% (9/50) were positive to one or more of the five serovars including Javanica, Ballum, Celledoni, Hardjoprajitno and Hurstbridge (Table 1). All the seropositive results were among the adult Malayan Porcupines in which the highest serum titre was found to be 1:40. The highest prevalence of leptospiral infection among the Malayan Porcupines was serovar Javanica at 8% (4/50), followed by Hurstbridge at 4% (2/50) and as for Ballum, Celledoni and Hardjoprajitno was at 2% each (1/50).

DISCUSSION

Based on the results, the serological prevalence of *Leptospira* in the Malayan Porcupines was determined to be 18%. This value is considerably high and comparable to rats. Mohamed-Hassan *et al.* (2010) reported a serological prevalence of 17.9% (30/168) of leptospiral infection in wild rats in Kelantan and Terengganu with 17.3% (14/81) and 18.4% (16/87) in the respective states. Similarly, a previous study by Siti-Aminah (2006) also reported a similar serological prevalence of 18.1% (6/33) of leptospiral infection among wild rats caught from Kuala Lumpur areas and almost 4% (1/27) were from wild rats from the Serdang area.

From this present study, Javanica was the major serovar detected at 8% (4/50) in the serum samples. The same serovar was found in majority of the rats from the study done by Siti-Aminah (2006). The positive serum samples were found only among the adult Malayan Porcupines. No positive MAT was seen in the juvenile porcupines tested, possibly because they have not yet been exposed to *Leptospira*. The serum titre of leptospiral antibodies was found to be 1:40. This low titre may mean the start of antibody production in an early acute infection, or a low level of antibodies persisting long after infection. There is also a possibility that the serovar infecting the Malayan Porcupines was not covered by the serovars in the test antigens.

From the findings, it is suggestive that the Malayan Porcupines could become carriers of leptospiral organisms and thus, farmers as well as other handlers are at risk of contracting leptospirosis from the Malayan Porcupines. Precautions should be taken during handling porcupines or their carcasses in preparation of their meat for human consumption. Farmers and handlers are advised to wear protective clothing such as boots, gloves and masks whenever handling such animals or during cleaning of their cages or pens. It is also important to wash or shower after exposure to urine splashes from these porcupines or from contaminated soil or water.

Table 1. The prevalence rate (%) of *Leptospira* infection in the 50 Malayan Porcupines

Serovars	No. of Positives	Prevalence Rate (%)
Javanica	4	8.0
Ballum	1	2.0
Celledoni	1	2.0
Hardjoprajitno	1	2.0
Hurstbridge	2	4.0
Total Positives	9	18.0
Total Serum Samples		50

From this study, the Malayan Porcupines are possibly carriers of leptospiral organisms which is expected as porcupines are of rodent species, similar to rats. To date, no study has been carried out on porcupines to implicate them as the source of leptospirosis to humans in Malaysia. Thus, further research is required to look into its association with human leptospirosis. Exposure of porcupine farmers and handlers to urine-contaminated environment put them at risk at contracting leptospirosis.

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Fatty Acid Composition of Nile Tilapia (*OREOCHROMIS NILOTICUS*) and Red Hybrid Tilapia (*OREOCHROMIS MOSSAMBICUS X OREOCHROMIS NILOTICUS*) Reared in Intensive and Extensive Systems

Siti Sarah Jamaudin, ¹MohamedAriff Omar & ^{1,2}Goh Yong Meng

¹*Department of Veterinary Preclinical Sciences*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract

Fish and marine mammals are among the richest sources of long-chain n-3 and n-6 polyunsaturated fatty acids (PUFA) in nature. As farmed fish becomes a major contributor to world fish supplies, hence it is important to maintain the high lipid nutritional quality of the product and to continue to provide large amounts of the health-promoting n-3 and n-6 PUFA to the consumers. Therefore, a study was conducted to examine the nutritional content, mainly fatty acid composition, of Nile (*Oreochromis niloticus*) and Red hybrid tilapia (*Oreochromis mossambicus x Oreochromis niloticus*), derived from intensive and extensive culture systems. Twenty-two samples of Nile tilapia and 16 samples of Red tilapia cultured intensively and 10 samples of extensively cultured Nile tilapia were used in this study. All samples were subjected to total fatty acids extraction and their fatty acid compositions were determined using gas liquid chromatography. Results showed no significant differences in the concentration of saturated fatty acids (SFA) between intensively and extensively cultured Nile tilapia. For monounsaturated fatty acids (MUFA), the concentration was higher ($p < 0.05$) in the intensively cultured Nile tilapia compared to the extensively cultured Nile tilapia. For n-3 PUFA, the concentration was higher ($p < 0.05$) in Nile tilapia from the extensive system compared to Nile tilapia from the intensive system. But for n-6 PUFA, the intensively cultured Nile tilapia had a higher ($p < 0.05$) concentration compared to the extensively cultured Nile tilapia. For n-6:n-3 PUFA ratio the intensively cultured Nile tilapia had a higher ratio ($p < 0.05$) than the extensively cultured Nile tilapia. The different fatty acid composition of Nile tilapia cultured in different systems could be due to the different types of feed consumed by the fish. Future research could be directed at increasing the PUFA level in tilapia through the manipulation of the PUFA level in the fish diet.

Keywords: tilapia, extensive culture system, intensive culture, polyunsaturated fatty acid, saturated fatty acid

INTRODUCTION

Fish and marine mammals are among the richest sources of n-3 poly unsaturated fatty acids (PUFA) in nature. Freshwater fish generally contained lower proportions of n-3 PUFA than marine fish but contain high proportions of n-6 PUFA. Fish supplies from

marine sources are dwindling due to uncontrolled harvesting, deterioration of the habitat and reduction in fish stock, thus making the catch of marine fish inadequate to meet the increasing demand of fish and fish products. Hence, aquaculture is seen as a significant option in ensuring continued fish protein supplies.

As farmed fish becomes a major contributor to world fish supplies, it is important to maintain the nutritionally high lipid quality of the product and to continue to provide large amounts of the health-promoting n-3 PUFA to the consumers. Studies showed that fish cultured in intensive systems were characterized by increased fat deposition of mainly saturated and monosaturated fatty acid and 18:2 n-6 whereas fish reared in the extensive system contained higher proportions of 18:3 n-3, 20:5 n-3 and 22:6 n-3, the favorable fatty acids for human consumption, higher n-3:n-6 PUFA ratios and lower proportions of 18:2 n-6. The fatty acid composition of the diet influenced the fatty acid composition of the fish muscle, although the differences in fatty acid composition of groups of fish were less than the differences in fatty acid composition of their respective diets (Robin *et al.*, 2003). Fatty acid composition can be changed through manipulation of diets, such as using different feed ingredients of oil seed origin such as linseed and flaxseed oils, or through manipulation of n-3 to n-6 PUFA ratio (Nguyen *et al.*, 2003). Therefore, this study was conducted to examine the nutritional content, mainly fatty acid composition, of Nile tilapia (*Oreochromis niloticus*) and Red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*), derived from intensive and extensive culture systems.

MATERIALS AND METHODS

Fish meat sample

Fifty tilapia fish samples were included in this study consisting of 30 Nile tilapia (*Oreochromis niloticus*); 20 fish from the intensive culture system and 10 fish from an extensive culture system, and 20 Red hybrid tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*) from an intensive culture system. All 40 tilapia samples from the intensive culture system with a bodyweight range of 350 to 450 g were purchased from the a wholesale market in Selangor, Malaysia and the fish originated from several farms that had adopted the intensive culture system using earth ponds, where the fish were fed commercial pellets and harvested at about 4 months of age. Another 10 Nile tilapia samples of body weight ranging 200 to 300 g were collected from a former mining pool (extensive system) where the fish were managed minimally and fed natural foods.

Fatty acid profile

All tilapias were filleted at the left dorsal cranial part of the body and shaped into standard size of 5 cm (long) × 3 cm (wide) × 1 cm (thick). Fillets were cut and 1 g samples from the middle portion were taken for total lipid extraction using the chloroform-methanol 2:2 (v/v) solvent system according to the method of Folch *et al.* (1957) modified by Rajion *et al.* (1985). Fatty acids were transesterified to methyl ester with 0.5 N NaOH in methanol and 14% boron trifluoride in methanol. In addition 100 µg of heneicosanoic acid (C 21:0) were added to the methylating tube as an Internal Standard (ISTD) prior to methylation. The methyl esters were quantified using gas

chromatograph GC (Agilent 7890N) on a 100m × 0.25 mm ID (0.20µm film thickness) Supelco SP- 2560 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microliter

of the sample was injected using an auto-sampler into a chromatograph, equipped with a flame ionization detector (FID) at a split ratio of 10:1. High purity nitrogen (99.999% from an atmospheric nitrogen gas generator) was the carrier gas at 40mL/minute. High purity hydrogen (Dominick Hunter, Parker Hannifin Ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used in the flame ionization detector for the gas-liquid chromatography. The injector temperature was programmed at 250°C and the detector temperature was 300°C. The column temperature programmed included an initial temperature of 150°C held for 2 minutes, and then warmed to 158°C at 1°C/minute which was then held at this temperature for 28 minutes, finally the column was warmed to 220°C at 1°C/minute, and maintained for 20 minutes to achieve optimum separation. The peaks of samples were identified and concentrations calculated based on the retention times and peak areas of known standards (Sigma Chemical Co., St. Louis, Missouri, USA). The fatty acid concentrations were expressed as the percentage of identified total fatty acids measured in each sample. Automatic expression of the peak areas as percentage of a detected fatty acid was obtained with a programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

Statistical analysis

Data of the fatty acid profile of tilapia fillets grouped by culture systems and species were analyzed for mean differences between groups by an independent sample t-test, using the Statistical Analysis System (SAS) 2000.

RESULTS AND DISCUSSION

The fatty acid profiles of Nile tilapia sampled from extensive and intensive culture systems and between species of Nile tilapia and Red hybrid tilapia are presented in Table 1. The fatty acid composition of Nile tilapia from extensive and intensive culture systems were similar to those reported earlier for tilapia and other fish species (Gonzalez *et al.*, 2006; Fuentes *et al.*, 2010). For the different genotypes of Nile tilapia and Red hybrid tilapia, only 3 types of fatty acids: 14:0, 24:0 and 18:3 n-3, were found to be significantly different. This finding is in agreement with the studies by Teoh *et al.* (2011) who also reported a significant effect of the tilapia genotype for 24:0, 18:3 n-3 and 22:1 n-9. Nile tilapia showed a significantly higher value for 14:0 and a significantly lower value for 18:3 n-3 than the Red hybrid tilapia. However, Teoh *et al.* (2011) showed that the Nile tilapia had lower 14:0 and significantly higher 18:3 n-3 compared to Red hybrid tilapia. They also observed that the net intake of fatty acids was not affected by the tilapia genotype but the dietary lipid sources had significantly affected the fatty acid composition.

The total percentage of SFA and PUFA were higher in extensively cultured Nile tilapia than the intensively cultured Nile tilapia, whereas the Nile tilapia from the intensive culture system showed a higher content of MUFA than Nile tilapia from the extensive culture system. This was probably due to the high content of MUFA in the diet

Table 1. Fatty acid composition of tilapia of different species and culture systems

Fatty acid	Fatty Acid (% Total Fatty Acid)		
	Intensive System		Extensive System
	Nile tilapia ²	Red hybrid tilapia ²	Nile tilapia ²
14:0	4.48 ^a ± 0.47	2.54 ^b ± 0.17	3.02 ^b ± 0.48
15:0	0.46 ^a ± 0.10	0.47 ^a ± 0.06	0.53 ^a ± 0.06
15:1	0.41 ^b ± 0.05	0.60 ^b ± 0.06	1.08 ^a ± 0.18
16:0	23.31 ^a ± 0.49	24.74 ^a ± 0.85	24.92 ^a ± 0.75
17:0	7.03 ^a ± 0.84	9.95 ^a ± 1.36	8.69 ^a ± 1.19
18:0	7.47 ^b ± 0.42	6.72 ^b ± 0.27	10.82 ^a ± 0.64
20:0	0.84 ^a ± 0.08	0.77 ^a ± 0.13	0.68 ^a ± 0.10
22:0	0.96 ^a ± 0.09	1.25 ^a ± 0.13	0.99 ^a ± 0.10
24:0	1.13 ^b ± 0.22	2.18 ^a ± 0.20	2.75 ^a ± 0.42
∑ SFA ³	46.10 ^a ± 0.78	46.40 ^a ± 1.16	48.10 ^a ± 1.18
18:1 n-9	29.45 ^a ± 1.28	26.47 ^a ± 1.41	15.93 ^b ± 2.46
24:1	0.79 ^b ± 0.07	0.59 ^b ± 0.10	2.56 ^a ± 0.36
∑ MUFA ³	31.17 ± 1.08	29.21 ^a ± 1.25	19.76 ± 2.03
18:3 n-3 (ALA) ⁴	0.89 ^b ± 0.08	1.34 ^c ± 0.11	2.69 ^a ± 0.29
20:5 n-3 (EPA) ⁴	1.56 ^b ± 0.13	1.21 ^b ± 0.09	2.41 ^a ± 0.27
22:6 n-3 (DHA) ⁴	5.02 ^b ± 0.50	4.86 ^b ± 0.50	11.90 ^a ± 1.54
∑ n-3 PUFA ³	8.27 ^b ± 0.65	8.01 ^b ± 0.74	19.57 ^a ± 0.65
18:2 n-6 (LA) ⁴	10.11 ^a ± 0.46	11.68 ^a ± 1.18	5.51 ^b ± 0.65
18:3 n-6 (GLA) ⁴	0.41 ^a ± 0.05	0.62 ^a ± 0.06	0.52 ^a ± 0.14
20:2 n-6 (AA) ⁴	3.71 ^b ± 0.62	4.03 ^b ± 0.36	6.58 ^a ± 0.64
22:5 n-6 (DPA) ⁴	1.56 ^b ± 0.13	1.21 ^b ± 0.09	2.41 ^a ± 0.27
∑ n-6 PUFA	14.35 ^a ± 0.60	16.32 ^a ± 1.23	12.55 ^b ± 0.77
∑ Total UFA ³	53.80 ^a ± 0.78	53.55 ^a ± 1.16	51.89 ^a ± 1.18
n-6 : n-3 ratio	1.88 ^a ± 0.10	2.24 ^a ± 0.20	0.74 ^b ± 0.10
UFA/SFA	1.17 ^a ± 0.03	1.17 ^a ± 0.06	1.09 ^a ± 0.05
PUFA/SFA	0.49 ^b ± 0.02	0.54 ^b ± 0.05	0.68 ^a ± 0.07

^{ab}Means with different superscripts within row are significantly differences at p <0.05

²Nile tilapia (extensive); n=10, Nile tilapia (intensive); n=22, Red tilapia (intensive); n= 16

³SFA= Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acid; UFA= Unsaturated fatty acid

⁴LA= Linoleic acid; GLA= γ-Linolenic acid; AA= Arachidonic acid;

DPA= Docosapentaenoic Acid; ALA=α-linoleic acid; EPA=Eicosapentaenoic acid;

DHA= Docosahexaenoic acid

of intensively cultured Nile tilapia. Oleic acid (18:1 n-6) was identified as the major MUFA in Nile tilapia from both intensive and extensive systems. The higher value of oleic acid in intensively cultured fish could be due to the high content of oleic acid in the commercial feed as reported by Fuentes *et al.* (2010) for the sea bass and Gonzalez *et al.* (2006) for the yellow perch.

In this study it was determined that the total SFA was higher ($P>0.05$) in extensively cultured Nile tilapia than intensively cultured Nile tilapia with total SFA of 48.13 and 46.14%, respectively. Palmitic acid (16:0) was the major fatty acid in the Nile tilapia from both extensive and intensive culture systems, followed by stearic acid (18:0). The stearic acid content was also found to be high in other fish species (Chen *et al.*, 1995; Fuentes *et al.*, 2010).

As for the PUFA content, extensively cultured Nile tilapia had a higher percentage of n-3 PUFA and n-6 PUFA compared with intensively cultured Nile tilapia. Arachidonic acid (20:4 n-6) was the major fatty acid in the n-6 PUFAs. Freshwater fish were reported to contain a higher concentration of arachidonic acid and linoleic acid than marine fish which could be due to dietary effects and saturation and/or elongation mechanisms. The higher concentration of arachidonic acid in Nile tilapia harvested from extensive culture systems could be attributed to the type of diet of the Nile tilapia in the extensive environment which comprised insect larvae, freshwater algae and crustaceans which have high content of linoleic acid and α -linolenic acid (Gonzalez *et al.*, 2006).

Besides that, for the n-3 PUFA, DHA (22:6 n-3) was the major fatty acid in this group, and the DHA and EPA (20:5 n-3) were higher in the extensively cultured Nile tilapia compared to intensively cultured Nile tilapia. This finding is in agreement with Karapanagiotidis *et al.* (2006) for tilapias and Fuentes *et al.* (2010) for the sea bass. For the n-3/n-6 ratio, the values for extensively cultured Nile tilapia were significantly lower ($P<0.05$) compared with intensively cultured Nile tilapia. This finding is contrary to previous reports for fish with n-3/n-6 ratio significantly higher ($P<0.05$) in fish from the extensive system (Fuentes *et al.*, 2010). Although the n-3 PUFA: n-6 PUFA ratio was low but the proportion of n-3 PUFA and n-6 PUFA was still significantly higher in extensively cultured Nile tilapia than in intensively cultured Nile tilapia.

With regards to the PUFA/SFA ratio, higher levels were detected in extensively cultured Nile tilapia compared to intensively cultured Nile tilapia with the values of 0.68 and 0.49, respectively and 0.54 for the Red hybrid tilapia. According to the nutritional guidelines of the Department of Health of the UK (1994), a ratio of 0.45 or more is recommended for a balanced fatty acid intake in a healthy diet.

CONCLUSION

The ratio of the n-3:n-6 PUFAs was higher in tilapia fish from the intensive culture system than the extensive culture system. This difference may be attributed to the dietary content of the Nile tilapia. There was no difference between different tilapia genotypes (Nile vs Red tilapia) for SFA, PUFA, PUFA/SFA and n-3:n-6 ratio since both genotypes were fed similar commercial pellets. It can be concluded that in order to have higher n-3:n-6 PUFA ratios and to have good quality tilapia fish with high content of n-3 PUFA (especially DHA and EPA) content, the content of PUFA in the diet could be increased. This is because fatty acid proportion of fish is very much dependent on the

type of fish diet. From the findings of this study it is evident that extensively cultured and intensively cultured Nile tilapias and also intensively cultured Red hybrid tilapias have high PUFA/SFA ratio that is beneficial to human health.

The knowledge of the fatty acid profile is important and this study provides alternative sources of healthy and high quality foods and the base data about the relationship between the type of management and genotype of tilapia fish with fatty acid concentration. Future research can be directed to increasing the PUFA level in tilapia by manipulating the PUFA level in the fish diet.

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DISEASE DETECTION OF BRUCELLOSIS IN GOAT POPULATION IN NEGERI SEMBILAN, MALAYSIA

Siti Sumaiyah Mohd Yusof,^{1,3} Abd. Wahid Haron & ²Siti Khairani Bejo

¹Department of Veterinary Clinical Studies

²Department of Veterinary Pathology & Microbiology

³Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

A serological study of brucellosis in goats caused by *Brucella melitensis* was conducted in the state of Negeri Sembilan, Malaysia. A total of 771 serum samples were collected from seven districts namely Rembau, Jelebu, Kuala Pilah, Seremban, Port Dickson, Jempol, and Tampin. At least two farms were selected and a minimum of 100 serum samples were collected from each district. All sera were tested for brucellosis using the Rose Bengal plate test (RBPT) and complement fixation test (CFT). In this study, only Rembau and Kuala Pilah showed seropositivity for *B. melitensis* with RBPT and CFT at 1.0 and 2.5%, respectively. The CFT was more sensitive than the RBPT because the serum antibodies against *B. melitensis* detected by CFT were twice higher than that detected by RBPT. As suggested by the Office International des Epizooties OIE, CFT was used as a confirmatory test for brucellosis. This test is also recommended as a prescribed test for international trade and is used in the control and eradication programmes.

Keywords: *Brucella melitensis*, RBPT, CFT, goats

INTRODUCTION

Brucellosis in goats is mainly caused by *Brucella melitensis*, a gram-negative coccobacillus or short rod. *Brucella melitensis* is pathogenic to sheep, goats and highly zoonotic (Erganis *et al.* 2005). The occurrence of the disease must be reported to the World Organization for Animal Health. Clinical manifestation of the disease is not specific. The definitive diagnosis of *B. melitensis* was usually made by isolation of the bacteria whereas the gold standard for serological methods was the complement fixation test (CFT). It was recommended by the Office International des Epizooties (OIE, 2008) to use a combination of the Rose Bengal plate test (RBPT) and the CFT for detection of brucellosis in small ruminants. The Rose Bengal plate test is being used as a screening test for brucellosis whereas the CFT is used as a confirmatory test for brucellosis. In addition, the RBPT is very cheap, dependable and give rapid results but the sensitivity and specificity of the RBPT in goats is still unclear (Blasco *et al.*, 1994; Erganis *et al.*, 2005). Brucellosis in goats and sheep still occur in Negeri Sembilan as reported by the

Department of Veterinary Services (DVS). Two serological diagnostic tests of RBPT and CFT are practiced by the DVS for detection of brucellosis. The data from the DVS of Negeri Sembilan revealed that each district has seropositivity towards *B. melitensis* with Rembau and Kuala Pilah being the highest. Control and prevention programmes were implemented in order to reduce the occurrence of the disease. The DVS is also practicing a stamping-out policy to all seropositive animals in order to control the disease.

The objectives of this study were to conduct serological testing using RBPT and CFT for the detection of brucellosis in goats, to determine current status of brucellosis in goats in Negeri Sembilan, and to determine the sensitivity and specificity of the RBPT and CFT for the detection of brucellosis in goats.

MATERIALS AND METHODS

The Rembau, Jelebu, Kuala Pilah, Seremban, Port Dickson, Jempol, and Tampin districts of Negeri Sembilan, Malaysia were selected for this study. A total of 771 serum samples were collected randomly from two goat farms from each district. A minimum of 100 goat serum samples were collected randomly from animals of more than one-year old from each district. The whole blood was collected into 6 ml plain sterile tubes. The serum was separated from the clotted blood and collected into new serum collecting tubes. The serum was stored at -20°C until required for serological tests. The RBPT was performed by adding 30 µL of test serum into 30 µL Rose Bengal antigen on white porcelain plate using a pipette and sterile tips and mixed thoroughly with a clean toothpick to produce a zone approximately 2 cm in diameter. The plate was rocked slowly for 3 minutes and any apparent agglutination is interpreted as positive.

All serum samples were also sent to the Veterinary Research Institute (VRI) for a confirmatory test using CFT. Briefly, the CFT involves inactivated brucella antigen, guinea pig serum containing a complement and indicator system which consisted of sheep erythrocytes sensitised with rabbit antibodies. A positive result is revealed by the absence of haemolysis by which the specific test serum contained a specific antibody which formed a complex with the inactivated antigen to bind with the complement. A negative result is revealed by the evidence of haemolysis when the complements freely bind to the sensitised sheep erythrocytes due to absence of the antigen-antibody complex.

Specificity and sensitivity

The specificity and sensitivity were calculated by 2 × 2 tables as in the formula below:

$$\text{Specificity} = \frac{\text{Total number of comparative test negative}}{\text{Total number of relative tests negative}} \times 100$$

$$\text{Sensitivity} = \frac{\text{Total number of comparative test positive}}{\text{Total number of relative test positive}} \times 100$$

RESULTS

The results of the *Brucella melitensis* antibody detection using RBPT and CFT are shown in Table 1. The RBPT detected 8 goats positive with *B. melitensis* antibodies while CFT detected 19 goats positive with *B. melitensis* antibodies. The data also revealed that only Rembau and Kuala Pilah showed seropositivity for *B. melitensis*. The prevalence in Rembau was 2.5% by RBPT and 5.6% by CFT while the prevalence in Kuala Pilah was 3.6% by RBPT and 9.1% by CFT. The CFT (2.5%) is more sensitive in the detection of *B. melitensis* antibodies than RBPT (1.0%). Table 2 shows the RBPT and CFT sharing 1 male serologically positive for *B. melitensis* antibodies while 7 females and 18 females were serologically positive for *B. melitensis* antibodies using RBPT and CFT. Based on Figure 1, the seroprevalence of brucellosis in year 2012 is higher compared to years 2010 and 2011 because the serum samples collected were from the farms with history of brucellosis. Comparison between Tables 3 and 4 revealed that CFT (85.7%) was more sensitive than RBPT (32%) while RBPT (99.9%) was more specific than CFT (98.3%).

Table 1. Rose Bengal Plate Test and Complement Fixation Test for *B. melitensis*

Districts	RBPT				CFT			
	+ve	-ve	Total	%	+ve	-ve	Total	%
Rembau	4	156	160	2.5	9	147	160	5.6
Kuala Pilah	4	106	110	3.6	10	96	110	9.1
Jelebu	0	98	98	0	0	0	98	0
Seremban	0	50	50	0	0	0	50	0
Port Dickson	0	118	118	0	0	0	118	0
Tampin	0	117	117	0	0	0	117	0
Jempol	0	118	118	0	0	0	118	0
Total	8	763	771	1.0	19	753	771	2.5

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test

+ve=positive; -ve=negative

+ve=positive; -ve=negative

Table 2. Seropositivity of *B. melitensis* between genders

Gender	Total	RBPT +ve (%)	CFT +ve (%)
Male	78	1 (1.3)	1 (1.3)
Female	693	7 (1.0)	18 (2.6)

RBPT=Rose Bengal Plate Test positive; CFT=Complement Fixation Test

+ve=positive; -ve=negative

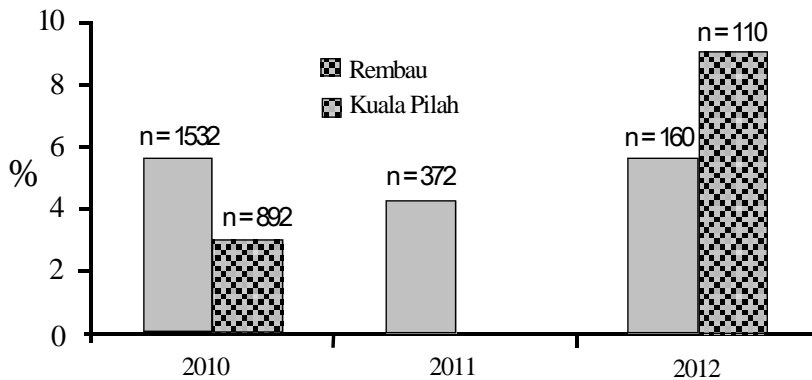


Figure 1: Serological Prevalence of Brucellosis in Goats in Rembau and Kuala Pilah using CFT (2010 – 2012) (Courtesy of Department of Veterinary Services, Negeri Sembilan)

DISCUSSION

Brucellosis in goats is caused by *B. melitensis* which is a gram-negative coccobacilli. The agent causes high economic losses in the host animal and is highly zoonotic to humans. The mode of transmission between animals is mainly through the ingestion of contaminated feed and water with uterine fluid and placenta expelled by infected cows while transmission to humans is mainly via ingestion of contaminated or non-pasteurized milk products and by-products. Typical signs of brucellosis are abortion and infertility.

From the annual report of the Department of Veterinary Services (DVS), Negeri Sembilan, Rembau and Kuala Pilah showed the highest prevalence of brucellosis in goats compared to other districts. The frequency of the disease monitoring contributed towards the decline in the prevalence of brucellosis in Rembau and Kuala Pilah from 2010 to 2011. In 2012, the seroprevalence of brucellosis was the higher than the previous years because the blood was sampled from goat farms with history of brucellosis. Both the districts may be the source of infections to other districts in Negeri Sembilan through the 'Pawah' scheme. Since the disease was not detected in the districts along the border of Negeri Sembilan, there is low probability that the disease wil spreading to the neighbouring states of Pahang and Selangor.

The RBPT and CFT are the most widely used tests for serological diagnosis of brucellosis in sheep and goats (MacMillan, 1990). The CFT was used as a confirmatory test for control and eradication programmes and are also recommended as serological tests prescribed for international trade (OIE, 2000). Studies have shown that there is possibility RBPT giving negative results with CFT positive when the infection is at the early stages or the animal had consumed colostrum. However, these animals were more than one-year old, thus colostrum consumption is not the likely reason. False negative can a result of improper storage of the RBPT antigen, because the antigen loses sensitivity at temperatures exceeding 4°C. In this project, the RBPT antigen used was properly stored, thus false positive result is not expected. Furthermore, one serum sample showed RBPT positive but CFT negative. This could be due to an antigen-antibody

cross-reaction from organisms sharing structurally similar genera such as *Yersinia enterocolytica* type 0.9.

To decrease the occurrence of brucellosis in Negeri Sembilan, continuous surveillance of the positive farms need to be carried out by the DVS as a control measure. This should be done routinely and the same time restricts and control animal movement. Awareness about brucellosis and its zoonotic potential can help farmers to understand the danger of the disease. This can make them more willing to volunteer for the disease surveillance and control programmes.

Table 3. Sensitivity and specificity of RBPT compared to CFT

	CFT +ve	CFT -ve	Total
RBPT +ve	6	1	7
RBPT -ve	13	751	764
Total	19	752	771

Sensitivity of RBPT= 32%; Specificity of RBPT= 99.9%

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test

+ve=positive; -ve=negative

Table 4. Sensitivity and specificity of CFT compared to RBPT

	RBPT +ve	RBPT -ve	Total
CFT +ve	6	13	19
CFT -ve	1	751	752
Total	7	764	771

Sensitivity of RBPT= 32%; Specificity of RBPT= 99.9%

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test

+ve=positive; -ve=negative

CONCLUSION

The serological detection of *B. melitensis* was successfully carried out by the RBPT and CFT. The prevalence of brucellosis in goats determined by the RBPT and CFT was 1.0% and 2.5%, respectively. These results reaffirmed what have been indicated previously that the CFT is more sensitive than the RBPT. The CFT which is used as a confirmatory test for the control or eradication programmes is also recommended as a test prescribed for international trade. This study also found that in Negeri Sembilan, only Rembau and Kuala Pilah were seropositive for brucellosis.

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IN VITRO EVALUATION OF NAPIER GRASS-OIL PALM FROND COMBINATION AS RUMINANT FEED

Wong Meng Joo, ¹Mohamed Ali Rajion, Leong Zi Ping, Mahdi Ebrahimi & ^{1,2}Goh Yong Meng

¹*Department of Veterinary Preclinical Sciences*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The effects of different combinations of Napier grass (*Pennisetum purpureum*) and oil palm (*Elaeis guineensis* Jacq) fronds on ruminal fermentation patterns *in vitro* in goats were investigated. Rumen liquor from three 2-year-old Kacang-crossbred goats was mixed with buffer and substrates. Four dietary treatments were compared namely 100% concentrates (CON), 50% OPF with 50% concentrates (OPF 50), 50% Napier grass with 50% concentrates (NP 50), and 25% Napier grass, 25% OPF and 50% concentrates (NP-OPF). Incubation of the mixture was carried out at 39°C for 24 h. Total gas production (GP) was recorded after 2, 4, 6, 8, 10, 12 and 24 h of incubation. Rumen fluid pH, methane gas, total volatile fatty acids and *in vitro* dry matter digestibility (IVDMD) were determined at the end of incubation. Long chain fatty acid (LCFA) profiles were obtained in separate runs to determine the apparent biohydrogenation (BH) of linoleic (C18:2n-6) and α -linolenic acids (C18:3n-3). Cumulative gas production was significantly higher for the CON group ($P < 0.05$) but not significantly different in the other groups. The NP 50 diet produced significantly higher methane ($P < 0.05$) while other groups did not differ significantly. For IVDMD, the NP-OPF group had a significantly higher digestibility than the NP 50 and OPF 50 groups. Rumen fluid pH, total VFA and apparent BH values for all treatments were not significantly different. In conclusion, the Napier-OPF combination represents a suitable feed for the small ruminant sector in Malaysia but more studies need to be done on effects of OPF on rumen biohydrogenation.

Keywords: *in vitro*, Napier grass, oil palm fronds, fermentation, biohydrogenation, goats

INTRODUCTION

The ruminant sector in Malaysia is not well developed, which is attributable to limited grazing grassland, suboptimum pasture quality and smallholder domination. Malaysia's food self-sufficiency did not show significant improvement in recent years. Malaysia produced only about 4.67% dairy products, 9.03% lamb/mutton and 24.07% beef (Abu Hassan *et al.*, 1993).

Napier grass (*Pennisetum purpureum*) has been shown to be the most adaptable and productive fodder grass under Malaysian conditions. Napier grass is an appropriate

perennial fodder recommended for intensively managed livestock systems. Cut-and-carry feeding management of the grass is also well practiced.

Malaysia is a leading country in palm oil production where about 3.87 million hectares of land was used for oil palm plantations. Oil palm (*Elaeis guineensis* Jacq) fronds are abundantly available agricultural byproducts, with around 26 million tonnes produced each year (Wan Zahari *et al.*, 2003). The OPF can be used as a source of alternative energy for ruminants. However, OPF cannot be used as sole feed due to its high lignin (205/kg DM) (Abdul Khalil *et al.*, 2006) and neutral detergent fiber content (700g/kg DM) (Ishida and Abu Hassan, 1997).

Combinations of Napier grass and OPF can be good ruminant feeds which may help solve the the feed insufficiency problem related to the low ruminant production level.

MATERIALS AND METHODS

Three 2 year-old fistulated goats (Kacang crossbred, male, 31.62 ± 1.66 kg bodyweight) were used. The animals were fed twice daily with a combination of OPF, Napier grass and concentrates at a 1:1:2 ratio at 2.5% of BW in dry matter throughout a 10-day adaptation period.

Rumen liquor was collected from rumen fistulated goats early in the morning pre-feeding, pooled and squeezed through four layers of cheese cloth. The rumen fluid/buffer solution was prepared by mixing the rumen fluid and phosphate and bicarbonate buffer.

Incubation of the rumen fluid/buffer was carried out at 38°C for 24 h. Gas production was recorded at 2, 4, 6, 8, 10, 12 and 24 hour of incubation. Rumen fluid pH, *in vitro* dry matter digestibility (IVDMD), methane production and total volatile fatty acids (VFA) were measured after 24 h incubation. The apparent biohydrogenation values of linoleic (18:2n-6) and α -linolenic acid (18:3n-3) were calculated according to Vlaeminck *et al.* (2008).

RESULT AND DISCUSSION

The effects of different feed combinations on cumulative gas production, methane production, IVDMD, rumen fluid pH and total VFA are presented in Table 1, Table 2, Table 3, Table 4 and Table 5, respectively. Apparent biohydrogenation for C18:2n-6 and C18:3n-3 is presented in Table 6.

The cumulative gas production for CON was significantly higher with no significant differences between the rest of the groups. In fact, The NP-OPF produced a higher gas production than OPF 50 and NP 50. This indicates that the NP-OPF combination is more fermentable than Napier grass or OPF alone.

Methane is produced by the reduction of carbon dioxide in the rumen, which cannot be further utilized and eventually removed by eructation. A higher methane production signifies higher energy losses by the animals. The NP 50 had yielded significantly higher methane gas than the CON. The groups that showed a significantly lower GP and IVDMD than group CON produced significantly higher methane. The levels of methane production in this study were comparable to reports by Navarro-Villa *et al.* (2011) who

found a negative relationship between *in vitro* methane output and amount dry matter degraded, i.e. there will be lower methane production in feed with better digestibility.

Table 1. Effect of different feed combinations on *in vitro* cumulative gas production

Treatment	Cumulative gas production (mL/g DM)
CON	148.67 ^a ± 5.98
OPF 50	85.85 ^b ± 4.32
NP 50	94.59 ^b ± 5.82
NP-OPF	96.97 ^b ± 4.46

All values are mean ± SE (n = 12)

* a, b, c values with different superscripts in a column differ significantly P < 0.05.

Table 2. Effect of different feed combinations on methane gas production

Treatment	Methane production (mL/g DM)
CON	6.53 ^b ± 0.48
OPF 50	7.08 ^{a,b} ± 0.47
NP 50	8.79 ^a ± 0.92
NP-OPF	7.24 ^{a,b} ± 0.58

All values are mean ± SE (n = 12)

a, b, c values with different superscripts within column differ significantly P < 0.05.

Table 3. Effect of different feed combinations on *in vitro* dry matter digestivity

Treatment	Digestibility (% DM)
CON	44.65 ^a ± 1.74
OPF 50	33.60 ^c ± 1.34
NP 50	34.53 ^{b,c} ± 2.70
NP-OPF	40.65 ^{a,b} ± 1.90

All values are mean ± SE (n = 12)

* a, b, c values with different superscripts within column differ significantly P < 0.05.

Table 4. Effect of different feed combinations on pH

Treatment	Digestibility (% DM)
CON	7.22 ± 0.02
OPF 50	7.24 ± 0.01
NP 50	7.24 ± 0.01
NP-OPF	7.24 ± 0.05

All values are mean ± SE (n = 12)

Table 5. Effect of different feed combinations on total volatile fatty acid

Treatment	Digestibility (% DM)
CON	24.91 ± 4.62
OPF 50	22.55 ± 4.78
NP 50	20.48 ± 4.85
NP-OPF	18.52 ± 4.89

All values are mean ± SE (n = 12)

Table 6. Effect of different feed combinations on apparent biohydrogenation

Treatment	Apparent Biohydrogenation	
	C18:2n-6	C18:3n-3
CON	0.75 ± 0.02	0.77 ± 0.06
OPF 50	0.70 ± 0.03	0.69 ± 0.05
NP 50	0.75 ± 0.03	0.78 ± 0.02
NP-OPF	0.68 ± 0.02	0.73 ± 0.03

All values are mean ± SE (n = 6)

The rumen fluid pH encountered ranged from 7.22 to 7.24 with no significant differences. The pH of the NP-OPF group was high and it was a good finding since various studies had suggested that a higher rumen pH will favour the growth and activity of cellulolytic bacteria, thereby enhancing fiber digestion, DM intake and growth performance (Zinn and Salinas, 1999).

In spite of the high apparent IVDMD and gas production observed, group NP-OPF yielded the lowest total VFA although there were no significant differences among all groups.

The treatment groups containing OPF had lower but non-significantly different apparent BH values than the other two groups. Previous studies showed that OPF contains tannin, which may favourably alter ruminal BH and promoting accumulation of healthy fatty acids. The lowered apparent BH values observed were probably due to the high tannin content of OPF. However, reports on the tannin effect on ruminal BH are few and contradictory. Khiaosa-Ard *et al.* (2009) had shown a positive effect on rumen vaccenic acid (VA) accumulation, while *in vivo* studies suggested non-significant or even negative effects (Benchaar and Chouinard, 2009).

The Napier grass-OPF combination can be a good ruminant feed since its use would reduce the farmer's feed costs and environmental waste, while producing even better results in certain aspects compared to the use of Napier grass and OPF alone.

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PRELIMINARY ASSESSMENT OF RAM'S HORN SNAIL (*GYRAULUS SP.*) AS LIVE FEED FOR COMMON CARP (*CYPRINUS CARPIO*)

Paramendra Duraisingam & ^{1,2}Hassan Hj. Mohd Daud

¹Department of Veterinary Clinical Studies

²Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Gyraulus sp. or Ram's horn snail (Figure 1) is a freshwater, air breathing snail of the family Planorbidae. It has a characteristic flat spiral shape shell in the shape of a Ram's horn. Snails from this family are hermaphrodites. They can be found abundantly in freshwater bodies such as lakes and ponds in Malaysia. Their diet consists of mainly of algae and dead plant matter. Many larger organisms such as water birds, turtles and freshwater fishes consume these snails as part of their normal diet. Currently, no studies have been conducted on the viability of this snail species as fish feed. Thus this study was conducted firstly to determine the protein content of the Ram's horn snail and its effects on the growth rate of common carp, an omnivorous freshwater fish and as an alternative source of fresh live-feed for freshwater fish aquaculture industry. Concurrently, the study also screen for the presence of parasites, fungus and pathogenic bacteria that may be carried by the snail. Three groups of ten carps, *Cyprinus carpio* fish were allocated into individual tanks supplied with individual filtration and aeration systems. Two groups of carps were fed Ram's horn snail at 5% body weight while the control group was fed commercial fish pellets at 5% body weight thrice daily. The growth rate of the fish was scored by measuring the weekly increment in length and body weights. A sample of whole snail was also sent for crude protein proximate analysis. Concurrently, the microbial burden of the snail was screened using the standard aerobic plate count (SPC) method from the snail's intestines. The bacteria colonies attained were sub-cultured and the species identified by biochemical tests. Samples of the snail were screened for parasites and fungus using squash smears preparations. This study showed that fish fed with Ram's horn snail had a daily growth rate of 0.8 to 1% body weight per day, while fish fed with commercial fish food had a daily growth rate of 1.6% body weight (Figure 2). The normal daily growth rate of common carp according to the FAO is 1.6% body weight. Proximate analysis done on the whole snail revealed a crude protein content of 11.3%. Bacteriology results revealed a bacterial content of $2.2 \text{ CFU} \times 10^6/\text{g}$ of intestinal tissues. *Aeromonas hydrophila* type II and *Pseudomonas oryzihabitans* were predominantly isolated from the snail's intestines. *A. hydrophila* is an opportunistic pathogen in fish that can cause 'motile aeromonas septicaemia' disease. However *A. hydrophila* and *P. oryzihabitans* are two of the most common bacteria in freshwater habitats throughout the world. Moreover, the experimental infective dose of *A. hydrophila*, type II was $1 \times 10^6 \text{ CFU/mL}$. No parasite or fungus was detected in the snail's body squash smears. No mortality associated with the feeding was recorded. In conclusion, the Ram's horn snail is cheap to culture, easily available, well-accepted by fish, had reasonably good protein content and could be incorporated into the diet of

common carp (cyprinids) and other species of cultured food and ornamental fishes without detrimental effect.

Keywords: Ram's horn snail, crude protein level, common carp, growth rate



Figure 1. Ram's horn snail (*Gyraulus* sp)

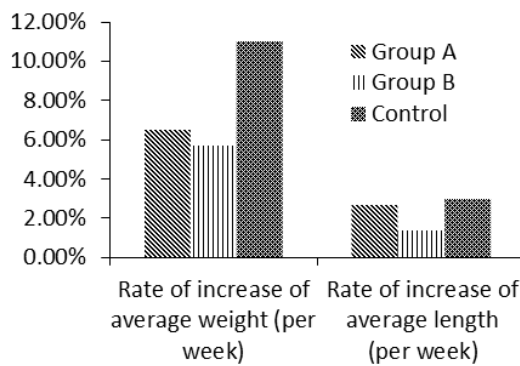


Figure 2. Growth rate of common carp fingerlings (average weight of 18.5 g) fed with Ram's Horn snail. Control group were fed with commercial fish pellets

POLYMERASE CHAIN REACTION DETECTION OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* IN MICE FOLLOWING ORAL INOCULATION WITH THE BACTERIA

**Ahmad Fauzan Muhdi,¹ Abdul Rahman Omar
& ^{2,3}Faez Firdaus Jesse Abdullah**

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

³*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Caseous lymphadenitis (CLA) is caused by aetiological agent *Corynebacterium pseudotuberculosis* which is an intracellular gram-positive, facultative anaerobic small curved rod bacteria. The infection of CLA may occur through the ingestion of contaminated food with the bacteria. Hence, this study was conducted to prove that the oral route will give same signs with other routes of transmission. This study was conducted to detect *C. pseudotuberculosis* using PCR from various organs in mice following oral route inoculation to demonstrate a natural route of the transmission of the bacteria in goat and sheep and the development of cheaper animal models to study *C. pseudotuberculosis*. A total of 8 mice were inoculated orally with the bacteria and 7 organs were collected for detection using PCR. PCR on DNA extracted using a pair of *C. pseudotuberculosis* specific primers was positive in some organs which were 3 samples of lymph node, 5 samples of brain and 1 sample of liver. Although the results in this study showed minimum detection of *C. pseudotuberculosis* in many organs, the PCR used in this study may successfully be applied for the detection and diagnosis of the bacteria in mice and oral inoculation or digestion transmission revealed presence of the bacteria and may produce effect in a chronic period.

Keywords: *Corynebacterium pseudotuberculosis*, PCR, oral inoculation

REPRODUCTIVE PERFORMANCE OF MURRAH CROSSED BUFFALO AT BUFFALO BREEDING CENTRE, TELUPID, SABAH

Ahmad Zulhizri Ahamad Suhaimi & ^{1,2}Mohd Zamri Saad

¹*Department of Veterinary Pathology & Microbiology*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

This study was conducted to determine the reproductive performance and the relationship between the rainfall pattern and the calf mortality of Murrah crossed buffalo managed in extensive farming system in Buffalo Breeding Centre Telupid Sabah. Retrospective data (2004-2011) of calving rate, pregnancy rate and calving interval were collected and analyzed using Microsoft Excel and SPSS software 16.0. Calf births and mortality data and the rainfall records from 2009 to 2011 were also collected and analyzed. The results showed that the mean calving rate was 25%; mean pregnancy rate was 43.6% and mean calving interval was 25.36 months. There was no significant difference detected between rainfall pattern and calf mortality although they were positively correlated ($r > 0$). Calf birth and mortality were highest during the rainy months each year (October to January). The buffalo also had a longer average calving interval of 740 days and this could be contributed by the nutrition provided and environmental condition of the farm (rainfall, ambient temperature) as well as the fertility of the bull and the dam themselves. It can be concluded that the buffalos in the extensive farm were not performing to their highest reproductive performance when compared with other reports on buffalo reproduction managed extensively.

Keywords: Murrah buffalo, calving interval, calf mortality, pregnancy rate, reproductive performance

QUANTITATIVE APPROACH IN SCREENING THE ANTIVIRAL PROPERTIES OF KANDIS HUTAN IN ANIMAL CELL CULTURE

Aziera Adnan,¹Zeenathul Nazariah Allaudin,³Sandy Loh Hwei San,
³Ting Kang Nee,¹Mohd Azmi Mohd Lila & ³Mohamed Ariff Omar

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Preclinical Sciences*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor

³*Faculty of Bioscience, University of Nottingham, 43500 Semenyih, Selangor Malaysia*

Abstract

This main aim of this study was to determine the concentration of Kandis Hutan leaf extracts that can inhibit the infectivity of pseudorabies virus (PrV) in Vero cells. The leaf extracts were crude and extracted with 3 organic solvents namely hexane, ethyl acetate and ethanol. The cytotoxic effect of extracts on Vero cells was assessed by both MTT assay and cell cytotoxicity scoring method. Two-fold serial dilutions of each extracts were prepared from the highest concentration of 1000µg/ml in 0.1% DMSO. For MTT assay, the highest cytotoxicity was found in the hexane extract ($CC_{50} < 1.25 \mu\text{g/mL}$), followed by ethyl acetate extract ($CC_{50} = 237.5 \mu\text{g/mL}$), whilst minimal cell cytotoxicity was observed in ethanol extracts ($CC_{50} = 555.0 \mu\text{g/mL}$). There was a significant correlation between cell scoring system and MTT assay in term of cell cytotoxicity whereby the least toxic was ethanol extracts, followed by ethyl acetate extract and the most toxic hexane extract. Four antiviral assays were conducted for each extracts, namely plaque reduction assay, cytopathic effect (CPE) reduction assay, inhibition assay and virucidal assay. The most promising result was obtained from the inhibition assay, in which ethyl acetate extracts produced 75% viral inhibition at 125 µg/mL concentration. In virucidal assay, both ethyl acetate and ethanol extracts produced 100% viral inhibition at 250 µg/mL. For plaque reduction assay, there was a significant dose dependent inhibition for ethyl acetate extract but not for ethanol extract and hexane extract. In comparison to CPE reduction assay, findings from plaque reduction assay showed better viral inhibition by ethyl acetate extract (47%) at concentration of 300 µg/mL. The estimated selectivity index (ESI) calculated from the inhibition assay showed the highest antiviral response by ethyl acetate extract (2.7) in comparison with ethanol extract (1.8) and hexane extract (0.1). Therefore, the most promising antiviral activity was produced by ethyl acetate extract which showed consistent viral inhibition in all tested antiviral assays. In contrary, hexane extracts showed the least antiviral efficacy among the tested extracts.

Keywords: pseudorabies virus (PrV), Kandis Hutan, antiviral assay, plaque reduction assay, inhibition assay, virucidal assay

**SEROLOGICAL PREVALENCE OF LEPTOSPIRAL INFECTION IN
CATTLE FROM LADANG ANGKAT
AND TAMAN PERTANIAN UNIVERSITI,
UNIVERSITI PUTRA MALAYSIA**

**Azlina Zulkapli,¹ Abdul Rani Bahaman,¹ Siti Khairani Bejo
& ^{2,3}Faez Firdaus Jesse Abdullah**

¹Department of Veterinary Pathology & Microbiology

²Department of Veterinary Clinical Studies

³Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract

The study was conducted to determine the serological prevalence of leptospiral infection in cattle from ladang angkat and Taman Pertanian Universiti, Universiti Putra Malaysia (UPM). Leptospirosis is known as an important disease in Malaysia which causes economic losses to the farmers as well as human infection. Previous studies revealed that there were high prevalence rates of leptospiral infection in Malaysia. One hundred fifty serum samples were collected from ladang angkat and Taman Pertanian Universiti, UPM. The samples were then tested for antibodies against 16 *Leptospira* serovars (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Djasiman, Hardjobovis, Hardjo, Hebdomadis, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Sejroe) using the microscopic agglutination test (MAT). Thirty (20%) of the 150 samples were positive on MAT. Of the 30 MAT-positive serum samples, 18 (12%) were positive against serovar Hardjo, 6 (4%) were positive against serovar Pomona, 2 (1.33%) were positive against serovars Bataviae and Autumnalis, and 1 (0.6%) was positive for serovars Celledoni, Hardjobovis, and Hebdomadis. Kedah-Kelantan breed was shown to have a high prevalence rate. Intensive system showed the highest prevalence rate when compared to the other management system. The age group of 1-2 years old also had the highest prevalence rate when compared to other age groups. Statistical analysis indicated a significant difference ($p < 0.05$) in serological prevalence among different breeds, farms, management systems, and age groups of cattle examined.

Keywords: Leptospirosis, cattle, ladang angkat, Taman Pertanian Universiti, MAT

**SELENIUM STATUS IN BEEF AND DAIRY CATTLE AT TAMAN
PERTANIAN UNIVERSITI, UNIVERSITI PUTRA MALAYSIA**

**Benny Gabriel Among, ^{1,2}Noordin Mohamed Mustapha
& ¹Hazilawati Hamzah**

¹Department of Veterinary Pathology & Microbiology

²Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Selenium (Se) nutrition forms an integral part of growth and immune function of man and animals. Deficiency of Se in cattle will inevitably lead to poor growth and mastitis causing an economic loss to a farm. This study was carried out to determine the level of Se in beef cattle and dairy cattle reared at the Taman Pertanian Universiti, Universiti Putra Malaysia. A total of 27 dairy and 36 beef cattle were randomly chosen in this study. The age of the cattle used ranged from 1 to 9 years old comprising of both sexes. Blood sample was collected into heparinized and plain tubes for the detection of glutathione peroxidase (GSH-Px) activity and aspartate aminotransferase (AST), creatine kinase (CK) and malonylaldehyde (MDA) concentrations, respectively. The activity of GSH-Px was detected using the dithio-bi-nitrobenzoic acid (DTNB) direct method assay. The concentrations of AST and CK were determined using standard diagnostic kits, while the MDA using the TBA (thio-barbituric acid) test. It was found that although no significant differences existed in the GSH-Px activity between breeds, their Se status were in the deficient state. This is in line with previous reports that the acidic soil of Malaysia renders low Se uptake by plants leading to deficiency state in grazing ruminants. The concentrations of AST and CK were higher in beef cattle due to much more overt muscular damage as the result of foraging and lack of supplementation compared to dairy cattle. It is suggested that diet of both breeds should be supplemented with Se to prevent such deficient status in order to increase the productivity of the farm.

Keywords: selenium, beef and dairy cattle, AST, CK, MDA, GSH-Px

THE EFFECTS OF MELOXICAM ON THE GROWTH AND VIABILITY OF MOUSE MAMMARY TUMOUR CELL LINES EMT6 AND 4T1

Cecilia Evelyn Cornelius & ¹Mohd Hezmee Mohd Noor

*¹Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

The *in vitro* growth of mouse mammary tumour cell lines EMT6 (benign type) and 4T1 (malignant type) were evaluated following treatment with three different doses of meloxicam. The viability and growth of these cells following treatment were determined via cell proliferation and live-dead assays. Cell proliferation assay for these cells showed the absorbance of treated cells was significantly ($p < 0.05$) higher than the control. The live-dead assay showed that the viability of the treated cells was significantly ($p < 0.05$) lower than the control for EMT6 cells while there was no significant difference for the 4T1 cells, however the viability of the treated cells was lower than the control. This study provides preliminary evidence that meloxicam may inhibit growth and viability of mouse mammary tumour cell lines EMT6 and 4T1.

Keywords: mouse mammary tumour, EMT6 and 4T1 cell lines, meloxicam, cyclooxygenase-2 (COX-2) enzyme

**EFFECTS OF INSULIN-LIKE GROWTH FACTOR-1 ON WEIGHT GAIN
IN WEANERS IN A PIG FARM,
PENANG, MALAYSIA**

Cheah Zi Herk, ¹Engku Azahan Engku Ahmed & ²Ooi Peck Toung

¹Department of Veterinary Preclinical Sciences

²Department of Veterinary Clinical Studies

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The aims of this study were to compare the effects of insulin-like growth factor-1 (IGF-1) on body weight, average daily gain, percentage of body weight gain, health status and faecal coliform counts in weaner pigs. Fifty commercial breed (Landrace and Duroc) weaner pigs were selected from a pig farm in Penang, Malaysia. The weaners were equally distributed into control and treatment groups. The control group was fed commercial diet while the treatment group fed commercial diet with IGF-I added. Body weights of weaners were recorded every 15 days. In this study, IGF-1 treatment group showed statistically significant ($p < 0.05$) improvement in body weight and average daily gain towards the end of the study (45 days). No significant difference in percentage body weight gain between control and treatment groups was observed. From the blood parameters, there was no sign of toxicity or infection in the control and treatment groups. The study showed that IGF-1 treated group had lower coliform count compared to controls, which indicates that IGF-1 may have beneficial effects on the digestive system of weaner pigs.

Keywords: insulin-like growth factor-1 (IGF-1), weaners, growth, faecal coliform count

EFFECT OF ENRICHMENT ON STRESS LEVEL OF LABORATORY RATS

Chia Kay Thuan, ¹Fuzina Nor Hussein & ¹Abdul Rahim Mutalib

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine

Univerisiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

In certain biomedical research protocols, there is a need to singly house experimental rats. Since rodents are classified as social animals in nature, housing rats singly will result in stress to the animals. The aim of this study was to determine the effect of enrichment to the stress leukogram, feed intake, weight gain and behavior of singly-housed rats as opposed to singly-housed rats without enrichment and pair-housed rats. Eighteen 5 weeks old, male Sprague-Dawley rats were randomly assigned to three groups; singly-housed without enrichment, singly-housed with enrichment (provided with enrichment nestlets), and pair-housed. The rats were acclimatized for 7 days before start of study. Blood samplings were done on days 0, 14 and 26 for complete blood and differential counts. Feed intake and weight gain were recorded every 4 days and on days 0, 14 and 26. Behavioral assessment was done every week for 10 minutes for each cage. Results showed significant neutropenia in the singly-housed rats with and without enrichment as opposed to neutrophilia in the pair-housed rats. Monocytosis and eosinophilia were also more evident in the singly-housed rats without enrichment than in either the pair-housed rats or singly-housed rats with enrichment. However, there was no significant difference in other blood parameters, feed intake and weight gain. Singly-housed rats without enrichment also showed significantly higher stereotypical behavior when compared to rats in the other two groups. Thus, it could be concluded that singly-housed rats did not show higher stress level than pair-housed rats in the short term. Enrichment nestlets too have been proven to be effective in reducing some degree stress, aggression and stereotypical behavior in singly-housed rats.

Keywords: Sprague-Dawley rats, enrichment, stress assessment

AN EVIDENCE-BASED ASSESSMENT OF VARIATION IN EQUINE HOOF CONFORMATIONS TO FARRIERY TECHNIQUES IN FEDERAL TERRITORY AND SELANGOR STABLES, MALAYSIA

Dayang Norhaizum Awang Kamaludin & ¹Bashir Ahmad Fateh Mohamed

*¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

Abstract

Equine hoof plays an important role in the performance of horses. There is minimal research done on hoof conformation in Malaysia. This study was carried out in 5 established stables in Federal Territory and Selangor, to determine how different farriery interventions could affect the conformation of the hoof, and to provide basic data on hoof measurements. A total of 54 actively shod and sound working horses were used to assess all limbs for hoof angle (HA), quarter width (QW), sole length (SL), frog length (FL) and width (FW), heel width (HW) and height (HH), toe length (TL), hoof pastern axis (HPA), sole concavity, and wear and tear of shoes. There were no significant differences in hoof assessments in horses managed by different farriers. However, this study showed that the common faults in farriery including low HA, long toes, low HH, and narrow frog. These farriery faults can eventually cause internal foot injuries and lameness in horses. Hence, proper farriery techniques should be implemented to produce sound and better performance horses.

Keywords: equine, hoof measurements, farriery techniques

MITRAGYNINE AS AN ANTHELMINTIC FOR CAPRINE STRONGYLES

Farah Hida Abdul Aziz,¹ Arifah Abdul Kadir & ^{2,3}Rehana Abdullah Sani

¹*Department of Veterinary Preclinical Sciences*

²*Department of Veterinary Pathology & Microbiology*

³*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Helminth infection is one of the most important causes of mortality and morbidity in small ruminant industries in Malaysia. Hence, helminth infection can lead to direct or indirect economic loss to farmers. Over the past 30 years, farmers have mainly relied on the use of commercial anthelmintics to control helminth infection. Unfortunately, heavy usage of the anthelmintics has led to the development of anthelmintic resistance. Therefore, an *in vitro* study was conducted to determine the anthelmintic properties of mitragynine (the major alkaloid in *M. speciosa*) against L3 stage larvae of strongyles. Five different concentrations of mitragynine were studied for their efficacy against L3 stage larvae of strongyles and five replicates for each concentration were prepared. The mortality was observed at 0, 2, 4, 6 and 24 hours post-treatment by observing the absence of motility of the larvae. The results revealed that the most effective and lowest effective concentrations of mitragynine within a 24 hour period were 0.4 and 0.2 mg/mL, respectively. Mitragynine also exhibited dose-dependent anthelmintic activity during the 24 hour period of observation.

Keywords: strongyles, anthelmintic, mitragynine

QUANTITATIVE *IN VITRO* ASSAYS FOR SCREENING ANTIVIRAL CHARACTERISTICS OF BEREMBANG BUKIT LEAF EXTRACTS

Farhah Zaidar Abdul Malik, ¹Zeenathul Nazariah Allaudin,
³Sandy Loh Hwei San, ³Ting Kang Nee, ¹Mohd Azmi Mohd Lila
& ²Mohamed Ariff Omar

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Preclinical Sciences*

Faculty of Veterinary Medicine

University Putra Malaysia, 43400UPM Serdang, Selangor

³*Faculty of Bioscience, University of Nottingham, 43500 Semenyih, Selangor Malaysia*

Abstract

Berembang Bukit or Megawasih, is a native of our tropical rainforest and other ASEAN countries. In a preliminary review, a qualitative screening on the antiviral activities of its leaf extracts revealed various degrees of antiviral potential. Therefore this study was done to evaluate its antiviral characteristics in a quantitative approach. The leaf extracts were prepared using hexane, ethyl acetate and ethanol extractions and dissolved in DMSO. DMSO cytotoxicity was initially evaluated to ensure a safe working concentration. A negligible cytotoxicity was observed at a concentration of $\leq 0.1\%$ DMSO. The cytotoxic effect of extracts on Vero cells was assessed by both MTT assay and cell cytotoxicity scoring method. Two-fold serial dilutions of each extracts were prepared from the highest concentration of 1000 $\mu\text{g/mL}$ in 0.1% DMSO. For MTT assay, the highest cytotoxicity was found in the ethyl acetate extract ($\text{CC}_{50} = 218\mu\text{g/mL}$), whilst minimal cell cytotoxicity was observed in both hexane ($\text{CC}_{50} = 833\mu\text{g/mL}$) and ethanol ($\text{CC}_{50} = >1000\mu\text{g/mL}$) extracts. However, there were no correlation between MTT and cell scoring for cytotoxicity in this study. A series of experiments including CPE reduction assay, plaque reduction assay, inhibition assay and virucidal assay were done to evaluate the total antiviral potential of the leaf extracts. The leaf extracts produced a dose-dependent antiviral response. Both ethyl acetate and ethanol extracts showed 100% plaque formation inhibition in plaque reduction assay, inhibition assay and virucidal assay. Hexane extracts showed absence of plaque inhibition in all the tested antiviral assays. In inhibition assay, the estimated selective index (ESI) for ethanol and ethyl acetate extracts were 8.3 and 1.9, respectively. Whilst in CPE reduction assay, the ESI for the respective extracts were 6.7 and 2.9. In conclusion, the ethanol extracts exhibited the highest antiviral efficacy among the tested extracts.

Keywords: antiviral assay, Berembang Bukit, plaque reduction assay, inhibition assay, virucidal assay

PATHOGENICITY OF AVIAN PATHOGENIC *ESCHERICHIA COLI* ISOLATES OF MALAYSIA IN SPECIFIC PATHOGEN FREE CHICKENS

Fida Fadzil & ¹Mohd Hair Bejo

¹*Department of Veterinary Pathology & Microbiology*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Avian pathogenic *Escherichia coli* (APEC) infections of poultry have been regarded to be a major infectious disease in birds and are economically important to poultry production worldwide. The infection is still widely believed to be opportunists or secondary to predisposing conditions. Current studies suggest that APEC is also potentially zoonotic. Existence of APEC in Malaysia is evident but its pathogenicity in poultry is still unknown. The objective of this study was to determine the pathogenicity of APEC of Malaysian isolates in specific pathogen free (SPF) chickens. Fifty-four, day-old SPF chicks were divided into three groups; A, B and C. Each group was further categorized into sacrificed and mortality subgroups. The chicks in sacrificed subgroup were used for sampling and those in the mortality subgroups were only observed for mortality with minimal handling. All chicks were fed an antibiotic-free diet and fresh water *ad libitum* throughout the study. Chicks in groups A and B were inoculated with 0.1 mL of 1×10^8 CFU APEC isolate A (UPM 1101) and APEC isolate B (UPM 1102), respectively whereby 0.05 mL 1×10^8 CFU each was given orally and intranasally. All chicks in group C were left uninoculated and served as controls. Three chicks were sacrificed prior to APEC inoculation. On days 1, 4, 7 and 14 post-inoculation (pi), three chicks each from the sacrificed subgroups of groups A, B and C were sacrificed. Body weights of the sacrificed chicks were recorded and cloacal swab, blood, and liver samples were taken for bacterial isolation and identification. The liver and trachea samples were also taken for histopathological examination. All chicks were observed for clinical signs and mortality throughout the trial. At necropsy, gross lesions were also examined. One dead chick (20%) was observed in mortality subgroup of group B. No mortality was recorded in mortality groups of groups A and C. Chicks in group A developed mild diarrhoea with faecal stains around cloacal area on day 4 pi, uneven body size distribution and ruffled feathers on day 14 pi. Chicks in group B showed moderate diarrhoea with faecal stains around cloacal area on day 4 pi, watery dark brownish diarrhoea, uneven body size distribution and ruffled feathers on day 14 pi. Chicks in group C remained healthy throughout the trial. There was no significant ($p > 0.05$) difference in mean body weight between the three groups. At necropsy, unabsorbed yolk was observed in all sacrificed chicks from groups A, B and C on day 1 pi. Unabsorbed yolk continued to be seen in chicks from group B on day 4 pi. Focal liver necrosis was observed in chicks from group A on day 7 pi. Histopathology revealed presence of lymphocytes and heterophils in submucosa of trachea of chicks from group B as early as day 1 pi and with mild deciliation on days 4, 7 and 14 pi. Lymphocytes and heterophils in submucosa of trachea were recorded in chicks from group C starting from day 4 pi. In group A, the presence of

lymphocytes and heterophils in submucosa of trachea with mild deciliation was observed on days 4, 7 and 14 pi. Area of degeneration and necrosis of liver were also observed on day 7 pi. It was concluded that the A (UPM 1101) and B (UPM 1102) APEC isolates of Malaysia are mildly and moderately pathogenic, respectively in SPF chicks.

Keywords: avian pathogenic *Escherichia coli* (APEC), specific pathogen free chickens (SPF), pathogenicity

HISTOPATHOLOGICAL EVALUATION OF VISCERAL ORGANS AND BRAIN OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO SUBLETHAL CONCENTRATIONS OF MALATHION

**Izzati Ali, ¹Intan Shameha Abdul Razak, ^{2,3}Hassan Hj. Mohd Daud
& ²Mohd Fuad Matori**

¹*Department of Veterinary Preclinical Sciences*

²*Department of Veterinary Clinical Studies*

³*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Organophosphate is a neurotoxin that inhibits acetylcholinesterase. Exposure to organophosphate toxicants can be through inhalation, absorption and ingestion of food treated with an organophosphate herbicide or insecticide. Malathion is among the most frequently used organophosphate insecticides. Malathion has been associated with reproductive malfunction, neurotoxicity, kidney and liver damage and birth defects. The objective of this study was to determine the effect of malathion on the catfish species *C. gariepinus*. One hundred and ten mature catfish, *C. gariepinus* with a body length of about 15cm were obtained from Taman Pertanian Universiti, Universiti Putra Malaysia. Prior to experimental trial, the fish were acclimatised for one week in a clean fibreglass tank provided with aeration, biophysical filtration and treated with Aqua-Med[®] to remove external bacteria and parasites. The fish were exposed to four different malathion concentrations of 1.6, 3.2, 6.4 and 8.5 ppm in a controlled laboratory condition. The LC₅₀ of malathion at 48 hours was determined to be 3.2 ppm. In the acute exposure study, 40 catfish with an average length of 18.0 ± 2 cm were divided equally into four tanks. Tank 1 contained the control group, where the fishes were bathed in dechlorinated water provided with aeration and biophysical water filter. The fish in the other three tanks were exposed to malathion concentrations of 0.8, 1.6 and 3.0 ppm for 48 hours. The fishes were then euthanized with an overdose of MS222 and their visceral organs viz., kidney and liver tissues were fixed in 10% buffered formalin and the brain tissue in Bouin's solution. All samples were histologically processed and stained with H & E stains. Histopathological evaluations were carried out using lesion scores of 0 to 3 and the significant differences were tested by one-way ANOVA. The kidneys of the malathion-treated group showed necrosis, infiltration of inflammatory cells, and capillary congestion while the controls did not. There was no difference (p>0.05) in kidney lesions between malathion-treated groups. Liver necrosis, infiltration of inflammatory cells, congestion and haemorrhage were evident in the malathion-treated group, which was greatest (p<0.05) in catfish treated with 1.6 ppm malathion. There was no difference (p>0.05) in other lesions among treated groups. The control catfish did not show any lesion. The malathion-treated catfish also showed brain necrosis, congestion and infiltration of inflammatory cell, which were not significantly (p>0.05) different among

treatment groups. The control group did not show any brain lesion. The study showed that at concentrations below LD₅₀ and exposure time of 48 hours, malathion is moderately toxic to *C. gariepinus*.

Keywords: malathion, visceral organs, histopathology, *Clarias gariepinus*

ROLE OF HEAT STRESS IN RED TILAPIA STREPTOCOCCOSIS

Jahwarhar Nazrin, ¹Md Sabri Mohd Yusoff, & ¹Noraini Omar

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Streptococcus agalactiae is one of the causative agents associated with warm-water streptococcosis that produces massive mortality in aquaculture. The emergence of disease in tilapia farms usually occurs during high temperature seasons, which suggested higher susceptibility of tilapia to infection under this condition. Thus, the objectives of this study were to determine the pathogenesis of streptococcosis in heat-stressed tilapia using various routes of infection and the role heat stress in the development of streptococcal infection in tilapia. Red tilapias, including the control group without heat stress, were inoculated with 10⁹ CFU/mL of *S. agalactiae* via intraperitoneal, immersion and immersion cut routes of inoculations and maintained at a water temperature of 34°C for 24 hours. Samples of brain, eyes and kidneys were taken and subjected to bacterial isolation, PCR, histological examination and immunoperoxidase test. Diseased fish showed typical signs of bacterial septicaemia including skin and fin haemorrhage and exophthalmia. The fishes were more susceptible to intraperitoneal route of infection, followed by immersion cut and lastly immersion. The bacteria was isolated and detected by PCR from all organs of fishes with and without heat stress. The lesions were more clearly seen first in fishes with heat-stressed than those without. Fifty percent mortality occurred in the heat-stressed group infected via intraperitoneal route. However, no mortality was observed in the group without heat stress. Post-mortem revealed that the lesions were more severe in the heat-stressed group infected via the intraperitoneal route than those infected via the immersion cut and immersion routes. The lesions observed were haemorrhage, presence of inflammatory cells and bacteria in the brain, eyes and kidneys. There is significant ($p < 0.05$) difference between route of infection and heat stress in most of the organs. However there is no significant ($p > 0.05$) difference was observed between routes of infection in most organs of fishes without heat stress. Immunoperoxidase test were positive in most organs. However, the intensity of the antigen-antibody reactions were greatest in the group infected via the intraperitoneal route followed by immersion cut and immersion groups. In conclusion, the severity of lesions observed in the brain, eye and kidneys are most marked in heat-stressed red tilapias infected with *S. agalactiae* via the intraperitoneal route, followed by the immersion cut and lastly the immersion route.

Keywords: Red tilapia, *Streptococcus agalactiae*, heat stress

PREVALENCE, GROSS AND HISTOPATHOLOGICAL FINDINGS IN THE LUNGS OF SUSPECTED HEARTWORM INFECTED CATS IN THE KLANG VALLEY, MALAYSIA

Julida Jalil, ¹Malaika Watanabe & ^{2,3}Noordin Mohamed Mustapha

¹Department of Veterinary Clinical Studies

²Department of Veterinary Pathology & Microbiology

³Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Feline heartworm disease is a potentially life-threatening disease of cats that most commonly manifests as heartworm-associated respiratory disease (HARD). Unlike canine heartworm disease, antemortem diagnosis is difficult due to low worm burdens, frequency of all male infections, and non-specific radiographic lesions. Thus, a combination of tests must be carried out and interpreted with care in order to make an accurate diagnosis of feline heartworm disease. Feline heartworm disease has yet to be studied in Malaysia, therefore there was a dire need to investigate and determine the prevalence rate of this disease in the country. Fifty cats comprising 14 strays and 36 pets were included in this study. Serological tests for antigen and antibody (SNAP® IDEXX and Heska ® Solo Step® FH respectively), was performed for all the samples. Knott's concentration technique, thoracic radiography, followed by necropsy and histopathology were also carried out for the stray cats. Feline heartworm disease was not detected following Knott's concentration technique or serological testing in the stray cat population. None of the pet cats were positive for feline heartworm disease following serological testing. The most common thoracic radiographic findings in the stray cats were interstitial and bronchoalveolar patterns of the lungs. The most common findings upon necropsy and histopathology included pulmonary oedema and pulmonary congestion and haemorrhage. Since there were no positive feline heartworm disease cases detected, no associations could be made between the radiographic and histopathologic findings and feline heartworm disease. The overall prevalence rate of feline heartworm disease in the Klang Valley, Malaysia was 0%.

Keywords: heartworm, lungs, feline, heartworm-associated respiratory disease (HARD), diagnosis

SEROPREVALENCE OF NEOSPOROSIS IN CATTLE AND ITS ASSOCIATED RISK FACTORS IN SELECTED FARMS IN SELANGOR, MALAYSIA

Juwairiyah Haji Jali & ^{1,2}Abd. Wahid Haron

¹*Department of Veterinary Clinical Studies*

²*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Neospora caninum is a protozoan parasite causing abortion and neonate mortality in cattle. It was discovered in 1988 and has been reported to be a major cause of abortion in many countries. Worldwide seroprevalence of neosporosis range from as low as 1 to over 90%. In Malaysia, a seroprevalence study has only been done in one farm. This preliminary study is conducted to determine the seroprevalence of neosporosis and risk factors associated with neosporosis in different farms in Selangor, Malaysia. One hundred and eighty serum samples were obtained from 9 different cattle herds from 8 farms. The samples were tested for the presence of *N. caninum* antibodies using the enzyme-linked immunosorbent assay (ELISA). A questionnaire was used to obtain information on the farm for the identification of risk factors associated with neosporosis. The association between the risk factors and neosporosis were evaluated using chi-square analysis. Seropositivity was observed in 2.8% (5/180) of the samples, which came from 3 farms. Chi-square analysis revealed that none of the risk factors is significantly associated with neosporosis. Although a seroprevalence status has been established in Selangor, further investigation on the epidemiology aspects of this disease is required.

Keywords: neosporosis, seroprevalence, cattle, ELISA, risk factors

IN VITRO EQUINE CAECAL FERMENTATION CHARACTERISTICS OF OIL PALM FRONDS

Koay Chen Phing & ^{1,2}Goh Yong Meng

¹*Department of Veterinary Preclinical Sciences*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Horses are non-ruminant herbivores that require forage in their diet. They depend on the microbial digestion in the caecum for nutritional needs. Therefore it is imperative that equine feeds have the necessary characteristics that promote healthy caecal digestion and fulfill the equine nutrition needs at a reasonable cost. Currently, the available equine feeds are costly. Oil palm fronds (OPF), being readily available locally and cheaper, is a good candidate to be considered for inclusion in equine feed formulation. Extensive studies on OPF as ruminant roughage has been carried out but there is scarce information on OPF utilization in the equine feed. The aims of this study were to investigate the dry matter (DM) digestibility of OPF in caecal content derived from horses based on the volatile fatty acids (VFA) production, protein digestion, total gas production and caecal pH changes in an *in vitro* model. Caecal content from a euthanised horse was used to digest the dried feed samples over 24 hours in a closed system syringes. Eight feed samples comprising oil palm fronds (OPF), alfalfa (*Medicago*) hay, timothy (*Phleumpratense*) hay, oaten chaffs, grains mixture and premix pellets: concentrate J (Equi-Jewel[®]), concentrate L (Pegasus[™]), concentrate P (Pegasus[™]) and concentrate S (Mitavite[®]) were used. The products of digestion measured were gas production and DM disappearance to determine the fermentability of feed, total VFA concentration as it is a portion of energy source, pH changes which affect the gastrointestinal flora habitat and ammonia nitrogen concentration to indicate protein digestion. Oil palm fronds had comparable ($p > 0.05$) DM digestibility ($30 \pm 2.93\%$) to commercial feeds evaluated in this experiment. However, it had significantly ($p < 0.05$) lower total VFA concentration (9.3 ± 0.67 mM), and greatest (51 ± 8.74 ppm) ammonia nitrogen concentration ($p < 0.05$) vis-à-vis other diets in this trial. The OPF yielded higher acetic acid to propionic acid proportion consistent in horses fed mainly with forage diets. In addition to that, OPF showed significantly ($p < 0.05$) better buffering ability (pH: 7.4 ± 0.03) to caecal content and least gas production ($p < 0.05$). The *in vitro* model managed to mimic the cellulolytic digestion of an equine caecum, which is crucial to understand digestion dynamics of various feeds in the equine caecum. In summary, the OPF feed showed to be a low cost feed with suitable characteristics to be included in equine diet.

Keywords: caecal fermentation, horse, oil palm fronds, *in vitro* digestibility, volatile fatty acids

IMMUNOTOXICITY OF PYRENE AND PHENANTHRENE IN EMBRYONATED CHICKEN EGGS

Koh Ee Zhan & ^{1,2}Noordin Mohamed Mustapha

¹*Department of Veterinary Pathology & Microbiology*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Pyrene and phenanthrene are members of polycyclic aromatic hydrocarbons (PAHs) which are ubiquitous environmental pollutant as a result of incomplete combustion of organic matter. Many PAHs are highly toxic, teratogenic, mutagenic, carcinogenic and immunosuppressive to animals and humans. However, toxicity data for pyrene (Py) and phenanthrene (Ph) are still limited especially for the concerns of the poultry industry. This study aimed to investigate the effects of Py and Ph in inducing immunotoxicity to the chicken embryos. Fifty 10-day-old embryonated chicken eggs were divided randomly into control, Py and Ph groups. The Py and Ph were inoculated into the embryonated chicken eggs at a dose of 15 mg/kg via the allantoic route, while the control group remained non-inoculated. All embryonated chicken eggs were incubated at 37°C and candled daily to observe for the embryonic mortality. After 11 days of incubation, sera were collected from the day-old chicks and the yolks collected from the dead embryos for the haemagglutinin-inhibition (HI) test to determine the Newcastle disease (ND) antibody titer. Then, all chick embryos were necropsied and lymphoid organs including thymus, spleen and bursa of Fabricius collected for histopathology examination. The chickens from the Py and Ph groups showed severe lymphocyte depletion in the thymus, spleen and bursa of Fabricius. Such changes in the lymphoid organs had led to decrease in antibody production in the treated chicks. The controls were not affected. This study showed that Py and Ph were able to cross the allantoic barrier or *in ovo* and be transferred to the chick embryos to induce immunosuppression by damaging the lymphoid organs.

Keywords: pyrene, phenanthrene, polycyclic aromatic hydrocarbons (PAHs), immunotoxicity, chick embryo

ANTIBIOTIC SUSCEPTIBILITY OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM ANIMALS

Maxine Chin Ying Sii & ¹Siti Khairani Bejo

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Klebsiella pneumoniae is an important opportunistic pathogen and a frequent cause of nosocomial infections. The bacteria are responsible for a variety of diseases in humans and animals. This study was conducted to determine the antibiotic susceptibility of *K. pneumoniae* isolates to twelve antibiotics. Forty-two isolates which were isolated between 2007 and 2012 by the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, were used in this study. These bacterial isolates were obtained from various animals including horses, cats, dogs, goats, avian species, gaur, cattle, exotic animals and wildlife. The samples included swab (18), organs (18), faeces (3) and urine (3). Isolates were subcultured and confirmed as *K. pneumoniae* using standard microbiology techniques. *Klebsiella pneumoniae* isolates were further subjected to antibiotic susceptibility testing using the Kirby-Bauer method. The susceptibilities of *K. pneumoniae* to amoxicillin-clavulanic acid, ampicillin, cephalothin, kenamycin, gentamicin, streptomycin, ciprofloxacin, tetracycline, doxycycline, trimethoprim-sulphamethoxazole, erythromycin and chloramphenicol were determined. *Klebsiella pneumoniae* was found to be highly resistant to erythromycin (98%) and ampicillin (95%) while it is moderately resistant to cephalothin (55%). Although 60% (25 of 42) of *K. pneumoniae* isolates were multidrug-resistant, the majority of isolates were sensitive to amoxicillin-clavulanic acid, gentamicin and ciprofloxacin with the same sensitivity rates of 71%.

Keywords: *Klebsiella pneumoniae*, nosocomial infections, Kirby-Bauer method, multi drug-resistance

EFFECT OF *ESCHERICHIA COLI* VACCINATION IN GILTS ON PIGLET PERFORMANCES IN A FARM IN PERAK, MALAYSIA

**Michelle Fong Wai Cheng,¹Ooi Peck Toung, Choo Pow Yoon
& ¹Ong Bee Lee**

*¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

This study aimed to determine the effect of *Escherichia coli* (Neocolipor vaccine – Merial) vaccination on neonatal diarrhoea score, growth parameters (average weight per piglet and average daily gain) and neonatal mortality rate in newborn piglets. A field trial was conducted in 35 litters of piglets from gilts selected from a farm in Perak, Malaysia. They were randomly allocated into treatment (16 litters from *E. coli* vaccinated gilts) and control (19 litters) groups. Body weights of the piglets were determined at days 1, 7, 14 and 21 of age and the episodes of diarrhoea and piglet mortality were monitored daily in each pen. The treatment group had significantly lower neonatal mortality rate, day-one neonatal diarrhoea percentage and diarrhoea in the overall period of 1- 21 days ($p<0.05$) when compared to the control group. However, there were no significant ($p>0.05$) difference in the overall diarrhoea percentages and weekly growth parameters between groups. Some of the limitations of the study include environmental stress, routine and random treatment of piglets with diarrhoea with antimicrobial and biasness towards the control groups, which thereafter may have affected the significance of the diarrhoea score and growth parameters. It is presumed that piglets in the control group experienced severe diarrhoea during the first week of life, leading to the significantly ($p<0.05$) higher mortality rates. It is also deduced that piglets of the treatment group with diarrhoea that survived the first week have lower growth rates. However, in terms of neonatal mortality, the result is reproducible as it is in agreement with other field trials, which indicate that *E. coli* vaccination in dams could be an alternative way of moderating mortality due to *E. coli*. In conclusion, this pilot study showed that *E. coli* vaccination in gilts significantly reduces first week piglet mortality and diarrhoeal percentages at day-one under farm conditions.

Keywords: *Escherichia coli*, diarrhoea score, mortality rate, growth parameters, body weight, average daily gain

ANTINOCICEPTIVE EFFECT OF *CRICA PAPAYA* L. LEAF AQUEOUS EXTRACT IN MICE

**Mohd Hafez Bahadom, Nor ‘Adilah Makhtar
& ¹Wan Mastura Shaik Mossadeq**

*¹Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

Papaya or *Carica papaya* L. is a type of herbaceous plant that is widely distributed around the world, mainly in the tropic and subtropic regions. It is a large perennial herb with rapid growth rate and interestingly, the male and female parts exist in different trees. The different parts of *Carica papaya* L. such as the fruit/pulp, leaves, seed, flower, latex and root are used as traditional medicine in many countries around the world. Recent studies showed that papaya leaves exhibited antitumor and anti-inflammatory properties but no scientific studies were carried out to assess the possibility of papaya leaves as potential analgesic agent. Hence, this study was conducted to investigate the antinociceptive property of papaya leaf aqueous extract (CPLA) in mice. The analgesic activity of CPLA was evaluated using the acetic acid-induced writhing test while the effect of extract on motor coordination and fatigue resistance in mice was assessed using the Rotarod performance test. Results showed that the intraperitoneal administration of extract (30, 100 and 300 mg/kg) significantly reduced the number of abdominal constrictions without inducing deficit in the motor activity of mice ($p < 0.05$). In conclusion, *Carica papaya* L. leaf aqueous extract exerted antinociceptive activity by acting through the peripheral nervous system without altering the motor coordination and balance in mice.

Keywords: *Carica papaya* L. leaf, aqueous extract, antinociceptive

STATUS AND EFFECT OF FASCIOLIASIS IN BUFFALOES KEPT EXTENSIVELY IN A FARM IN SABAH, MALAYSIA

**Mohd Ridzuan Noorzahari,^{1,2} Mohd Zamri Saad
& ^{1,2}Rehana Abdullah Sani**

¹*Department of Veterinary Pathology & Microbiology*

²*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

This study was carried out to determine the status and possible effect of fascioliasis among buffaloes kept extensively in the Buffalo Breeding and Research Centre at Telupid, Sabah, Malaysia. Forty adult four-year old buffaloes from a herd of 250 animals were selected for the study. Faecal samples were obtained from the animals and subjected to sedimentation technique to determine the presence of *Fasciola* sp. ova. Blood sample from each animal was collected into a plain tube and serum obtained for determination of AST and albumin concentrations. The helminth burden was determined in these animals to obtain information on the prevalence of gastrointestinal helminthiasis in the farm. None of the faecal samples had *Fasciola* sp. ova. However, the serum ALT concentrations in these animal were significantly ($p < 0.05$) higher than the upper limit of the reference range. The study suggests that that the buffaloes may have been exposed to diseases that caused liver damage.

Keyword: fascioliasis, AST, albumin

**POLYMERASE CHAIN REACTION DETECTION OF *PASTEURELLAMULTOCIDA*
TYPE B IN MICE FOLLOWING ORAL INOCULATION WITH THE
BACTERIA**

**Mohd Syamil Mohd Yusof, ¹Abdul Rahman Omar
& ^{2,3}Faez Firdaus Jesse Abdullah**

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

³*Ruminant Diseases Research Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract

Pasteurella multocida type B is an etiological agent of haemorrhagic septicaemia in cattle and buffaloes. The disease is commonly fatal and considered as one of the most economically important cattle disease in Southeast Asia including Malaysia. This study described the detection of *P. Multocida* type B using Polymerase Chain Reaction (PCR) from the organs of the mice inoculated orally with the bacteria. In this study, 16 male and healthy mice aged 3-weeks were selected; they were divided into two equal groups of 8 mice each. Mice in group 1 were inoculated orally with 10⁹ CFU of *P. multocida* type B, while the mice from group 2 were inoculated orally with PBS, pH 7. The mice were observed for 5 days (120 hours). Post-mortem was conducted on mice which died within 5 days of the experimental period and organs such as heart, lung liver, spleen, stomach, small intestine and large intestine were collected and subjected to isolation and identification of the bacteria on blood agar. The organs collected from the mice showed positive results using PCR which detected *P. multocida* type B. On the contrary, *P. multocida* type B was not isolated from the organs of the surviving mice which were culled at the end of the experiment.

Keywords: *Pasteurella multocida* type B, oral inoculation, polymerase chain reaction (PCR), mice

PREVALENCE AND RISK FACTORS OF CASEOUS LYMPHADENITIS IN GOATS FROM SELECTED FARMS IN SELANGOR, MALAYSIA

Muhamad Shahrman Ismail, ¹Siti Zubaidah Ramanoon & ¹Ong Bee Lee

*¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

Caseous Lymphadenitis (CLA) is not well-studied in Malaysia despite the fact that the disease is common in goats. Therefore, this study was conducted to determine the prevalence and the risk factors for CLA in goats from eight selected farms in Selangor, Malaysia. In total, 159 serum samples were collected once via jugular venipuncture and subjected to the agar gel immune-diffusion (AGID) test to estimate the prevalence of CLA. Information on the potential risk factors for CLA at the animal and farm levels was collected through a questionnaire. The prevalence of CLA was estimated at 28% in goats and 75% in farms in Selangor. Goats with body condition score (BCS) of lower than 2, older than one year old, and female were significantly associated with higher prevalence of CLA ($P < 0.05$). Eighty-eight percent (7/8) of farms were under intensive system, 63% (5/8) had less than three workers, 63% (5/8) with history of CLA, 50% (4/8) were at least 6 years old, 50% (4/8) had more than 150 animals; 50% had male:female of 1:11-20, 50% had isolation pens, 13% (1/8) had sharp objects in pens, all had perimeter fence, 50% practised farm quarantine, 38% (3/8) had added new animals, 25% (2/8) imported new animals, 13% (1/8) had foot-dip, 75% (6/8) had treated cases of CLA, 75% had deworming programmes, and none practiced CLA vaccination programme in their farms. In conclusion, the high prevalence of CLA in goats from the selected farms in Selangor that could be due to poor management practices, lack of biosecurity measures and vaccination programmes. Therefore, these factors should be taken into consideration when formulating programmes for effective prevention and control of CLA in goats.

Keywords: goat, caseous lymphadenitis, agar gel immunodiffusion test, questionnaire, biosecurity

IN VITRO EVALUATION OF ANTIVIRAL PROPERTIES OF EDIBLE BIRD NEST EXTRACT AGAINST FELINE INFECTIOUS PERITONITIS VIRUS

Muhammad Daud Derwin Sepiri, ¹Siti Suri Arshad, Parvaneh Mehrbod, ¹Mohd Kamaruddin Awang Isa, Faruku Bande & Fadzilah A'ini Abdul Kadir

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine,

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

An *in vitro* study was carried out to evaluate the antiviral properties of edible bird nest extract (BNE) against feline infectious peritonitis virus (FIPV). Cytotoxicity assay was conducted towards BNE test material in Crandell Feline Kidney (CrFK) cells using MTT assay to determine the 50% cytotoxic concentration (CC₅₀) values. For antiviral test, three treatments were used to determine the antiviral inhibition effect by BNE extract. Co-treatment [(V+E) +C] was done by mixing the virus(V) and extract(E) together before inoculating into cells(C). Pre-treatment [(E+C) +V], involved treatment of extract before inoculation of the cells with virus. Post-treatment [(V+C) +E] was done by inoculating the virus first into the cells before inoculation of extract. Ten-fold dilutions of BNE were used to determine the CC₅₀ until 8th doubling dilutions. The FIPV dose was fixed for 100TCID₅₀. Cytotoxicity assay showed that all concentrations could be used for antiviral assay except for the stock solution. The results also showed that the extract was non-toxic to the cells. For antiviral assay, all treatments showed inhibitory effects on virus multiplication in the cells where pre-treatment showed the highest effect compared to the other two treatments. However, the finding was not statistically significant from the control treatment groups (P<0.05).

Keywords: bird nest extract, FIPV, CC₅₀, TCID₅₀, antiviral assay, MTT assay, inhibitory effect

COMPARISON OF ANAESTHETIC EFFECT OF KETAMINE-MEDETOMIDINE, KETAMINE-MEDETOMIDINE-MORPHINE AND KETAMINE-MEDETOMIDINE-TRAMADOL IN RED-EARED SLIDERS (*TRACHEMYS SCRIPTA ELEGANS*)

Muhd Taqiyudin Zainal Ulum,¹ Arifah Abdul Kadir,² Chen Hui Cheng,

³Hazilawati Hamzah & ⁴Choy Foon Seng

¹*Department of Veterinary Preclinical Sciences*

²*Department of Veterinary Clinical Studies*

³*Department of Veterinary Pathology & Microbiology*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor

⁴*Taipung Zoo and Night Safari, 34000 Taiping, Perak*

Malaysia

Abstract

Anaesthesia in chelonian species has always been a challenge for most veterinarians due to slow metabolism of anaesthetic drugs and difficulty in obtaining vascular access. Combination of ketamine-medetomidine can be administered intramuscularly and the effect can be antagonized with atipamezole. Morphine and tramadol in chelonians was found to provide antinociceptive effect, but with profound respiratory depression. Therefore, the objective of this study was to evaluate anaesthetic effects of ketamine-medetomidine (KM), ketamine-medetomidine-morphine (KMM) and ketamine-medetomidine-tramadol (KMT) in the red-eared sliders (*Trachemys scripta elegans*). Six red-eared sliders were randomly divided into three groups. Using a 3X3 Latin square crossover design, each group of animals was subjected to all the three treatment protocols that were KM, KMM, and KMT, with two weeks wash-out period in between each of the treatment protocol. Anaesthesia parameters such as heart rate, palpebral reflex, muscle relaxation, jaw tone and ease of intubation were accessed for 60 minutes. Blood was sampled via the subcarapacial sinus for pre- and post-anaesthesia complete blood count and serum biochemical analyses. All the three treatment protocols rendered the turtles to reach a level of anaesthesia that was sufficient for endotracheal intubation procedure. There were no significant differences in heart rate, muscle relaxation and palpebral reflex score for the three treatment protocols. Treatment with KMM resulted in significantly prolonged return of spontaneous breathing after reversal with atipamezole ($p < 0.05$) as compared to KM and KMT. Treatment with KMM also resulted in prolonged recovery while KMT showed incidences of renarcotization. In conclusion, KM is the drug combination of choice to induce general anaesthesia in chelonians. For invasive procedures, opioid in combination with KM may be considered with close monitoring of post-anaesthesia.

Keyword: ketamine-medetomidine, morphine, tramadol, red-eared sliders

A RETROSPECTIVE STUDY OF CANINE MONOCYTIC EHRlichiosis IN UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

Ng Che Giap, ¹Hazilawati Hamzah & ²Malaika Watanabe

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The purpose of this retrospective study was to determine the risk factors and clinical disorders of canine monocytic ehrlichiosis at University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). Medical records of dogs admitted to the UVH from year 2007 to 2011 were reviewed and the sole inclusion criterion was dogs tested for *E. canis* antibodies using dot-ELISA, Immunocomb® Canine Ehrlichia Antibody Test kit. Data on signalment (age, sex and breed), occupation of dog (police and non-police), preventive healthcare status, Immunocomb® antibody titer, tick infestation, clinical and clinicopathological findings were obtained. Over the 5 years, only 27 dogs admitted to UVH tested for *E. canis* antibody. Twenty-two dogs were *E. canis* seropositive while the remaining 5 dogs were *E. canis* seronegative. No statistically significant association was found between *E. canis* seropositivity and sex, age, breeds, occupation of dogs and tick infestation. The most common clinical findings were anorexia, lymphadenopathy, pyrexia, lethargy, ocular signs and splenomegaly. Thrombocytopenia, lymphopenia, anaemia (mainly non-regenerative anaemia), hypoalbuminaemia and hyperglobulinaemia were the predominant clinicopathological abnormalities. *E. canis* seropositive dogs were at risk to develop thrombocytopenia and lymphopenia ($p < 0.05$).

Keywords: canine monocytic ehrlichiosis, *E. canis*, retrospective study, thrombocytopenia

OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SP. IN SNAKES IN SINGAPORE

Ng Wen Chee, ¹Zunita Zakaria & ¹Rasedee Abdullah

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Snakes are known to be asymptomatic *Salmonella* carriers that can be a source of infections to humans. Treatment of salmonellosis may become complicated with the rise of the antibiotic resistance among the *Salmonella* strains. A study was conducted to determine the prevalence of *Salmonella* and its resistance towards antibiotics in snakes at the Singapore Zoo. Cloacal samples were collected from snakes at the zoo and were subjected to isolation and identification of *Salmonella* sp. and its resistance to commonly used antimicrobial agents. *Salmonella* sp. was present in 20 (65%) of 31 snakes sampled. A total of 9 different serovars were found, and the predominant serovars were *S. Mountpleasani* (15%) and *S. Cerro* (15%); followed by *S. Rissen* (10%), *S. Lohbruegge* (10%), *S. Lansing* (5%), *S. Hvitvingfoss* (5%), *S. Sachsenwald* (5%), *S. Pomona* (5%) and *S. Lindern* (5%). Twenty percent were unknown *Salmonella* serovars. The antibiotic susceptibility test revealed that all serotypes except for one were susceptible to six different antibiotics tested which included enrofloxacin (100%), marbofloxacin (100%), ceftiofur (100%), cephalexin (100%), chloramphenicol (95%) and amoxicillin (95%). The only serotype that was not susceptible to antibiotics was *S. Lohbruegge*. It was isolated from a corn snake and has an intermediate sensitivity towards chloramphenicol while resistant towards amoxicillin. The study showed snakes are infected with bacteria that could potentially transmit the infection to handlers and visitors. Thus precaution is advised when handling snakes.

Keywords: *Salmonella* sp., snakes, prevalence, antibiotic resistance

INVESTIGATION OF MORTALITY IN *MACROBRACHIUM ROSENBERGII* LARVAE ASSOCIATED WITH HATCHERY MANAGEMENT AT UNIVERSITI PUTRA MALAYSIA MARINE SCIENCE RESEARCH CENTRE, PORT DICKSON, MALAYSIA

Noorashimah Roslim & ^{1,2}Hassan Hj. Mohd Daud

¹Department of Veterinary Clinical Studies

²Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Since the middle of year 2011, high mortalities in *Macrobrachium rosenbergii* larvae at Universiti Putra Malaysia Marine Science Research Centre, Port Dickson, Malaysia were regularly observed, which had caused severe economic losses. This research was carried out to investigate the biological and non-biological factors in the hatchery that contributed to disease development in order to postulate the relationship of the factors involved in larvae mortality and to examine the appropriate culture management approach to reduce mortality. Six experimental trials were carried out, namely physical examination of water quality parameters in the hatchery, microbiological studies of culture water, larvae, feed (including *Artemia nauplii*, egg custard, and blood cockles), and parasitological and mycological studies of culture water and larvae in hatcheries. The samples were taken twice a week. Physical quality of water was checked twice a day, in morning and evening using portable hand-held water quality kit. For bacteriology study, the isolation of bacteria were done using TSA and TCBS agar and identification using commercial test kits, BD BBL Crystal™ and API 20E™. Methylene blue stain was used for routine parasitology and mycology studies. The water quality in all tanks were within the recommended range, where the temperature was between 28 - 31°C for larvae and 27 - 32°C for brood stock. The pH for brood stock culture was between 7 - 7.2 while for the larvae it was between 7.2 - 7.4. The salinity ranged between 12 - 16 ppt for larvae and 3 - 5 ppt for brood stock. Two main bacteria were isolated during this study, which were *Aeromonas hydrophila* type II and *Bukholderia cepaciae*. *A. hydrophila* type II, *B. cepaciae* and *Enterobacter* spp. were normal flora of water and also opportunistic bacteria but could cause septicemic diseases. No parasite or fungus was detected. In conclusion, early mortality in *M. rosenbergii* prawn larvae was closely related to hatchery management i.e. the use of green water system, feed preparation and sanitary procedures.

Keywords: *Macrobrachium rosenbergii*, larvae, water quality, *Artemia*, egg custard, blood cockles, *A. hydrophila* type II, *B. cepaciae*

OCCURRENCE OF *CAMPYLOBACTER* AND *SALMONELLA* SPP. IN DUCKS AND DUCK EGGS

Nor Faiza Sarif & ¹Saleha Abdul Aziz

¹*Department of Veterinary Pathology & Microbiology*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Seventy-five cloacae swab samples from ducks were collected for *Campylobacter* spp. and *Salmonella* spp. isolation and 32 duck eggs were purchased for *Salmonella* isolation. The samples were collected and purchased from two backyard and a government farms. The shells and contents of the eggs were examined for *Salmonella* spp. All cloacae samples were cultured for *Campylobacter* spp. by direct plating on *Campylobacter* selective agar, mCCDA agar and for *Salmonella* isolation, preenrichment and enrichment were done before plating on XLT4 agar. The isolates of *Salmonella* and *Campylobacter* were identified by colony morphology, gram staining, cell morphology and biochemical tests. Nine samples from 3 farms (12%) were positive for *Campylobacter* spp. A total of 12 samples (8.9%) were positive for *Salmonella* spp. The eggs were negative for *Salmonella* spp. This study revealed that ducks are carriers for *Campylobacter* spp. and *Salmonella* spp. Thus proper hygiene management of the animals and farms may help to control infection. This is to avoid contamination of carcasses during processing. These bacteria are zoonotic and may cause gastroenteritis in humans.

Keywords: ducks, duck eggs, *Campylobacter* spp., *Salmonella* spp.

COMMON SPECIES OF CYCLORRAPHAN DIPTERA IN A RED JUNGLEFOWL FARM, SELANGOR, MALAYSIA

Nor Hidayah Ibrahim,^{1,2} Shaik Mohamed Amin Babjee
& ^{1,2}Reuben Sunil Kumar Sharma

¹Department of Veterinary Pathology & Microbiology

²Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

A study on the prevalence of Cyclorrhaphan Diptera species was carried out in a Red Junglefowl Farm, Dengkil, Selangor, Malaysia. Trappings were done twice a day, in the morning and evening, using hand nets and baited traps over a duration of 7 days. Chicken intestines were used as bait to attract the flies. The mean temperature and relative humidity during the period of sample collection were recorded. The flies were counted and the identification of species was based on standard taxonomic keys. Eleven species of flies were detected on the farm, representing eight genera; *Chrysomya megacephala*, *Chrysomya rufifacies*, *Lucilia cuprina*, *Hemipyrellia liquorriens*, *Musca domestica*, *Musca sorbens*, *Musca ventrosa*, *Parasacrophaga*, *Boettcherisca*, *Seniorwhitea princeps* and *Chrysomela cf. aenea*. The most prevalent fly species was *Chrysomya megacephala*, accounting for 80% of the total catch, followed by *Musca domestica* (5.15%). The other species were present in less than 5% of the catch. There was no significant correlation between temperature and the active fly population ($r=-0.324$, $n=14$, $p=0.258$). Similarly, the relative humidity did not have a significant effect on the number of flies on the farm ($r=0.257$, $n=14$, $p=0.376$). The number of female flies on the farm was high, comprising 64.42% of the total fly population. Certain species of flies displayed temporal activity. *Chrysomya cf. aenea* and *Seniorwhitea princeps* were active in the morning, while *Boettcherisca* sp. was only encountered in the evening. The correct identification of the fly species, and knowledge on their temporal distribution and sex ratios, are important in order to ensure that control measures can be carried out in a more effective manner.

Keywords: Cyclorrhaphan diptera, Red Junglefowl

REPRODUCTIVE PATHOLOGICAL CHANGES IN MICE ASSOCIATED WITH *BRUCELLA MELITENSIS* AND ITS LIPOPOLYSACCHARIDES

Norasiah Nik,¹ Abdul Aziz Saharee & ^{1,2}Faez Firdaus Jesse Abdullah

¹Department of Veterinary Clinical Studies

²Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Brucella melitensis is the gram-negative, aerobic bacteria that cause brucellosis in goats. Brucellosis causes abortion in wild and domestic animals resulting in enormous financial losses. There is still lack of knowledge of host cell response towards the immunogen of *Brucella melitensis* and the response of mice towards the LPS immunogen. In this experiment 3 groups of male mice were used and each group was inoculated with 1 mL PBS (pH 7), 1×10^9 *Brucella melitensis* and 1 mL lipopolysaccharides from 10^9 colonies of *Brucella melitensis* intraperitoneally to examine the clinical signs like mobility, eye discharge, closed eyes, ruffled hair and responsiveness exhibited in the mice. All 3 groups of mice that were inoculated with immunogens were observed for histopathological changes in the male reproductive organs like testes, vas deferens and seminal vesicle. The duration of observation was 5 days. After the fifth day all the mice that were still alive were euthanised by cervical dislocation and the histopathology lesions examined. Mice that were inoculated with the *Brucella melitensis* showed severe clinical signs. Mice infected with *Brucella melitensis* started to die from sixth hour post-inoculation. For the LPS group, the mice showed less movement beginning at 2 hours post-inoculation but became normal again after 5 hours post-inoculation. The control group exhibited normal clinical signs. Histopathology results showed mice inoculated with *Brucella melitensis* had moderate to severe atrophy of the spermatocytes of the testes and degenerative necrosis of the pseudostratified epithelium of vas deferens, while mice that were inoculated with LPS showed mild to moderate atrophy of the spermatocytes of the testes and moderate to severe degenerative necrosis of the pseudostratified epithelium of vas deferens.

Keywords: *Brucella melitensis*, brucellosis, lipopolysaccharides, atrophy, spermatocytes, vas deferens

PATHOGENICITY OF FOWL ADENOVIRUS ISOLATES IN SPECIFIC PATHOGEN-FREE EMBRYONATED CHICKEN EGGS

Norfitriah Mohamed Sohaimi & ¹Mohd Hair Bejo

¹Department of Veterinary Pathology & Microbiology

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Fowl adenovirus (FAdV) is the primary pathogen in inclusion body hepatitis (IBH) in chickens and causes sudden onset of mortality in broiler and layer chickens. Inclusion body hepatitis outbreak has a worldwide distribution and has recently been reported in the region. The clinical signs of the infection were weakness, dehydration, ruffled feather and paleness of comb in the affected chickens. Upon necropsy, the infected chickens showed swollen liver with petechial to focal haemorrhages, hydropericardium, and gizzard erosion and haemorrhages. Isolation of the virus from clinical cases is needed to determine the pathogenicity of the avian adenovirus. Thus the objective of this study was to determine the pathogenicity of recent FAdV isolates in specific pathogen-free (SPF) embryonated chicken eggs. Two isolates of FAdV were obtained from recent outbreaks of the disease in layer (Group A) and broiler (Group B) chicken farms. Isolate from each liver sample was processed and inoculated in SPF eggs. The eggs were harvested and liver of the embryo collected for preparation of FAdV inocula. Sixty 9-day-old SPF embryonated chicken eggs were used in the study. They were divided into three major groups, namely, Group A [A1 (sacrifice) and A2 (mortality)], B [B1 (sacrifice) and B2 (mortality)] and C [C1 (sacrifice) and C2 (mortality)]. Five eggs each from Groups A2, B2 and C2 were labeled as mortality groups and observed for mortality throughout the trial. Twenty eggs from Groups A and B were inoculated with 0.1 mL FAdV isolates A and B, respectively. Twenty eggs from Group C were not inoculated and served as the control group. The eggs were candled twice daily and mortality recorded. Three eggs each from Groups A1, B1 and C1 were sacrificed at days 1, 3, 6, 9 and 12 post-inoculation (pi). At necropsy, the gross lesions were recorded and liver, gizzard and chorioallantoic membrane (CAM) samples were fixed in 10% buffered formalin for histological examination. The study showed 100% mortalities in Groups A and B within 1 to 9 days pi for the mortality group. The control group had no mortality throughout the trials. The number of dead embryo from the sacrifice group was 7 and 11 in the groups A and B, respectively. Control group did not show mortality. Gross lesions in the sacrifice group of Group A were mainly observed in the CAM, liver and gizzard. The CAM became thickened and cloudy beginning day 3 pi. Lesions in the liver revealed enlarged, pale, petechial haemorrhages with multifocal area of necrosis, which were first observed on day 6 pi. The gizzard was congested at day 9 pi. The gross lesions observed in Group B were mainly in the CAM and liver. The lesions were observed as early as day 3 pi with thickening and cloudiness of the CAM as well as enlargement, pale to yellowish liver. The control group remained normal throughout the trial. Histologically, typical intranuclear inclusion bodies were observed in the CAM, liver and gizzard in Group A.

The lesions were confined to the CAM and liver in Group B. It was concluded that FAdV is highly pathogenic to SPF embryonated chicken eggs and the embryonic liver should be used for isolation and propagation of the virus.

Keywords: fowl adenovirus (FAdV), specific pathogen free (SPF) embryonated chicken eggs, pathogenicity, liver, chorioallantoic membrane (CAM)

MICROBIOLOGICAL PROFILE OF ORAL CAVITY OF AND OCCURRENCE OF *SALMONELLA* SPP. IN SUGAR GLIDERS

Nur Diana Hassan,¹Saleha Abdul Aziz,¹Siti Khairani Bejo
& ^{2,3}Azlan Che' Amat

¹Department of Veterinary Pathology & Microbiology

²Department of Veterinary Clinical Studies

³Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Sugar gliders (*Petaurus breviceps*) are popular pocket pets in Japan, Canada and United States and now gaining popularity in Malaysia. The close relationship between humans and pets may lead to possible health hazards if the animals are infected with zoonotic pathogens. To date, not much is known about the potential health hazard of keeping sugar gliders as pet. The objectives of this study were to evaluate the microbiological profile of oral mucosa in sugar gliders, to determine the occurrence of *Salmonella* spp. in sugar gliders and to determine the antibiotic resistance of the isolates against six types of antibiotics namely enrofloxacin, trimethoprim-sulfamethazole, tetracycline, ceftiofur, penicillin G and neomycin. Thirty-seven oral swabs and 37 faecal samples were collected from 18 individual owners and a breeder. Nine species of bacteria isolated were *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Pasteurella* spp., *Staphylococcus* spp., *Klebsiella pneumonia*, *Staphylococcus delphini* and *Streptococcus viridans*. Most of the isolates were normal microflora in other pet animals, such as cats, dogs, and parrots. Eight (22.9%) faecal samples were positive for *Salmonella* spp of which only 1 (12.5%) was from individual owner, while the other seven (87.5%) were from the breeder. Two serovars of *Salmonella* spp. isolated were *Salmonella Albany* (62.5%) and *Salmonella London* (37.5%). The high occurrence of *Salmonella* in the animals suggested that they are of potential risk in disease transmission to humans. All the *Salmonella* spp. isolates were susceptible to three types of antibiotic, Enrofloxacin, Trimethoprim-Sulfamethazole, and Tetracycline. Of these isolates, 33.3% were sensitive to Ceftiofur and the rest were intermediately sensitive. However, all were resistant to Penicillin G and Neomycin. The presence of *Salmonella* spp. in these pet animals poses a health risk to the owners. Owners are advised to wash their hands thoroughly after handling these animals, clean the cages frequently, bring these animals for regular veterinarian check-ups, and for immunocompromised people, to avoid handling these animals.

Keywords: Sugar gliders (*Petaurus breviceps*), oral microflora, faecal microflora, *Salmonella*

PREVALENCE OF *MANNHEIMIA HAEMOLYTICA* AND *PASTEURELLA MULTOCIDA* IN GOATS FROM SELECTED FARMS IN SELANGOR, MALAYSIA

Nuraini Simaa Always,¹ Abdul Rahim Mutalib & ²M. Murugaiyah

¹*Department of Veterinary Pathology & Microbiology*

²*Department of Veterinary Clinical Studies*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Mannheimia haemolytica and *Pasteurella multocida* are natural inhabitants of the upper respiratory tract of healthy animals and can cause pneumonic pasteurellosis in stressed animals. Healthy animals are able to control the multiplication of these bacteria and the inhaled bacteria, if they migrate to the lungs, will be cleared by the host defense mechanisms. Stress and other infections will cause the breakdown of the host defense mechanisms. These will lead to multiplication of the bacteria and colonisation of the lungs. Outbreaks of pneumonia occur in 10 to 14 days post-stress exposure. These bacteria are gram-negative, facultative anaerobes and have rod-shaped morphology. The special characteristic of these bacteria is that they show bipolar staining characteristic under Giemsa and Wright's stains. *Mannheimia haemolytica* is haemolytic on blood agar and O-nitrophenyl- β , D-galactopyranoside (ONPG) positive while *Pasteurella multocida* is non-haemolytic on blood agar and ONPG negative. Ninety six (96) nasopharyngeal swab samples were taken from 4 goat farms in Selangor, Malaysia and bacterial isolation and identification were carried out. Presumptive isolates were identified by biochemical tests. Out of the 96 samples, 3 were positive for *Mannheimia haemolytica* and 11 positive for *Pasteurella multocida*. This gives a 3.13% and 11.46% prevalence rate for *Mannheimia haemolytica* and *Pasteurella multocida*, respectively. The antibiotic sensitivity tests done on both isolates showed 29% were resistant to streptomycin and 21% resistant to compound sulfonamide. All isolates were sensitive to ampicillin and amoxicillin-clavulanic acid whilst 93% was sensitive to oxytetracycline and enrofloxacin. Even with low prevalence of *Mannheimia haemolytica* and *Pasteurella multocida*, it is important to control and prevent their infections and also to prevent further development of antimicrobial resistance as the disease is associated with these bacteria and have high economic impact.

Keywords: goat, *Mannheimia haemolytica*, *Pasteurella multocida*, prevalence rate, antibiotic sensitivity test

HISTOPATHOLOGICAL CHANGES OF SKIN AND GILLS OF CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO ORGANOPHOSPHATE TOXICANT

**Nurul Najwa Mohd Sharipudin, ¹Intan Shameha Abdul Razak,
^{2,3}Hassan Hj. Mohd Daud & ²Mohd Fuad Matori**

¹*Department of Veterinary Preclinical Sciences*

²*Department of Veterinary Clinical Studies*

³*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The usage of pesticide in the industry will help increase crop yield and meet consumer demand for Malaysia, a country that is undergoing economic revolution through the agricultural. However, the excessive and inappropriateness use of pesticides can affect the environment, water quality and public health. In this experiment, 40 adult African catfish (*Clarias gariepinus*) were divided and exposed into sublethal concentrations of Malathion (0.8, 1.6 and 3.0 ppm) and a control group for 48 hours. At the end of the study, all fish were sacrificed before skin and gill samples were collected to determine the effect of malathion on the gills and skin. The histopathological lesions in the gills and skin were scored and statistically analysed to compare between group. Histological examination of the gill showed epithelial lifting at secondary lamella, hyperplasia of primary epithelium, fusion of secondary lamella and infiltration of inflammatory cells with peeling and rupture of epithelial cells of secondary lamellae. In the skin, the changes observed were hyperplasia of mucus cell and shrinkage of the club cells after the exposure to malathion. There were significant differences between treatment and control groups. Thus, malathion causes concentration-independent and nonspecific lesions in the gills and skin of African catfish.

Keyword: pesticide, Malathion, histopathology, gill, skin, catfish

OCCURRENCE OF *CAMPYLOBACTER* SPP. AND *ARCOBACTER* SPP. IN GOATS

Nurul Shakira Mohd Noh, ¹M. Murugaiyah & ²Saleha Abdul Aziz

¹*Department of Veterinary Clinical Studies*

²*Department of Veterinar Pathology & Microbiology*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Arcobacter spp. and *Campylobacter* spp. belong to the family Campylobacteraceae but they differ in that *Arcobacter* can grow at temperature below 30°C and aerotolerance. *Campylobacter* is a zoonotic foodborne pathogen and can be divided into thermophilic and non-thermophilic *Campylobacter*. Thermophilic *Campylobacter* has become a major cause of bacterial gastroenteritis. *Arcobacter* is an emerging group of zoonotic foodborne and waterborne pathogens in which there are about 13 recognised species. To date, limited studies have been done in Malaysia to determine the occurrence of these bacteria in goats. Twenty goats each from 3 different goat farms in Selangor, Malaysia were randomly selected. Two sets of rectal swabs were collected from each goat. One set of the rectal swab was placed in Cary Blair transport medium and the other set agitated in *Arcobacter* broth. Isolation and identification were done in order to isolate *Campylobacter* and *Arcobacter* spp. from the samples. For *Campylobacter* spp., the samples were cultured via direct plating to the CCDA. Out of the 60 goats, 9 (15%) goats were positive for *Campylobacter* spp. of which 5 (8.33%) were *C. jejuni* and 4 (6.67%) were *C. coli*. For *Arcobacter* spp., enrichment in *Arcobacter* broth followed by culture on the blood agar was done. Nine (15%) goats were found positive for *Arcobacter* spp. This study showed that the occurrence of *Campylobacter* spp. and *Arcobacter* spp. in goats were low, but prevention and control measures are still needed to be taken into consideration because both bacteria have zoonotic importance.

Keywords: goats, occurrence, *Campylobacter* spp., *Arcobacter* spp.

INCIDENCE OF EQUINE HOOF DERANGEMENTS IN SELANGOR AND FEDERAL TERRITORY OF KUALA LUMPUR AND PUTRAJAYA, MALAYSIA

Nurul Syuhada Zainal Abidin & ¹Bashir Ahmad Fateh Mohamed

¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

Abstract

The study was carried out to investigate the various environmental conditions responsible for abnormal hoof conformity and lameness in horses from various establishments as the result of poor management. The hooves of the horses in five establishments in Selangor, Putrajaya and Kuala Lumpur were examined for abnormal hoof conditions ranging from thrush, soft sole, seedy toe, hoof crack, hoof ring, chip hoof and displaced coronet. The management and nutrition statuses were recorded via observation and personal communication. Fifty-three horses of different breeds and usage were selected and categorised as clinically healthy, actively working and shod. These horses comprised of 20 Thoroughbreds, 21 Arabs, 2 each of Anglo-Arabs, Warmbloods, Percherones, Andalusians and 1 each of Appaloosa, Crossbred, Stock Horse and Quarter Horse. Factors that were compared in relation to incidence of hoof derangements were breed, use of the horse, hoof pigmentation, management and nutrition. Hoof derangements were scored according to severity. Six types of hoof derangements recorded were chipped hoof, thrush, hoof ring, grass crack, horizontal crack and sand crack. The incidence rate for chipped hoof was the highest with 52 cases (53.52%). The lowest incidence rate recorded was sand crack where only one case was recorded (0.96%). Other hoof derangements were grass crack (41.74%), Thrush (40.20%), hoof ring (39.00%) and horizontal crack (3.37%). The high incidence of hoof derangements in this study was due to poor management and inappropriate nutrition. The study also showed that there is a relationship between use of horse and thrush and chipped hoof conditions.

Keywords: hoof derangements, incidence, thrush, chipped hoof, hoof ring, hoof crack

ANTINOCICEPTIVE EFFECT OF *MOMORDICA CHARANTIA* FLESH AQUEOUS EXTRACT IN MICE

Siti Aminah Yusob, Nor 'Adilah Makhtar
& ¹Wan Mastura Shaik Mossadeq

¹*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

Momordica charantia (Cucurbitaceae), also known as “Peria” in Malaysia and as “Kerala” in Bengala has traditionally been used in folk medicine for its antidiabetic, anti-inflammatory and antioxidant properties. However, there is little information available regarding the analgesic activity of the plant. Therefore, this study was carried out to investigate the analgesic effect of *Momordica charantia* flesh aqueous extract (MCA) using a chemical model of nociception in mice. The analgesic effect was assessed using acetic acid-induced writhing test, while the possible effect of the extract on motor coordination or fatigue resistance in mice was assessed using the Rotarod performance test. In this study, the intraperitoneal (i.p.) administration of extract (30-300 mg/kg body weight) strongly and dose-dependently inhibited the acetic acid-induced writhing in mice ($p < 0.05$). In addition, results from the Rotarod performance test showed no significant difference between the control and treatment group (300 mg/kg body weight), suggesting that the extract did not produce apparent motor impairment in all the animals tested. Together, the present data suggests that MCA was effective against pain at the peripheral level most probably through the inhibition of cyclooxygenase (COX) and/or lipooxygenase (LOX) and other inflammatory mediators. Furthermore, the analgesic activity induced by MCA is unlikely to be secondary to its depressant and/or muscle relaxant effect.

Keywords: *Momordica charantia*, aqueous extract, antinociceptive

EVALUATION OF GRASS QUALITY IN GRAZING AND UNGRAZING PADDOCKS IN A BUFFALO BREEDING AND RESEARCH CENTRE, TELUPID, SABAH, MALAYSIA

Siti Hafizah Mohd Salleh,^{1,4}Md Zuki Abu Bakar,^{2,3}Mohd Zamri Saad & ^{3,4,5}Azhar Kassim

¹ *Department of Veterinary Preclinical Sciences*

² *Department of Veterinary Pathology & Microbiology*

³ *Ruminant Diseases Research Centre*

⁴ *Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

⁵ *Department of Animal Sciences, Faculty of Agriculture*

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Buffalo production is dependent almost entirely on forages. Thus, adequate nutrition plays important role in the productivity of buffaloes. In Buffalo Breeding and Research Centre, Telupid, Sabah, Malaysia, Signal Grass (*Brachiaria decumbens*) is fed to buffaloes. Since the growth of the buffalo depends on the nutritional quality of grass provided to them, this study was undertaken to evaluate the nutrient contents of signal grass to determine its quality. In addition, a comparison was made on the nutrient contents between signal grass obtained from the grazing and non-grazing areas. Six grass samples each was obtained from grazing and non-grazing areas. The grass samples were taken using the quadrat and all the samples were air-dried and sent to Universiti Pertanian Malaysia for proximate analysis. The dry matter (DM) and crude protein (CP) were determined. The values of Van Soest Fibre were also determined, to include neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). The results revealed that the DM and CP were significant ($p < 0.05$) higher in grass from grazing than non-grazing area. There was also significant ($p < 0.05$) negative correlation between DM and CP contents of signal grass. In conclusion, the grass in grazing area has better nutritive value compared to grass in non-grazing area. In addition, the relationship between DM and CP was inversely related, that is as DM increases CP decreases. The results of the current study could be used to improve the performance of farm and as a reference for future study.

Keywords: *Brachiaria decumbens*, proximate analysis, Van Soest Fibre

**CLINICAL RESPONSE AND PATHOLOGICAL CHANGES
ASSOCIATED WITH *PASTEURELLA MULTOCIDA* TYPE B INFECTION
THROUGH ORAL ROUTE INOCULATION
IN MICE**

Syahirah Ahmad Affandi,^{1,3}Faez Firdaus Jesse Abdullah,

¹Abdul Aziz Saharee & ²Jasni Sabri

¹Department of Veterinary Clinical Studies

²Department of Veterinary Pathology & Microbiology

³Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Haemorrhagic septicaemia (HS) is caused by *Pasteurella multocida* type B in Asia (*P. multocida* type B). It is an important cattle and buffalo disease in Malaysia as well as other Southeast Asian countries due to its nature of acute and highly fatal disease which have an economic impact on the affected countries. This study describes the clinical signs and histopathological changes in mice following oral route inoculation with *P. multocida* type B. In this study, sixteen mice were selected and divided into two groups of 8 mice each. Mice in group 2 were inoculated with 1.0 mL 10⁹CFU/mL *P. multocida* type B orally while group 1 with PBS orally. The entire challenged group showed significant clinical signs ($P < 0.05$) where ruffled fur, laboured breathing, less responsive and dullness together with eye discharge were observed. Six out of 8 mice died between 24 to 50 hours post-inoculation. Histopathological lesions observed include haemorrhage, congestion, degeneration and necrosis and infiltration by the inflammatory cells in the lungs, kidney, heart, liver and small intestine. The study showed that experimental *P. multocida* infection in mice caused oedema the lungs and infiltration of numerous Kupffer cells in the liver.

Keywords: *Pasteurella multocida* type B, oral inoculation, histopathology, clinical signs, mice

EFFECT OF ROUTE OF INFECTION ON DEVELOPMENT OF STREPTOCOCCOSIS IN RED TILAPIA

Syuhaidah Abdullah, ¹Md Sabri Mohd Yusoff, & ¹Noraini Omar

¹*Department of Veterinary Pathology & Microbiology*

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The effect of route of infection on the development of streptococcosis in the red tilapia was assessed using histopathological, PCR, microbiological and immunohistochemical methods. Three hundred healthy, adult Red tilapias (*Oreochromis* spp.) were obtained from a commercial hatchery. Forty-eight fish were randomly taken from the same age and weight (150 g) per group. Fish were clinically examined and screened one week prior to the experiment. The experiment was divided into three groups with duplicates. Group 1 was exposed to *Streptococcus agalactiae* intraperitoneally (IP), Group 2 was exposed to *S. agalactiae* through an immersion broth, and Group 3 was exposed through a skin cut immersion. The fish were necropsied at the end of 24 hours exposure, and the brain, eye and kidney samples were taken for microbiological, histopathological examinations and indirect immunoperoxidase test. Statistical analysis (ANOVA) from the histopathological lesions revealed that there was significant ($p < 0.05$) difference between the route of infection in the brain but not in the eye and kidney, while IP route showed severe lesions at 8 hours post-inoculation. In conclusion, *S. agalactiae* was pathogenic to Red tilapia causing septicaemia and severe pathological changes through different routes of infection.

Keywords: *Streptococcus agalactiae*, immunoperoxidase, histopathology, *Oreochromis* sp.

DETECTION OF THE JAPANESE ENCEPHALITIS VIRUS IN WILD BOARS AND COMPARISON OF BLOOD PROFILES BETWEEN WILD BOARS AND DOMESTIC PIGS IN SELANGOR, MALAYSIA

Tan Wei Cheit, ¹Hazilawati Hamzah & ²Ooi Peck Toung

¹Department of Veterinary Pathology & Microbiology

²Department of Veterinary Clinical Studies

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

The Japanese encephalitis virus (JEV) RNA was detected in mosquito vectors in Selangor, Malaysia almost two decades ago. However, the JEV status in wild boars, a potential reservoir, has yet to be investigated. Blood profiles can be used for health monitoring however data on the wild boar blood profile is limited. This study was performed to detect the presence of JEV RNA in wild boars in Selangor, Malaysia and to determine the haematological and serum biochemical values of the wild boars. The blood profiles of domestic pigs were also determined for comparison. Thirty-five wild boar tonsils were collected for RNA extraction while 21 wild boar and 40 domestic pig blood were collected for analyses. The RNA extraction was performed using the QIAamp RNA Blood Mini Kit (Qiagen, USA) while reverse transcription and PCR amplification were performed using the i-JEV Detection Kit (iNtron Biotechnology, Korea). The blood analyses were performed using standard protocols in the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The JEV RNA (genotype I and/or III) was detected in one of 21 (4.8%) tonsils; gene sequencing can be done to plot a phylogenetic tree to identify the origin of virus. The positivity may be higher if a vigorously optimized multiplex RT-PCR was used in the detection JEV genotypes. The leucocyte, segmented neutrophil counts and serum globulin concentration of the wild boars were significantly ($p < 0.05$) lower than in domestic pigs and the existing reference data. Serum AST and CK concentration of the wild boars were significantly higher than that of domestic pigs, which might be attributed the physically more active wild boars.

Keywords: JEV RNA, PCR, blood profile, tonsil, wild boar

EFFECT OF DIETARY PROTEIN LEVEL DURING EARLY BROODING PHASE ON SUBSEQUENT GROWTH PERFORMANCE AND MORPHOLOGICAL DEVELOPMENT OF DIGESTIVE SYSTEM IN CROSSBRED KAMPUNG CHICKEN

**Tarmisal Alimin, ¹Engku Azahan Engku Ahmed, ²Azlina Azma, I.A.
& ¹Yusof Hamali Ahmad**

*¹Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

²Strategic Livestock Research Centre, MARDI, GPO Box 12301, 50774 Kuala Lumpur

Abstract

A study was undertaken to investigate the growth performance and morphological development of the digestive system in the crossbred kampung chicken, in response to changes in dietary protein levels during the early brooding phase. Nine hundred day-old crossbred kampung chicks were utilised in the study which lasted over 28 days. The chicks were randomly divided into three equal treatment groups. Three dietary protein (CP) treatments were imposed from the age of one day till 12 days, namely high protein (25% CP), medium protein (21% CP) and low protein (17% CP) feeds. This was followed by a common commercial starter feed (21% CP) given to all birds until the termination of the study on day 28. The growth parameters investigated were feed intake, body weight gain and feed conversion ratio (FCR). Gross morphology of the digestive system was assessed on sampled birds at ages 14 and 28 days. The digestive organs considered included the crop, proventriculus, gizzard, liver, gall bladder, pancreas, duodenum, jejunum, ileum, caecum, and colon. Over the study periods of 1-12, 12-28, and 1-28 days, significant differences in body weight gain and feed intake were observed among the three dietary treatment groups while no differences were noted in terms of feed conversion ratio. Birds on the high protein diet gained more weight and consumed more feed than the birds on the lower protein treatment. In general, no significant effects of dietary protein levels on the morphological development of the digestive system were observed. It is concluded that within the limits of the experimental conditions imposed, provision of high dietary protein during the early brooding phase resulted in an improved subsequent growth performance of the crossbred kampung chicken. However, the high dietary protein consumed apparently did not help to improve the morphological development of the digestive system of the birds.

Keywords: dietary protein level, brooding phase, growth, digestive system morphology, crossbred kampung chicken

CHARACTERISATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS IN SELECTED FARMS IN MALAYSIA

Vania Kiu Tse Ling & ¹Ooi Peck Toung

*¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

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. The PRRS virus can be divided into two antigenically and genetically different strains: Type I (European) and Type II (North American). In this study, 120 sera were collected from 12 farms in 6 states in Malaysia for the seroprevalence study. Ten sera from apparently healthy sows/gilts, finishers and growers were collected from each farm and tested using IDEXX HerdChek ELISA for both strains. All the farms tested were seropositive with an overall seroprevalence of 89.2%. Tissue samples were collected and PRRSV isolated were genotyped using nested-PCR (with reported primer pairs targeting ORF7) that enabled the differentiation of Type I and Type II PRRSV by producing different sizes of PCR products. Out of 27 tissue samples collected from 11 farms, 12 were positive for PRRSV. All the PRRSV genomes from the 12 PRRSV-positive tissue homogenates were of Type II PRRSV, whereas no Type I PRRSV was detected. These data indicate that PRRS is endemic in the farms tested with a high possibility of subclinical infections.

Keywords: porcine reproductive and respiratory syndrome (PRRS), seroprevalence, PRRSV strain, nested-PCR

EFFECTS OF OLFACTORY ENRICHMENT ON DIURNAL ACTIVITY AND STEREOTYPIC BEHAVIOUR OF CAPTIVE MALAYAN TIGERS (*PANTHERA TIGRIS JACKSONI*)

**Vishwanee Kolandaiveloo,^{1,2} Reuben Sunil Kumar Sharma
& ^{2,3}Sumita Sugnaseelan**

¹*Department of Veterinary Pathology & Microbiology*

²*Wildlife Research & Conservation Centre*

Faculty of Veterinary Medicine

³*Department of Animal Science, Faculty of Agriculture*

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract

Environment enrichment using olfactory stimuli is an emerging facet of zoo animal management, and has been shown to be effective in increasing behavioural diversity and reducing stereotypies in captive animals. This study was undertaken to document the effects of olfactory enrichment on the diurnal activity patterns and stereotypic repertoire in captive Malayan Tigers (*Panthera tigris jacksoni*). The diurnal behaviour of three pairs of adult *P.t. jacksoni* was observed at a local zoo using instantaneous scan sampling. The experimental protocol comprised three temporal blocks (pre-enrichment, enrichment and post-enrichment) lasting for five days each. A synthetically derived product (perfume) and a natural product (lime) were used as olfactory stimuli, and were applied on the walls of the enclosure following a fixed alternating routine. The frequency of behaviours and stereotypies observed for each tiger were scored following a defined ethogram. The diurnal activity budgets of the tigers were dominated by locomotion and stance (47%) and resting (33%). Pacing and patrolling were the major stereotypies exhibited by the tigers accounting for 8% of the diurnal activity budget. Investigative behaviour and scent marking significantly increased during the enrichment period. A reduction in locomotor activity was noted and the tigers spent more time resting in the presence of the olfactory stimuli. The frequency of stereotypic pacing and patrolling did not differ significantly between the pre-enrichment, enrichment and post-enrichment phases. However, the tigers did exhibit a trend of decreasing stereotypic behaviour pattern when the olfactory enrichment items were introduced. The highest contact frequency of the olfactory items was during the morning and evening. Olfactory stimuli have the potential to be used as a form of enrichment in captive tigers to increase their behavioural diversity and encourage investigative activities. Experimenting with varieties of olfactory stimuli may reveal preferences in captive tigers, and may prove effective in alleviating stress and reducing stereotypies in captive populations.

Keywords: Malayan Tigers, *Panthera tigris jacksoni*, stereotypies, olfactory enrichment

TOXICOLOGY STUDY ON USAGE OF DORAMECTIN IN RED-EARED SLIDERS (*TRACHEMYS SCRIPTA ELEGANS*)

**Vivian Lee Ker Chuon,^{1,3}Goh Yong Meng,^{2,3}Noordin Mohamed Mustapha
& ³Choy Foon Seng**

¹Department of Veterinary Preclinical Sciences

²Department of Veterinary Pathology & Microbiology

³Ruminant Diseases Research Centre

Faculty of Veterinary Medicine

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

³Taiping Zoo and Night Safari, 34000 Taiping, Perak, Malaysia

Abstract

Ivermectin has been widely acknowledged to cause neurotoxicity in chelonians even at a dose of 50 µg/kg body weight. Interestingly, another member of the macrocyclic lactone family namely milbemycin, did not induce any observable toxic effects when administered to chelonians at therapeutic doses. However, injectable milbemycin is currently not available in the market and has to be prepared from technical grade materials. There is a need for injectable endo- and ecto-parasiticides that can be used safely in chelonians, which have a unique anatomy that makes it difficult to administer oral medication. The objective of this study was to determine whether or not doramectin given at twice the usual therapeutic dose (600 µg/kg body weight) will result in toxicity in the red-eared sliders. For this study, the sliders were distributed into 4 groups and doramectin was administered intramuscularly to the sliders according to dose group; low dose group (100 µg/kg body weight, n=4), medium dose group (300 µg/kg body weight, n=4), and a high dose group (600 µg/kg body weight, n=4), while the control group (n=4) was given 0.9% NaCl injections. They were monitored for signs of neurotoxicity, particularly paresis and paralysis for 4 weeks following doramectin administration. Blood samples were taken before treatment and at the conclusion of the study. The red-eared sliders were then humanely euthanised and samples of the liver and brain examined histopathologically. No clinical sign was observed throughout the 4-week study, and the blood results and histopathology did show significant findings. This indicates that doramectin can be used with no obvious adverse effects in red-eared sliders even at a high dose of 600 µg/kg body weight.

Keywords: doramectin, red-eared slider

EFFECT OF DEXAMETHASONE ON GROWTH AND VIABILITY OF MOUSE MAMMARY TUMOUR CELL LINES, EMT6 AND 4T1

Wan Maryani Wan Hassan & ¹Mohd Hezmee Mohd Noor

*¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Abstract

The in-vitro growth of benign (EMT6) and malignant (4T1) mouse mammary tumour cell lines were evaluated following treatment with doses of 0.1, 0.01 and 0.001 mg/mL dexamethasone. The viability and growth of these cells following dexamethasone treatment were determined by cell proliferation and live:dead assays. In cell proliferation assay, both cells were rapidly proliferating in serum-free media after treatment with different concentrations of dexamethasone over 24 hours. Following treatment with 0.1 mg/mL dexamethasone the proliferation of EMT6 cells were significantly increased while the proliferation of 4T1 cells was only slightly significantly increased. The effect was not dose-dependent. Using the live:dead assay, the results for both cell lines were consistent with the that of cell proliferation assay. This study provides preliminary evidence that dexamethasone may induce growth and viability of mouse mammary tumour cell lines, EMT6 and 4T1.

Keywords: mouse mammary tumour cell lines, EMT6, 4T1, dexamethasone

EVALUATION OF THE SEMEN QUALITY OF A BUFFALO BULL IN THE BUFFALO BREEDING AND RESEARCH CENTRE, TELUPID, SABAH, MALAYSIA

Zuhairah Mohamad Nasir, ¹Md Zuki Abu Bakar, ^{2,3}Mohd Zamri Saad & ^{3,4,5}Azhar Kassim

¹ Department of Veterinary Preclinical Sciences

² Department of Veterinary Pathology & Microbiology

³ Ruminant Diseases Research Centre

⁴ Wildlife Research & Conservation Centre

Faculty of Veterinary Medicine

⁵ Department of Animal Science, Faculty of Agriculture

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract

Breeding Soundness Examination (BSE) is an evaluation of fertility of male animals. Semen quality and scrotal circumference of buffalo bulls were the parameters determined during BSE. This study was conducted at the Buffalo Breeding and Research Centre Farm, Telupid, Sabah, Malaysia to evaluate semen quality of buffalo bulls and determine the correlation between scrotal circumference with age. Nine buffalo bulls were selected of which 6 were Murrah-Swamp crosses and 3 local Swamp. The age of bulls ranged between 1 to 10 years with body weight ranging between 346 and 500 kg. Semen collection was done using an electroejaculator from only two swamp buffalo bulls of age 3 and 4 years. Semen was collected twice at one week interval. Scrotal size circumference was measured using a measuring tape. Semen was evaluated based on ejaculated volume, sperm motility, live sperm and sperm morphology. Semen volumes collected from the two buffalo bulls were 0.2 and 0.5 mL, respectively. The mean semen volume was 0.4 mL, general sperm motility was 77.5%, individual progressive sperm motility was 77.5%, live sperm was 66.0% and sperm abnormality was 9.25%. The measurement scrotal size showed that the Murrah-Swamp crosses had mean scrotal circumference of 27.16 cm while for the Swamp buffalo it was 23.33 cm. There was no significant ($p>0.05$) difference in scrotal circumference between the two breeds. However, correlation between scrotal circumference and age of bull were significant. In conclusion, semen characteristics of swamp buffalo bull in this farm meet the requirements for breeding soundness evaluation of the buffalo bull and as the buffalo bulls get older the circumference of the scrotum increases.

Keywords: breeding soundness evaluation, semen evaluation, scrotal size circumference, buffalo

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