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Faculty of Veterinary Medicine UPM

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Rasedee Abdullah • Mohamed Ariff Omar • Abdul Rahim Mutalib
Abdul Rani Bahaman • Saleha Abdul Aziz • Mohamed Ali Rajion
Mohd Azmi Lila • Siti Suri Arshad • Gayathri Thevi Selvarajah

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PREFACE

The series of the Proceedings of the Seminar on Veterinary Sciences has now gone into its ninth year. The editors are elated because this year we have a record number of 39 extended abstracts, which is more than double that of last year in the 8th proceedings. We believe the faculty members realise the importance of the proceedings as a record of research activities conducted by the final students. The proceedings also serve as a reference and guide for students and academicians to be used in deciding on future researches. Like previous years the studies conducted by the students covered many areas of veterinary science. It is quite encouraging to see that students are interested in animal behaviour, particular in the wild. This type of research should be encouraged to ensure appreciation and preservation of our wildlife for generations to come.

The editorial board is most grateful to the students and their supervisors for writing these articles. The period during which the students were required to produce the articles is among the busiest of their five-year study. However, the proceedings are now becoming a tradition for the faculty, which should be preserved. To ensure its continuity we need to inject new blood into the editorial board. It is most fortunate that we now have two new members who volunteered to serve as editors. We hope they will pick up the reign and be responsible for future publications of the proceedings.

We wish to also express our gratitude to the Faculty of Veterinary Medicine, Universiti Putra Malaysia and all government and commercial organisations for their contributions to the final year projects. Their participation had played a significant role in the success of the final year projects and publication of the Proceedings.

May Allah bless us all.

The Editors
Rasedee Abdullah
Mohamed Ariff Omar
Abdul Rahim Mutalib
Abdul Rani Bahaman
Saleha Abdul Aziz
Mohamed Ali Rajion
Mohd Azmi Lila
Siti Suri Arshad
Gayathri Thevi Selvarajah

PLEURAL EFFUSION IN CATS PRESENTED TO THE UNIVERSITY VETERINARY HOSPITAL UNIVERSITI PUTRA MALAYSIA

Abu Huzaifah Md Redzuan, [1]Puteri Azaziah Megat Abdul Rani & [2]Rasedee Abdullah

[1]Department of Companion Animal Medicine and Surgery
[2]Department of Veterinary Laboratory Diagnosis
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Pleural effusion is one of the important respiratory conditions commonly reported in cats. The retrospective study of pleural effusion in cats over the period of January 2008 until December 2012 was carried out at University Veterinary Hospital, Universiti Putra Malaysia. The objectives were to identify the occurrence, common aetiology and risk factor of pleural effusion in cats presented to UVH. Sixty-six cases were reported during this period of study, of which 69% (46/66) were caused by infection while 31% (20/66) were of non-infectious origins. The most common disease for the infectious group is FIP (67%, 31/46) and neoplasia disease (45%, 0/20) for the non-infectious group. The risk factor of age, sex, breeds, vaccination status, and single and multiple cat household, outdoor and indoor management of cats showed no significant relationships with infectious and non-infectious diseases. In conclusion, feline pleural effusions caused by infectious origins had higher incidence compared to those of non-infectious origins.

Keywords: feline pleural effusion, infectious, non-infectious, risk factor

INTRODUCTION

Pleural effusion is an abnormal accumulation of fluid within the pleural space and it is a clinical manifestation of various conditions (Nelson, 2009). Feline infectious peritonitis (FIP), heart failure, pyothorax and neoplasia are common causes of pleural effusion in cats (Davies *et al*., 1996). Therefore, determining the underlying aetiology is the key to appropriate management in pleural effusion cases. A systematic investigation approach is crucial to determine the aetiology and to plan the management of pleural effusion cases. The data obtained from signalment, history, physical examination are vital to generate a solid differential diagnosis (Beatty *et al.*, 2010).

Anecdotal evidence shows that pleural effusion is one of the respiratory distresses for feline cases presented to the Universiti Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). Therefore, the compilations of information from previous cases are crucial to assist the investigation and management of feline pleural effusion cases in the future. Therefore, this study should provide information on pleural effusion in cats, which will form the database for clinical management of these cats.

MATERIALS AND METHODS

This study was conducted using a retrospective approach to identify the cases of feline pleural effusion in cats referred to UVH, UPM from January 2008 until December 2012. Medical records of feline cases were obtained if the diagnosis of pleural effusion was done through radiograph and thoracocentesis. The information obtained from each case included age, sex, breed, vaccination status, history information, indoor versus outdoor status in cats management, single versus multiple cat household, physical examination findings, radiographic findings, haematology and biochemistry findings, pleural fluid analysis findings and other diagnostic work-ups.

For most cases, the underlying disease was determined based on diagnostic findings. These cases were then classified into two groups: infectious origin and non-infectious origin of pleural effusion.

RESULTS AND DISCUSSION

Out of 88 cats with pleural effusion, 65 had a diagnosis while 23 cases without. Based on Table 1, the most frequent conditions encountered in association with pleural effusion were FIP, pyothorax, congestive heart failure (CHF) and neoplasia. These findings confirmed earlier reports where FIP, pyothorax, CHF and neoplasia were the most common findings in pleural effusions (Beatty *et al*., 2010).

The prevalence of pleural effusions due to infectious origins (69%, 46/66) was 2.2 times higher compared to the prevalence of non-infectious origins (31%, 20/66) (Table 2). This prevalence of infectious effusions observed in this study was high compared to other studies in Germany (39%) and USA (42%) (Davies *et al*., 1996; Beatty *et al*., 2010). It is possible different climatic region caused be one of the cause in the difference in prevalence in infectious effusion.

The common findings upon physical examination were dyspnea, dehydration, poor body condition and tachypnea. Most cats with pleural effusion also had a history of inappetance and lethargy. This pattern was similar to another report where non-specific findings were common in cats diagnosed with pleural effusion (Beatty et al., 2010).

Most of the risk factor of age, sex, breed, single and multiple cat household, and management of cats were not significantly related to infectious and non-infectious

pleural effusion, although previous studies indicated that the multiple-cat household significantly increased the occurrence of pyothorax and FIP (Waddell *et al*., 2002; Chan, 2012).

Based on the laboratory findings, the association between infectious and non-infectious pleural effusions and leukocyte parameters were not signifcant. However the total protein, globulin and albumin concentrations showed significant ($p<0.05$) positive associations more with infectious than non-infectious origin.

Table 1: Diseases reported in cats associated with pleural effusion

Diseases	Frequency	Percentage
Feline infectious peritonitis	30/65	46.2
Pyothorax	15/65	23.1
Congestive heart failure	5/65	7.7
Neoplasia	9/65	13.8
Chylothorax	3/65	4.6
Mediastinal mass	2/65	3.1
Trauma	1/65	1.5

Table 2: Infectious and non-infectious pleural effusion at University Veterinary Hospital, Universiti Putra Malaysia

	Frequency	Percentage
Infectious	45/65	69
Non-infectious	20/65	31

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EVALUATION OF ELECTROLYTE COMPONENTS IN MILK OF COWS WITH SUBCLINICAL MASTITIS

Aisyah Munira Mohd Amir, [1,4]Abd Wahid Haron,
[1,4]Faez Firdaus Jesse Abdullah, [2]Mohamed Ariff Omar & [3]Hazilawati Hamzah
[1]Department of Veterinary Clinical Studies
[2]Deparment of Veterinary Preclinical Sciences
[3]Department of Veterinary Pathology and Microbiology
[4]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The aim of this study was to determine the sodium ion (Na^+) and potassium ion (K^+) concentrations in milk collected from cows with subclinical mastitis (SCM). Thirty-seven dairy cows were used in this study and categorised as positive and negative based on California mastitis test. The somatic cell count (SCC), Na^+ and K^+ concentration were calculated. The results showed significant increase ($p<0.05$) of $Log_{10}SCC$ and Na^+ and reduction of K^+ concentration in the milk of cows with SCM. The K^+ concentration was negatively correlated ($p<0.01$) with Na^+ concentration, and $Log_{10}SCC$. Therefore, Na+ and K+ concentration evaluation can be beneficial in differentiating milk from healthy cows and SCM cows. The CMT result scoring can be estimated from SCC, Na^+ concentration and K^+ concentration of milk.

Keywords: dairy cows, subclinical mastitis, somatic cell count, sodium, potassium

INTRODUCTION

Milk is a nutrient fluid produced by the mammary gland of many mammals for the nourishment of their young. Clinical mastitis is mastitis with observed abnormalities of the udder or its secretion. Subclinical mastitis is a form of mastitis in which the udder and milk appear normal.

Mastitis especially the subclinical form, is one of the most economically costly and menacing diseases of the dairy industry worldwide, and stands as the major obstacle towards healthy milk production. There are several reports on how mastitis affects the electrical conductivity (EC), pH and electrolyte content in cow milk, but the work done on evaluating them as putative indicators for detecting subclinical mastitis (SCM) has not been widely done (Guha and Gera 2012).

Major mastitis pathogens are generally the bacteria commonly associated with clinical mastitis. These are generally accepted to be *Streptococus agalactiae*,

Staphylococcus aureus, *Streptococcus dysgalactiae*, *Streptococcus uberis*, coliforms (such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.) and *Pseudomonas* spp (Biggs, 2009).

The gold standard for mastitis diagnosis is bacteriological analysis (Biggs, 2009). But a study by Pilla *et al*. (2013) concluded that of lymphocyte, macrophage and polymorphonuclear cell counts can identify inflammatory processes in quarters with low somatic cell count (SCC) that are otherwise considered healthy. They also stated that both cytometric differential cell counting and SCC could represent an excellent diagnostic method to identify inflammatory processes in the mammary gland while avoiding bacteriological analysis.

MATERIALS AND METHODS

Thirty-seven apparently healthy dairy cows were selected from 5 farms in the state of Selangor. The milk from the different quarters were tested with California Mastitis Test (CMT) and these animals were divided into two groups based on the test results: milk samples with noticeable clumping were categorised as positive and those not showing sign of clumping were categorised as negative result. The SCC was counted by direct microscopy method after staining with Wright's stain. Sodium (Na^+) and potassium (K^+) were estimated using an absorption spectrophotometer (AAS) on filtered 1:100 diluted samples.

Comparison of means was done using T-test, Mann-Whitney U test, multivariate test and Kruskal-Wallis test. Pearson's correlation analysis was also conducted.

RESULTS AND DISCUSSION

The difference between cows with positive and negative CMT results were significant ($p<0.05$) for $Log_{10}SCC$, Na^+ content and K^+ content (Table 1). There was also significant ($p<0.05$) difference between the normal, $S1^+$, $S2^+$ and $S3^+$ for variables $Log_{10}SCC$, Na^+ and K^+ content (Table 2). These results showed significant differences ($p<0.05$) between CMT-N and CMT-P with increased Na^+ and decreased K^+ contents. There is also significant ($p<0.05$) difference in $Log_{10}SCC$ between the milk from normal and subclinical groups. The $Log_{10}SCC$ between subclinical groups showed no significant difference and this could be contributed to the varible SCC of milk (cisternal, alveolar or striping) obtained at a single milking (Sølverød *et al.*, 2005). It is assumed that foremilk and residual milk samples have the highest SCC. Due to the limitations in sampling, the samples taken in this study were mostly residual milk after milking; only a few samples were from foremilk. The results for Na^+ and K^+ milk content are in agreement with that of Guha and Gera (2012).

The Pearson's correlation coefficients between $Log_{10}SCC$, Na^+ and K^+ contents are shown in Table 3. These results correspond to that obtained by Guha and Gera (2012), where the $Log_{10}SCC$ had positive correlation with Na^+ and negative

correlation with K^+ content. There was a significant ($p<0.01$) correlation between K^+ content and $Log_{10}SCC$ and Na^+ content.

Table 1: Sodium and potassium content of milk from California Mastitis Test-negative cows.

Parameters	CMT-N	CMT-P
$Log_{10}SCC$	$4.72^a \pm 0.10$	$5.35^b \pm 0.06$
Na^+ content (mean rank)	14.06^a	23.20^b
K^+ content(mg/L)	$1954^a \pm 40$	$1226^b \pm 76$

^{ab}Means between columns with different superscripts differ significantly at $p<0.05$.
CMT-N = California Mastitis Test negative; CMT-P = California Mastitis Test positive.

Table 2: Sodium and potassium content of milk from cows with subclinical mastitis

Parameters	Subclinical mastitis groups			
	Normal	$S1^+$	$S2^+$	$S3^+$
Log10SCC	$4.72^a \pm 0.10$	$5.24^b \pm 0.09$	$5.30^b \pm 0.11$	$5.57^b \pm 0.09$
Na^+ content (mean rank)	14.06^a	20.50^{ab}	21.28^{abc}	29.90^{bc}
K^+ content (mg/L)	$1954^a \pm 40$	$1222^{bc} \pm 187$	$1370^b \pm 79$	$972^c \pm 212$

^{a,b}Means with different superscripts with column differ significantly at $p<0.05$.
$S1^+$, $S2^+$ and S^{3+} = Subclinical mastitis 1^+, 2^+ and 3^+, respectively.

Table 3: Pearson's correlation between $Log_{10}SCC$, sodium and potassium contents

Pearson's correlation	$Log_{10}SCC$	Na^+	K^+
$Log_{10}SCC$	-	0.270	-0.436**
Na^+	0.270	-	-0.688**
K^+	-0.436**	-0.688**	-

** significant correlation at $p<0.01$

CONCLUSION

The study revealed that SSC, Na^+ and K^+ contents of milk can be used as indicators for subclinical mastitis in cows. $Log_{10}SCC$ was found to be significantly correlated with Na^+ and K^+ content and K^+ content could be used as a good predictor of $Log_{10}SCC$.

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PREVALENCE OF COCCIDIOSIS AND IDENTIFICATION OF *EIMERIA* SPP. IN SELECTED GOAT FARMS OF *LADANG ANGKAT*, FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA

Anis Athira Mauli, Rozaihan Mansor & Shaik Mohamed Amin Babjee
[1]*Department of Farm and Exotic Animal Medicine and Surgery*
[2]*Department of Veterinary Pathology and Microbiology*
[3]*Wildlife Research Centre*
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Coccidiosis in goats is a protozoan disease caused by several species of the genus *Eimeria* and of great economic importance. A study was conducted to determine the prevalence of coccidiosis and the *Eimeria* species associated with disease. Thirty faecal samples were collected from goats of 3 different age groups; ≤4 months old, 5 to 11 month old and ≥1 year old, from five selected *Ladang Angkat* goat farms. Among the 150 faecal samples collected, 121 were positive for *Eimeria* oocyst. The prevalence of coccidiosis was 80.7%, ranging from 30 to 100% in these farms, while the prevalence of coccidiosis in each age group ranged from 76 to 86%. Goats aged ≤4 month old had the highest mean oocyte per gram among age groups. The *Eimeria* spp. identified were *E. arloingi* (46.8%), *E. hirci* (14.4%), *E. ninakohlyakimovae* (13.2%), *E. alijevi* (7.6%), *E. pallida* (5.2%), *E. christenseni* (4.8%), *E. jolchijevi* (4.4%), *E. caprovina* (2.8%) and *E. caprina* (0.8%). The most pathogenic species, *E. ninakohlyakimovae* showed the third highest prevalence among *Eimeria* spp. in these *Ladang Angkat*.

Keywords: coccidiosis; protozoan; *Eimeria* spp; goat; *Ladang Angkat*

INTRODUCTION

Coccidiosis is caused by *Eimeria* spp. from the class of *Sporozoasida* under Family of Eimeriidae. Coccidiosis is commonly diagnosed by clinical findings, laboratory diagnosis as well as necropsy examinations. The clinical signs in coccidiosis include diarrhoea, poor weight gain and inappetance. The laboratory techniques typically done for diagnosis of coccidiosis are the modified McMaster and faecal floatation techniques. Healthy animals may have >100,000 oocyte per gram in the faeces (Blood and Radostits, 1989). There were 17 *Eimeria* spp. reported in

different parts of the world (Kheirandish *et al.*, 2014). However, it is very difficult to distinguish the geographical distributions between these *Eimeria* spp. (Chartier and Paraud, 2012). In Selangor, Malaysia nine *Eimeria* spp. were found, which were *E. pallida*, *E. alijevi*, *E. hirci*, *E. ninakohlyakimovae*, *E. arloingi*, *E. caprina*, *E. caprovina*, *E. jolchijevi* and *E. christenseni*. The most prevalent *Eimeria* spp. is *E. arloingi* (71%), followed by *E. ninakohlyakimovae (*67%) and *E. alijevi* (61%) (Jalila *et al.*, 1998). Although the disease caused by *Emeria* can cause mortality in young animals, the main economic importance of this disease is the lower productivity caused by coccidiosis (Blood and Radostits, 1989). This disease caused economic losses through mortality, reduction in growth performance, decreased in productivity and increased treatment costs (Abo-Shehada and Abo-Farieha, 2003; Kheirandish *et al.*, 2014) of livestock. This study was undertaken to determine the prevalence coccidiosis and to identify *Eimeria* spp. in goats in Malaysian farms.

MATERIALS AND METHODS

Farms

Five goat farms of the *Ladang Angkat* of the Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) were included in this study. Farm A is located in Labu, Negeri Sembilan, Farm B in Pulau Meranti, Cyberjaya, Farm C in Dengkil, Selangor, Farm D located in Nilai, Negeri Sembilan and Farm E in Hulu Langat, Selangor, Malaysia.

Animals

Goats of Saanen, Jamnapari and Feral breeds were randomly sampled (n=30) from each farm. The goats were of 3 age groups; more than one-year-old (n=10), 5 to 11 months old (n=10) and kids <4 months old (n=10). One hundred and fifty faecal samples were obtained per rectum from these goats.

Parasitology

Determination of oocyst count and oocyst per gram (OPG) was done by the modified McMaster technique. To facilitate sporulation of oocysts, 2.5% potassium dichromate solution was mixed with 2 g faecal sample. The mixture was sieved and spread thinly on a petri dish and left at room temperature for 2 to 3 days (MAFF, 1986). Sporulated oocysts were examined microscopically using the microscope analyser moticam live module 2.0 at 40 × magnification. The characteristics of the oocyte determined included micropyle cap, length and width of the oocysts. These characteristics are essential for the differentiation of *Eimeria* spp.

Statistical Methods

Two-Way ANOVA was used to determine the significant difference between the OPG and goat age groups. One-way ANOVA was used to determine the difference between the mean of length and width of oocysts among *Eimeria* spp.

RESULTS AND DISCUSSION

Prevalence of coccidiosis in selected goat farms of *Ladang Angkat* was 80.7%. Out of 150 fecal samples, 121 fecal samples were positive with coccidia oocysts.

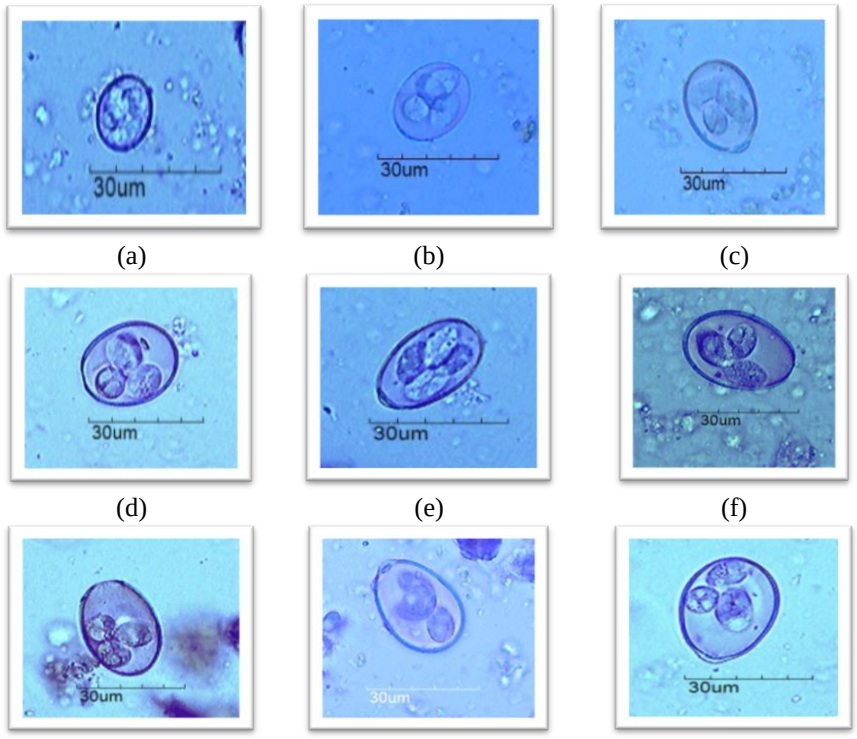
Farm A had the highest prevalence of coccidiosis at 100%, followed by Farm B (96.7%), Farm C (90%), Farm E (86.7%) and finally Farm D (30%). The prevalence of coccidiosis in Farm A was the highest because of high stocking density and high productivity. The prevalence coccidiosis in Farm D was the lowest among the five farms and this could be due to use of anticoccidial drugs in the feed as well as good hygiene practices in that farm (Jalila *et al*., 1998; Harper and Penzhorn, 1999). The prevalence of coccidiosis varied between farms because of variability of factor in these farms that contribute to the coccidial infection. Among these factors were sanitation procedures and ecological conditions in these farms.

Prevalence of coccidiosis in ≤4 month age group of goats was the highest which was 86%, followed by 5 to 11 month age group at 80% and ≥1 year old age group at 76%. The high coccidiosis prevalence in the ≤4 month old goats was due to low resistance towards coccidial infection or low immunity to the *Eimeria* spp. in that age group goats. However, older goats in this study (≥1 year old goats) showed the lowest prevalence due to their well-establishment of immunity towards coccidial infection (de la Fuente and Alunda, 1992). The goats in the 6 to 12 months age group had showed low oocysts output because their immune system was still developing (Daud, 1991).

The mean OPG of ≤4 month old goat was the highest among the goats in the study at 23,344 OPG. This may be is due to these goats being at the early weaning period, when oocyst excretion is heavy (Koudela and Boková, 1998; Chartier and Paraud, 2012). Apart from the stress of weaning, poor hygiene in the pens was also an important contributor to the high mean OPG in the goats and this could result in outbreak of coccidiosis in younger goats (Jalila *et al.*, 1998). The mean OPG of the 5 to 11 month old goat was 8130. The ≥1 year old goats had the lowest OPG at 1424, which is attributable to better immune status in these goats (de la Fuente and Alunda, 1992).

Nine *Eimeria* spp. were identified in this study, which were *E. pallida* (5.2%), *E. alijevi* (7.6%), *E. hirci* (14.4%), *E. ninakohlyakimovae* (13.2%), *E. arloingi* (46.8%), *E. caprovina* (2.8%), *E. caprina* (0.8%), *E. jolchijevi* (4.4%) and *E. christenseni* (4.8%). The sporulated oocysts of the *Eimeria* spp. are shown in Figure 1. The *E. caprovina, E. caprina* and *E. jolchijevi* were the least prevalent in th goats due to the rarity of these species (O'Callaghan, 1989).

The mean of length and width of *Eimeria* sp. as well as its shape is shown in Table 1. These mean length and width of oocysts were compared to MAFF, (1986). One-Way ANOVA analysis revealed there was significant difference in mean of length and width between the different *Eimeria* spp. It can be concluded that each *Eimeria* sp. can be differentiated by the length and width of the oocysts other than the typical morphological characteristics of the oocysts.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 1: Sporulated oocysts of *Eimeria* spp. in goat faeces. (a) *E. pallida*; (b) *E. alijevi*; (c) *E. hirci*; (d) *E. ninakohlyakimovae*; (e) *E. arloingi*; (f) *E. caprovina*; (g) *E. caprina*; (h) *E. jolchijevi*; (i) *E. christenseni*

Table 1: Characteristics of oocyts of *Eimeria* spp.

Eimeria sp.	Shape of oocyst	Length (µm)	Width (µm)
E. pallida	Ovoid	14.02±1.09	10.32±0.57
E. alijevi	Subspherical	17.48±0.67	14.64±1.42
E. hirci	ellipsoidal	20.67±1.82	15.75±1.63
E. ninakohlyakimovae	ellipsoidal	22.68±1.93	17.45±2.86
E. arloingi	ellipsoidal	25.36±4.03	17.45±2.86
E. caprovina	ellipsoidal	29.67±3.23	24.56±2.64
E. caprina	elongated	31.80±1.56	22.20±1.27
E. jolchijevi	broad ovoid	26.96±1.50	19.96±0.69
E. christenseni	ovoid	37.25± 2.18	23.50±1.38

Values are mean ± Std. Dev

Studies on prevalence of coccidiosis and *Eimeria* spp. in specific age groups in goats of *Ladang Angkat* should be considered in future. A larger number of sporulated oocysts are necessary to increase the reliable measurement of length and width of oocysts.

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EFFECTS OF SHORT-TERM OMEGA-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION ON ANXIETY-RELATED BEHAVIOR IN MICE

Arzadtul Bakis Zolkaply, [1]Wan Mastura Shaik Mossadeq & [1]Hafandi Ahmad
[1]Department of Veterinary Preclinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Dietary omega-3 polyunsaturated fatty acid is associated with improvement of brain functions in animals. The best dietary sources of omega-3 fatty acid are fish from ocean and fish oils. Recently, several reports concerning various brain functions including mood variations, depression and underlying mechanisms. Although many studies have been conducted on the effect of omega-3 fatty acid on anxiety and depression in animals, the findings for short-term effect are limited. Therefore, we investigated the effect of short-term dietary omega-3 fatty acid supplementation on anxiety-related behavior in mice. Twenty-four male BALB/c mice were randomly divided into 4 groups. The mice were fed normal pellet added with 6.66% fish oil (Group 1, n=6), normal pellet supplemented with 6.66% soybean oil (Group 2, n=6), normal pellet supplemented with 10% butter (Group 3, n=6), and normal pellet only (Control group, n=6). All mice were fed with the respective treatment diets for 3 weeks and then tested for anxiety behaviour using an elevated plus-maze test. Mice fed omega-3 fatty acid supplementation showed less anxiety ($p<0.05$), whereby they spent more time exploring the open than the close arms of the elevated plus-maze. This indicates that omega-3 fatty acid supplementation activates receptors in the limbic system or facilitates the release of inhibitory neurotransmitters to induce the anxiolytic effect in mice. In conclusion, animals fed with omega-3 fatty acid supplementation exhibited low level of anxiety or high level of calmness. Therefore, omega-3 fatty acid is recommended as a dietary supplementation to induce sedation or relaxing effect in either humans or animals.

Keywords: omega-3 polyunsaturated fatty acid, mice, anxiety-related behavior

INTRODUCTION

Anxiety can be defined as a demonstration of a feeling of uneasiness, apprehension or dread (Blood *et al*., 2007). Stress is normal and can also serve as a prompt to deal with difficult situations. Anxiety and depression can impact individuals of any age. People with depression frequently suffer from anxiety. In animals, anxiety is an

essential emotion, which is conserved during evolution. Animals are adapted to reaction when confronted with danger or threat (Solomons *et al*., 2009). Thus, anxiety enables an individual to escape from dangerous situations and avoid them in the future.

Chemical treatment may be used to treat anxiety in both human and animals but treatment is often accompanied by side-effects such as sleeplessness and reduced appetite (Ferguson, 2001). Therefore, the search for new anxiety-reducing agents from diet supplement has attracted attention of researchers worldwide. An example of dietary supplement that has potential anxiolytic effect is fish oil. Fish oil is a great source of omega-3 fatty acid, which may exert significant influence on major depression via cytokine modulation. These cytokines can have direct and indirect effects on the central nervous system, which include lowered neurotransmitter precursor availability, activation of the hypothalamic-pituitary axis, and alterations of the metabolism of neurotransmitters mRNA (Maes *et al*., 1998).

Therefore, this study was conducted to evaluate the short-term effects of dietary omega-3 fatty acid supplementation on the behavioral measures of anxiety in mice.

MATERIALS AND METHODS

Twenty four male BALB/c mice were randomly divided into 4 groups. The mice were fed normal rodent pellets supplemented with 6.66% fish oil (Group 1, n=6), normal pellet supplemented with 6.66% soybean oil (Group 2, n=6), normal pellet supplemented with 10% butter (Group 3, n=6), and normal pellet only (Control group, n=6). All mice were fed with the respective treatment diets for 3 weeks and then tested for anxiety behaviour using an elevated plus-maze test. The principle of this test was; less anxious mice will explore the open arms more often than the close arms of the elevated plus-maze.

RESULTS AND DISCUSSION

Mice fed with omega-3 fatty acid supplementation showed less anxiety ($p<0.05$) whereby these animals spend more time exploring the open arms compared to closed arms. There was a significant ($p<0.05$) difference in the total open arms entries between groups at with average total entries for fish oil group of 6.45, 5.28 for soybean oil group, 5.67 for the butter group 1.83 for the control group. The result was also significantly ($p<0.05$) different in the total time spent in open arms of the elevated plus-maze, among treatment groups; fish oil group = 45.39 seconds; soybean group = 31.06 seconds; butter group = 31.28 seconds; and control group = 16.17 seconds, which indicate that there were differences in level of anxiety in mice given diets with different supplementations.

The results suggested that dietary omega-3 fatty acid supplementation activates receptors in the limbic system or facilitates the release of inhibitory

neurotransmitters to induce the anxiolytic effect in mice. Dietary omega-3 fatty acid supplementation also reduced the anxiety in mice by decreasing the corticosterone level, which then prevents the hyperactivation of the hypothalamic-pituitary-adrenal axis). Omega-3 fatty acid supplementation also increased the serotonin level which then reduces the bed nucleus of the stria terminal is neuronal excitability thus giving the calming effect to the mice. In addition, the elevated plus-maze is known to increase the concentrations of plasma stress hormones (File *et al*., 1994; Rodgers *et al*., 1999). Exposure to the elevated plus-maze exposure has also significantly increased the adrenocorticotropic hormone response. Thus, these results imply that dietary omega-3 fatty acid sources such as fish oil have an acute effect on anxiety-like behavior in mice.

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PREWEANING GROWTH AND STRONGYLE PATTERN OF BOER GOATS

Azlina Antasha Azman, [1]Mohamed Ariff Omar, [2]Rehana Abdullah Sani & [3]Stefen N.K. Kosmas

[1]Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia,
[3]Boer Goat Breeding Centre, Korporasi Pembangunan Desa,
88999 Kota Kinabalu, Sabah

ABSTRACT

A study was conducted to evaluate the pre-weaning growth and strongyle patterns of two generation groups of Boer goats; F_3 group consisting of F_3 and earlier generations with less than 87% Boer and F_4 group were made up F_4 and later generations with more than 93% Boer composition. Body weight records from birth to weaning of 80 Boer × Feral kids from the two generations were used. Faecal samples of 15 female goats were collected to determine faecal egg count (FEC). Higher body weight and ADG were shown by F_4 than F_3 kids ($p<0.05$), possibly due to the higher Boer percentage in later generation goats. Correlation coefficient among body weight parameters showed positive and strong relationship ($P<0.05$). Faecal egg count of F_3 (126.8 epg) was lower ($p<0.05$) than the F_4 (1286.8 epg) kids. In conclusion the Boer×Feral kids with higher percentage of Boer have better growth rate and conversely, crosses having lower percentage of Boer have lower FEC, which may reflect resistance to strongyle infestations.

Keywords: Boer, pre-weaning, growth, helminth resistance, filial generation

INTRODUCTION

From 2006 to 2011, Malaysia registered a self-sufficiency level for mutton of only 12.9% (DVS, 2013a, b). Consequently, the country imported almost 90% of domestic demand for goat meat. The Government has targeted to increase the self-sufficiency level to 35% by 2015. In order to achieve this target, the industry needs at least 1.5 million does and 50,000 bucks by that year (Mustafa, 2011).

The introduction of exotic goat breeds such as Etawah, Boer and Shami goats from other parts of the world into Malaysia is with the aim of improving the local goat industry. Genetic improvement has been a fundamental part of the many goat

development programmes in developing countries. Malaysia has developed many Boer goat farms owned by government agencies and private enterprises with variable success.

The Boer goat (*Capra hircus*) is a popular meat-type breed well-known for its rapid growth, excellent meat quality and high fertility (Greyling, 2000; Malan, 2000). Mature body weights for bucks and does are 90 to 130 kg and 80 to 100 kg, respectively (Ariff, 2010). These body weight measurements varied because of the influence of genetics, nutrition, health and disease, and management system.

Information on growth and strongyle patterns in Boer goats is scarce and it is postulated that later filial generations have higher helminth resistance than purebred parental generation due to acquired immunity developed. A study was conducted to evaluate the pre-weaning growth performance and strongyle pattern of different generations of Boer goats.

MATERIALS AND METHODS

The present study was conducted using animals sampled from a Boer goat breeder farm owned by Korporasi Pembangunan Desa (KPD) in Papar district in Sabah, Malaysia. The goats were semi-intensively managed in houses with open raised floors. The bucks were 100% Boer imported from Australia while the does were Boer and Feral crosses of different generations. Two groups of animals available in the KPD farm were of F_3 and F_4 groups; F_3 consisting of F_3 and earlier generations with less than 87% Boer whilst F_4 group were made up F_4 and later generations with more than 93% Boer composition. The goats have not been dewormed for at least 6 weeks before the start of the study. Goats were fed concentrate pellets in the morning and then allowed to browse on native pasture around the houses. Cut Napier grass was provided to the goats in the houses for the rest of the day.

Records of birth and weekly pre-weaning body weights from 40 kids each of F_3 and F_4 groups were used in the study. Strongyle pattern was studied in 30 females of F_3 and F_4 groups by determining the faecal egg count (FEC) using Modified McMaster Method from three fresh faecel samples taken at 7-day intervals. Data on growth parameters (body weight and ADG) were analysed with independent t-test to detect differences between generational groups. Strongyle FEC data were analysed with t-test on log10 transformed mean FEC. All analyses were performed using IBM SPSS Statistic Version 22. Chicago, IL: IBM.

RESULTS AND DISCUSSION

F_4 group showed significantly heavier ($p< 0.05$) weight at all ages compared to F_3 group (Table 1). Mean birth weight was 3.2 and 3.8 kg and mean pre-weaning ADG was 131.6 and 249.6 g for F_3 and F_4 groups, respectively. There was significant difference in FEC between F_3 and F_4 generational groups where FEC of F_3 group

was lower ($P<0.05$) than F_4 generation (Table 2). This indicated that F_3 group was more resistant to strongyles.

It is believed that F_4 generation showed higher growth rate than F_3 generation due to higher Boer percentage in the crosses. Boer goats have been reported to show high growth rate in other farms (Malan, 2000; Erasmus 2000; Lu 2002). The F_3 group that possessed lower FEC had lower Boer percentage which could confer higher resistance to strongyles. This is because resistance is a dynamic process of parasite regulation by the host and the faecal egg production is one of the variables which reflect this regulation (Gruner and Lantier, 1995). This also means that Boer goats may not adapt well to our local climate and environmental challenges. This is similarly seen by Browning *et al*. (2010) when they suggested that the general hardiness of the Boer goats across diverse environment has been overstated. Additionally, breeds that are selected for rapid growth and larger body mass may be disadvantaged when resources are inadequate and overall fitness is compromised.

CONCLUSION

It appears that crosses having higher percentage of Boer have better growth rate and conversely, crosses having lower percentage of Boer have lower FEC, which may reflect resistance to strongyle infestation. Foreign breeds of goats purportedly superior to local goats in meat production are likely to require increased management to overcome a lack of hardiness in stressful environments.

Table 1. Body weight and ADG of Boer goats in Koperasi Pembangunan Desa farm

Age/ Parameter	Body weight (kg)	
	F_3 (n=40)	F_4 (n=40)
Birth*	3.2±0.60	3.8 ± 0.77
1 Month*	7.6±1.11	9.5±1.56
2 Months*	11.3±1.74	17.4±1.89
Weaning*	15.0±2.45	26.3±1.91
ADG (g)*	131.6±25.53	249.6±17.02

Values are mean±Std dev. * significant between generations at $P<0.05$. F_3, F_4 = Goats of 3rd generation or earlier and 4th generation or later, respectively.

Table 2. Faecal Egg Count of different generational groups of Boer goats

Generation	N	FEC				p value
		(Mean±SD)	Median	Minimum	Maximum	
F_3	14	126.8±2.01	115.5	33.1	363.1	< 0.05
F_4	15	1286.8±1.97	1366.8	398.1	5248.1	< 0.05

Values are mean±Std dev. FEC = Faecal egg count; F3, F_4 = Goats of 3rd generation or earlier and 4th generation or later, respectively.

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ASSESSMENT OF IMMUNOMODULATORY PROPERTIES OF EXTRACELLULAR AND CLARIFIED PRODUCTS FROM HEAT-KILLED AQUATIC BACTERIA IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*, BURCHELL, 1822)

Halimatu Assaadiah Muhammad, [1,2]Hassan Hj. Mohd Daud & [1]Mohd Fuad Matori

[1]Department of Veterinary Clinical Studies
[2]Aquatic Animal Health Unit
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor,Malaysia

ABSTRACT

The purposes of this study was to assess in vivo the potential of extracellular and clarified ultrafiltration products from *Aeromonas hydrophilla* type 2 and *Vibrio alginolyticus* to enhance immunity in African catfish (*Clarias gariepinus*) by evaluating the survivability of experimentally infected fingerlings. These products were evaluated after inoculation via intramuscular and intraperitoneal routes into the African catfish fingerlings. It was found that *Aeromonas hydrophila* type 2 product caused lower fingerling survivability than *Vibrio alginolyticus* product. The ultrafiltration products of these bacteria caused higher mortality than the concentrated products. Some histopathological lesions were found in the control groups, thus there could be pre-infection in the fingerlings. There was no significant difference between routes of administration. Both bacterial products had the potential to be immunomodulators but further study is needed to identify the types of toxoid and steps to eliminate as many confounding factors as possible.

Keywords: *Clarias gariepinus*, *Aeromonas hydrophilla* type 2, *Vibrio alginolyticus*, ultrafiltration products.

INTRODUCTION

Aeromonas hydrophila is a causative agent known to infect freshwater fish and many tropical or ornamental fish. The pathogenicity of the organism is related to its extracellular product properties (Howard *et al*., 1987, Hirono and Aoki, 1991) and the lipopolysaccharides (Dooley *et al*., 1985). *Vibrio alginolyticus* is usually isolated from finfish, shellfish, seawater and sediment (Gjerde and Boe, 1981); however, it may infect freshwater fish, although the mechanism of its virulence has

not yet been extensively studied. The most probable pathogenicity factors for the organism are collagenase and several extracellular proteases (Blake *et al*., 1980). Thus the objective of this study was to evaluate the potential immunomodulator of *Aeromonas hydrophila* products for the development of alternative treatment and preventive measures for the bacterial catfish diseases.

MATERIALS AND METHODS

Bacterial products that were harvested from *Aeromonas hydrophilla* type 2 and *Vibrio alginolyticus* were designated as clarified ultrafiltration and concentrate extracellular products. Ultrafiltration products were harvested by filtering the tryptic soy broth (TSB) growth medium at 48 hours after bacterial inoculation using 0.22 µm membrane filter and vacuum pump while the concentrate was obtained by washing the membrane filter with sterile TSB and collecting the washed products into sterile eppendorf tubes. While the concentrate was subsequently heat-killed in a 100℃ water bath for 5 minutes followed by three series of freeze-thawing and refrigerated centrifugation at 10,000 rpm and 10℃ for 15 minutes.

The bacterial products were injected either via intraperitoneal or intramuscular routes into 15 fingerlings (average body weight: 10 grams) per group. The control groups were not given any product. All groups were observed for 14 days. Daily mortality was recorded and dead fishes were randomly subjected to post-mortem and the tissues analysed histopathology. Non-parametric statistical analysis were done using SPSS version 20.

RESULT AND DISCUSSION

The fingerlings treated with bacteria products were observed to have reduced swimming activity and appetite, erratic swimming and black body discolouration. A few hours post-treatment, some fingerling showed sloughing of mucus layer and finrot. This observation was similarly reported by Lee *et al*., (1996) and Balebona *et al*., (1998). The main gross findings of dead fishes were petechial haemorrhage at the dorsal and lateral fins and swollen abdomen after treatment with the bacterial products from *A.hydrophila* type 2 and *V.alginolyticus.* The typical lesions found in the abdominal cavity were congestion of the visceral organs mainly the liver, intestines and the kidneys and some ascites. The gills did not shown any gross lesion. These findings were seen in almost all treated fingerlings, but less frequently in the control group. In addition, some dead fingerlings from the group treated via intramuscular route showed various degrees of tissue reaction at the injection site. Some of these findings were also seen in a previous study (Rey *at al*., 2009). Two fingerlings treated with *V. alginolyticus* products showed blood-stained fluid in the anus. The similarity in gross findings between treated and control fingerlings were presence of two to five subserosal cream coloured nodules on either the liver or the

kidneys, which increased with duration post-treatment. Random sample of tissues revealed mononuclear inflammatory cells infiltration that was also seen in the control group. Haemorrhagic lesions were inconsistently observed in these tissues, even within treatment group. A previous study showed that the introduction of extracellular products of *A. hydrophila* into white cachama (*Piaractus bracgypomus*) and hybrid tilapia (*Oreochromis* sp.) caused degenerated erythrocytes with rounded hyaline appearance particularly in the spleen and kidney, plus degeneration and necrosis of other tissues such as the pancreas and the muscular layers of the gut (Rey *et al*., 2009). In the present study, the products of *V. alginolyticus* only caused hyaline degeneration in the kidney tubules.

A. hydrophila caused higher mortality rate than *V. alginolyticus* in treated fingerlings. Since *A. hydrophila* is ubiquitous in freshwater and *V. alginolyticus* is found in marine habitat, this suggest that *A. hydrophila* is an important organism that could infect freshwater fishes.

Ten of 15 fingerlings from the control group treated intraperitoneally with TSB died. The cause of mortality in this group of fingerlings is not known. However, it is suggested that the fingerlings died from nonspecific underlying infection. When bacterial cultures were made from the creamy pus-like subserosal lesion seen during post-mortem, the organism identified was *Citrobacter freundii*.

CONCLUSION

In summary, the extracellular and ultrafiltration products of *A. hydrophila* type 2 and *V. alginolyticus* have the potential to enhance immunity in African catfish. These products are suitable candidates for alternative treatment and preventives measures to be used in fish bacterial diseases.

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BACTERIAL CULTURE FROM MILKING MACHINE IN DAIRY FARMS IN SELANGOR, MALAYSIA

Izza Nadiah Abas & [1]Siti Zubaidah Ramanoon
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Bacterial contamination in milk may originate from infected udder and milking equipment during and after milking. The objective of this study was to determine the presence of bacteria in milking machine cups and on the teats of cows. Two dairy cattle farms in Selangor enrolled as *Ladang Angkat* of the Faculty of Veterinary Medicine (FPV), Universiti Putra Malaysia (UPM) were selected for the study. Swab samples from cups of milking machine and teats of milking cows before (BM) and after milking (AM) were taken aseptically. The total number of samples were 65 cups (BM: 32; AM: 33) and 71 teats (BM: 35, AM: 36). All samples were subjected to culture at the Bacteriology Laboratory, FPV. Subsequently, isolation and identification of the bacteria were done on culture positive samples. The culture results from cups samples were 100% negative BM and 94% positive AM. The bacterial isolates from cups samples AM were predominantly coagulase-negative *Staphylococcus* (27.3%) followed by *Staphylococcus aureus* (24.2%), *Streptococcus agalactiae* (12.1%), *Escherichia coli* (9.1%), *Staphylococcus intermedius* (9.1%), *Klebsiella pneumonia* (6.1%), *Bacillus* sp (6.1%) *and* no growth (6.1%). Teats samples were 94% culture positive BM and 97.2% AM. Coagulase-negative *Staphylococcus* and *Staphylococcus aureus* were predominant in teats samples samples BM and AM. In conclusion, the findings showed that both farms practice adequate premilking cleaning for milking machines but not for the teats of milking cows. The bacteria isolated in this study indicate that the sources could be from the environment, teat skin and infected milking cows. Thus these farms are potentially at risk of mastitis problem. Therefore, the implemention of preventive udder health pogramme is recommended to the farmers.

Keywords: dairy, cattle, milking machine, bacteria, coagulase negative Staphylococci, *Staphylococcus aureus*, mastitis

INTRODUCTION

Milking machine allows farmers to milk more cows than the traditional hand-milking method. Milking machines are generally used at least twice daily and for several hours at a time and so they are probably one of the most used equipment in

dairy farms. Despite this familiarity that dairy farmers have with their milking machines and the fact that they are generating high income from its use, the farmers often neglect the maintenance of this equipment. The cleanliness of milking machines can affect milk quality and influence the development of mastitis in dairy cows by the exacerbation of existing infection or facilitation of intramammary infections (Edmonson, 1997).

This study was undertaken to determine the presence of bacteria in milking machine cups and on the teats of cows.

MATERIALS AND METHODS

Two dairy cattle farms in Selangor enrolled as *Ladang Angkat* of the Faculty of Veterinary Medicine (FVM), Universiti Putra Malaysia (UPM) were selected for the study. Both farms use static automatic milking machines to milk their cows. The practice of cleaning cups of the milking machines was similar for the farms. The machines were cleaned by the circulation method where the detergent is added into the milking line. It is done in three steps that is rinsing, washing and final rinsing. In the study swab samples from the milking machine cups and teats of milking cows before (BM) and after milking (AM) were taken aseptically. The total number of samples were 65 cups (BM: 32; AM: 33) and 71 teats (BM: 35, AM: 36). All samples were stored at 4°C until pending analysis at the Bacteriological Laboratory, FVM, UPM to culture, isolate and identify bacteria. The samples were cultured on blood agar plates by incubating for 18 to 24 hours at 37°C for the bacteria to growth. The bacteria were subcultured to obtain primary cultures and incubated at 37°C for 24 hours. In case of no growth, the plates were reincubated for another 18 to 24 hours at 37°C. Plates that still did not show growth were discarded. Gram staining was done on plates with bacteria growth to differentiate the bacterial species.

RESULTS AND DISCUSSION

The cup BM samples were 100% negative while the AM samples were 94% positive. The bacterial isolates from AM cups samples were predominantly coagulase-negative *Staphylococcus* (27.3%) followed by *Staphylococcus aureus* (24.2%), *Streptococcus agalactiae* (12.1%), *Escherichia coli* (9.1%), *Staphylococcus intermedius* (9.1%), *Klebsiella pneumonia* (6.1%), *Bacillus* sp (6.1%) *and* no growth (6.1%). Teats samples that were positive for bacteria at BM were 94%, and at AM were 97.2%. Coagulase-negative *Staphylococcus* and *Staphylococcus aureus* in order of severity were predominant in both the BM and AM teats samples. Negative bacterial culture of samples from the cups of milking machine before milking indicates that there was adequate cleaning of the milking equipments in both farms.

CONCLUSION

In conclusion, the study showed that both farms practice adequate premilking cleaning for milking machines but not for the teats of milking cows. It is suggested that the sources of bacteria isolated in this study include the environment, teat skin and infected milking cows. Thus these farms have potential risk of facing problems of mastitis. Therefore it is recommended that the farmers adopt good animal husbandry practice and udder health monitoring programme to prevent and control mastitis problem in their farms in the future.

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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM ORAL CAVITY AND CLOACA OF HOUSE CROWS (*CORVUS SPLENDENS*) FROM URBAN AREAS OF SELANGOR, MALAYSIA

Kavindaran Krisnasamy, [1,3]Abdul Rani Bahaman & [2,3]Reuben Sunil Kumar Sharma

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Laboratory Diagnosis
[3]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This study aimed to identify pathogenic bacteria from house crows (*Corvus splendens*) inhabiting urban areas. Oral and cloacal swabs were taken from 21 house crows trapped in housing areas by the Klang Municipal Council Health Department, Selangor, Malaysia. Standard bacterial isolation and identification protocols revealed the presence of 12 and 10 genera of bacteria from the oral cavity and cloaca, respectively. Among the organisms isolated, *Staphylococcus* spp. (76.2 %), *Bacillus* spp. (52. 4 %) and *Corynebacterium* sp. (23.8 %) were the highest in the oral cavity, whereas from the cloaca, *Escherichia coli* (66.7 %), *Staphylococcus* spp. (42. 9 %), *Proteus* spp. (33.3 %) and *Enterococcus* sp. (23.8 %) were predominant ones. The important pathogenic bacteria to humans isolated from both the oral cavity and cloaca were *Escherichia coli* (66.7 %), *Staphylococcus intermedius* (19.0 %), *Pasteurella multocida* subsp. *multocida* (14.3 %) and Y*ersinia* sp. (4.7%). These bacteria were subjected to antibiotic sensitivity test against 6 commonly used antibiotics in humans in Malaysia, namely Gentamicin, Benzylpenicillin, Vancomycin, Ceftriazone, Ciprofloxacin and Rifampicin. The sensitivity test revealed that all the *E. coli* isolates were multidrug-resistant. Close interactions between humans and crows occur in may parts of the country. The zoonotic potential of bacterial diseases from these birds is therefore of prime concern and should be further investigated.

Keywords: crow (*Corvus splendens*), pathogenic bacteria, oral, cloacal, swab

INTRODUCTION

Corvus splendens or commonly known as the house crow originates from central Asia in parts of India, Iran and Sri Lanka and also some parts of Southern China and

Thailand. It is believed that they were initially introduced to Malaysia into oil palm estates as a biological control against the Rhino beetles. Since then, their presence changed from being a biological control to become an agriculture nuisance where they are known to strip fruit trees in orchards and ruin grain crops and even devouring chicks of domestic poultry. Studies have shown that the house crows establish their habitat in urban area close to human activities (Soh *et al.*, 2002). Their nature of being fearless brings them closer to humans. The house crow's population propagated mainly due to the ever-increasing amount of rubbish generated by humans, which the crows use as source of food. House crows pose a threat as carriers of diseases to humans and animals. It has been reported that they can be carriers of viruses such as the West Nile Virus and paramixovirus which can cause Newcastle Disease (Csurhes, 2010). Besides that, many pathogenic bacterial agents have been isolated from crow species. There were reports of humans suffering from episodes of diarrhoea, abdominal pain and fever as a result of consuming food contaminated with crow droppings. In Malaysia, house crow studies have isolated *Campylobacter* spp. and *Mycoplasma* from their gastrointestinal and upper respiratory tract (Ganapathy *et al.*, 2007). Isolation and identification of common aerobic flora in the oral cavity and cloaca of house crows would enable the identification pathogenic bacteria that could be potential zoonotic agents. This would enable the taking of precautionary measures against the dangers of close proximity of crows to humans and domestic animals, from the pathogenic bacteria that they carry. Thus the objectives of present study are to isolate and identify pathogenic bacteria from the oral cavity and cloaca of house crows (*Corvus splendens*) and to determine the antibiotic sensitivity pattern of pathogenic bacteria isolated from the oral cavity and cloaca of these crows.

MATERIALS AND METHODS

Sampling

Twenty-one house crows were trapped in 3 separate locations in the urban areas within the Klang District, Selangor, Malaysia. The traps were prepared by the Health Department of the Klang Municipal Council. Each bird was restrained by their wings and feet. Their oral cavity and cloaca were swabbed using sterile cotton-tipped swabs. The samples were immediately brought to the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for analysis.

Bacterial isolation and identification

Each sample was primarily cultured onto blood agar and MacConkey agar followed by examination of colony and cellular morphology using gram-staining. Various colonies were subcultured onto individual blood agar to obtain pure cultures. The pure cultures were then subjected to the biochemical tests as recommended in *A Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology* by Jang *et al.* (2008) to identify the pathogenic bacteria.

Antibiotic Sensitivity Test

The identified bacteria were subjected to antibiotic sensitivity tests using the Kirby Bauer disc diffusion method. Six antibiotics commonly used in human medicine in Malaysia (National Antibiotic Guideline (2008) of Malaysia) were chosen for the tests. These antibiotics were Gentamicin (CN, 10 µg), Vancomycin (VA, 30 µg), Penicillin (P, 10 µg), Rifampicin (RD, 5 µg), Ciprofloxacin (CIP, 5 µg) and Ceftriazone (CRO, 30 µg).

RESULTS AND DISCUSSION

Twelve and 10 genera of bacteria were isolated from the oral cavity and cloaca of 21 house crows respectively. Tables 1 and 2 show the bacterial genera from the oral cavity and cloaca, respectively. All the isolates were further differentiated into the pathogenic and nonpathogenic bacteria for humans. Tables 3 and 4 presents the pathogenic isolates from the oral cavity and cloaca, respectively.

Table 1: Bacterial Isolates from Oral cavity of 21 House crows

Bacteria	No. of isolates	Occurrence (%)
Staphylococcus spp.	16	76.2
Streptococcus sp.	1	4.8
Corynebacterium sp.	5	23.8
Bacillus spp.	11	52.4
Escherichia coli	4	19.0
Proteus sp.	4	19.0
Yersinia sp.	1	4.8
Chryseobacterium spp.	3	14.3
Acinetobacter sp.	1	4.8
Pasteurella sp.	3	14.3
Galibactirium sp.	4	19.0
Bordetella sp.	1	4.8
Total	54	

House crows in Malaysia are carriers of various intestinal and oral cavity pathogenic bacteria such as *Staphylococcus intermedius*, *Pasteurella multocida* subsp. *multocida* and *Escherichia coli.* Two *Yersinia* sp. were isolated from the oral cavity and the cloaca from two different birds. Biochemical identification of the isolate from the cloaca isolate failed to identify the exact species of the *Yersinia* genus. However, Zartash *et al.* (2013) suggested the *Yersinia enterocolitica* as a possible cause of food-borne disease. *E. coli* were found to be one of the pathogenic bacteria that were multidrug-resistant towards antibiotics commonly used in human

medicine in Malaysia. House crows comes in contact with humans either directly or indirectly, by attacking humans as they walk pass their nest or soiling residential areas with their droppings. Therefore, these crows can easily transmit pathogenic bacteria in their oral cavity and droppings to humans, making them an important agent in the transmission of diseases to humans.

In conclusion, it is imperative that the house crow population in the urban areas be controlled to minimise spread of zoonotic diseases.

Table 2: Bacterial Isolates from Cloaca of 21 House crows

Bacteria	No. of isolates	Occurrence (%)
Staphylococcus spp.	9	42.9
Enterococcus sp.	5	23.8
Corynebacterium sp.	2	9.5
Bacillus spp.	4	19.0
Escherichia coli	14	66.7
Proteus sp.	7	33.3
Yersinia sp.	1	4.8
Acinetobacter sp.	1	4.8
Enterobacter sp.	1	4.8
Klebsiella sp.	3	14.3
Total	47	

Table 3: Occurrence of Pathogenic Bacteria from the Oral Cavity

Bacteria	No. of isolates	Occurrence (%)
Staphylococcus intermedius	1	4.8
Escecherichia coli	4	19.0
Pasteurella multicida subsp. *multocida*.	3	14.3

Table 4: Occurrence of Pathogenic Bacteria from the Cloaca

Bacteria	No. of isolates	Occurrence (%)
Staphylococcus intermedius	1	4.8
Escherichia coli	14	66.7

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C5a ANTAGONISM ON FLEA ALLERGEN-INDUCED DERMATITIS IN MICE

Ku Nurul Atiqah Ku Azir, [1]Mohd Hezmee Mohd Nor & [2]Malaika Watanabee
[1]Department of Veterinary Preclinical Sciences
[2]Department of Companion Animal Medicine and Surgery
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

In this study, the effect of complement 5a (C5a) on mice treated with canine flea antigen was investigated. Mice were randomly divided into four treatment groups and exposed once to 100 µg/mouse flea antigen via intramuscular and intradermal injections. Four groups of 5 male and 5 female mice each were used. Group A was only exposed to flea antigen (negative control). Groups A, B and D were injected subcutaneously, daily beginning day 1 of antigen exposure, with 0.1mg/kg bwt C5a antagonist, 0.1mg/kg bwt prednisolone, and 0.1mg/kg bwt anti-histamine, respectively. Total leucocyte count and percentages of lymphocyte, neutrophil, eosinophil count did not show significant ($p>0.05$) difference among treatment groups. No physical lesions developed in any of the mice while skin samples of the negative control mice revealed a significant hyperplasia of the epidermal layer. The C5a antagonist- and antihistamine-treated mice showed a significant ($p<0.05$) reduction in thickness of the epidermal layer. In conclusion, C5a antagonists may reduce the allergic reaction in animals infected with flea.

Keywords: flea allergy dermatitis, C5a antagonist, leucogram, hyperplasia

INTRODUCTION

Flea allergy dermatitis, also known as flea bite hypersensitivity, is pruritic dermatitis due to sensitisation to antigenic material in the flea saliva by *Ctenocephalides* spp (Wilkerson *et al*., 2004). The allergic inflammation is triggered when bitten by flea with saliva containing histamine-like compounds, proteolytic enzymes and anti-coagulants, which activate the hypersensitivity type I and IV reactions (Lam and Yu, 2009) followed C3 and C5complement activation (Monk *et al*., 2007). Hezmee *et al*. (2012) believed that by inhibiting C5a activation through C5a receptor (C5aR), the severity of the inflammation could be reduced. It has also been suggested that severity of inflammation can most effectively be reduced by blocking upstream inflammation cascade (Mollnes and Kirschfink,

2005). Thus, the objective of this preliminary study was to determine the potency of C5a antagonist in reducing the allergy inflammation in flea allergy dermatitis.

MATERIALS AND METHODS

Twenty male and 20 female 6-week old BALB/C mice were purchased from the Animal Resource Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia and equally divided into 4 groups of 5 male and 5 females each. Six mice (3 males and 3 females) from each group were exposed to 100 µL/ killed flea antigen mouse (cat no B22-66) intramuscularly while 4 from each group were injected intradermally with 50µL of the antigen at the shaved and aseptically cleaned (alcohol) left flank of the mice. The degree of allergic reaction after intramuscular treatment were assessed according to the method previously (Zhao *et al*., 2006) within 3 days and graded according to the following scale; 0 = no clinical sign, 1 = mild, 2 = moderate and 3 = severe clinical signs. In mice treated intradermally, wheal formation was observed immediately after exposure. The scoring of the lesion was based on three points at the edge of the wheal at pre- and post-treatment periods. On day 14 post-flea antigen injection, all mice were subcutaneously treated for 7 days as follows; group A was not treated (negative control), group B was injected with 0.1 mg/kg bwt C5a antagonist, group C with 0.2 µL/mouse prednisolone (Prednikel, 10 mg/mL) and group D with 0.2 µL/mouse with chlorphenoramine maleate (Histamil, 10mg/mL) (positive control). Blood was collected intracardiacally from the mice immediately upon euthanasia. Skin tissues were stained with H&E, examined and the thickness of the epidermis layer was measured using a N-S Element D3-2 under microscope.

RESULTS AND DISCUSSION

The haematology findings showed no significant ($p>0.05$) difference among treatment groups (Table 1). The lack of haematological response may be due the flea strain used and/or duration and method of treatment that may have been insufficient to mount a blood leucocyte response in the treated mice.

The expected allergy reaction toward the flea antigen after intramuscular injection of flea antigen did not occur in this study. However, dermatological lesions were first noted immediately after the intradermal flea antigen injection with formation of wheals of approximate 0.5 cm in diameter in the treated mice. The wheals regressed at 1 day post-treatment. In the treatment group, the wheal were absent even at day 3 post-treatment while it was absent in the control group even at day 4 days post-treatment. All mice showed histopathological changes with hyperplastic epidermis layer, spongiosis with weakening of the tissue structure and infiltration of inflammatory cells, which is, similar to that of an earlier study (Zhao *et al*., (2006). There was a significant ($p>0.05$) difference in the thickness of the

Table 1: Haematological parameters of C5a antagonist- and antihistamine-treated BALB/C mice.

	Group A			Group B			Group C			Group D		
Parameter	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Total Leucocyte (10^9/L)	1.79	2.8	0.1	1.21	2.6	0.2	2.43	6.9	0.1	3.41	5.7	0.9
Lymphocyte (%)	61.13	76	34	69.63	80	42	72.86	83	62	74.43	87	60
Neutrophil (%)	33.00	46	23	28.13	42	18	23.43	39	13	24.29	39	13
Eosinophil (%)	1.38	6	0	1.00	3	0	3.00	6	0	43	2	0
Monocyte (%)	2.38	7	0	1.25	2	0	1.14	4	0	0.86	4	0

Treatments - Group A; negative control, Group B; 0.1mg C5a antagonist /kg bwt, Group C; 0.2 mL prednisolone /kg Bwt, Group D; 0.2 mL chlorphenoramine maleate / kg bwt

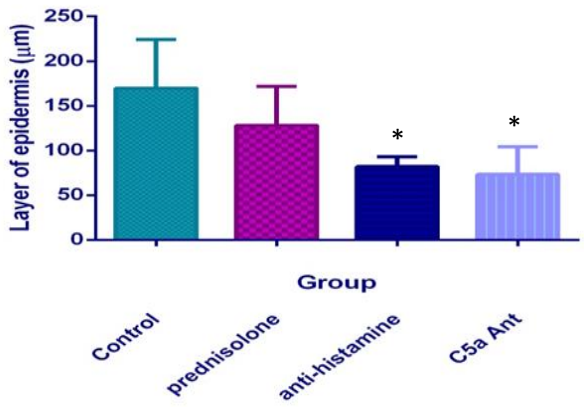

Figure 1. Thickness of the layer of epidermis.
* Significant ($p<0.05$) difference with control.

epidermis layers between the C5a-treated and control group (Figure 1). By comparing the severity of the lesions, it can be concluded that C5a antagonist treatment could reduce the inflammation in mice treated with flea antigen.

In conclusion, C5a antagonist agents are effective in controlling allergic reaction in animals with flea allergic dermatitis. It is suggest that studies on complement inhibition as a method of treating flea allergy dermatitis warrants further investigation.

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EFFICACY OF AN OEDEMA DISEASE VACCINE IN YOUNG PIGS

Jin-Ee Lee, [1]Bee Lee Ong, [1]Raymond Pow Yoon Choo, [2]Mohamed Ariff Omar, [2]Mohamed Ali Rajion, [3]Wei Hoong Loh & [3] Chiou Yan Tee

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Rhone Ma Malaysia (M) Sdn. Bhd., 46100 Petaling Jaya, Selangor, Malaysia.

ABSTRACT

Oedema disease in pigs is caused by Shiga toxin-producing *Escherichia coli* (STEC). Outbreaks of the disease are characterised by swelling of the eyelids, ataxia and sudden death in rapidly growing, healthy post-weaned pigs. Several outbreaks have occurred in Malaysian pig farms about 2 to 3 years ago and control measures taken did not yield satisfactory results. Therefore, a field trial was initiated to test the efficacy of an imported vaccine, *EdemaVac*™, in controlling oedema disease in a commercial pig farm. Forty-eight piglets aged 20 to 25 days were randomly selected from 6 litters and divided equally into vaccinated (single dose oral vaccine) and non-vaccinated (control, placebo) groups. The piglets in the vaccinated group showed increased body weight gain, which was significantly ($p<0.02$) higher by 22% than piglets in the control group. However, survivability rate of the vaccinated group (87.5%) was not significantly ($p>0.05$) higher than the control group (75%). *EdemaVac*™ vaccine appeared to increase body weight gain but not survivability of the pigs.

Keywords: oedema disease, vaccine, pigs

INTRODUCTION

Oedema disease in young pigs is caused by Shiga toxin-producing *Escherichia coli* (STEC). A recent investigation reported outbreaks of clinical oedema disease in 17 farms in Malaysia (Muniandy *et al*., 2014). The outbreaks were characterised by swelling of the eyelids, ataxia and sudden death with high mortality in post-weaned pigs with postmortem lesions of oedema in multiple organs, particularly the colon and stomach. Pure growth of haemolytic *E. coli* possessing F18ab fimbriae and Stx2e gene were isolated from 13 (76%) of these farms. Although many intervention procedures were undertaken by the farmers to include change in feeding strategy and improved hygiene and antimicrobial therapy, the outcomes were not satisfactory.

MATERIALS AND METHODS

EdemaVac™ is a lyophilised freeze-dried vaccine comprising of an avirulent live Stx2e, F18ab+ and non-enterotoxigenic *E. coli* M171 strain. An efficacy trial using vaccine *EdemaVac*™ was initiated in a commercial pig farm with a previous history of clinical diagnosis of oedema disease and positive for STEC isolates. Forty-eight piglets of 20 to 25 day-old from 6 litters derived from mid-parity sows were selected based on age, weight and sex as control (n=24) and vaccinated pigs (n=24) within the litter. The vaccinated group was administered with a dose of reconstituted *EdemaVac*™vaccine given orally, as recommended by the manufacturer. The weight of pigs was recorded at day 1, 17 and 35. The animals were observed daily for mortality and clinical signs of typical oedema disease which included sudden death, swollen eyelids and ataxia.

RESULTS AND DISCUSSION

Vaccinated piglets showed increased body weight gain, which was significantly ($p<0.02$) higher by 22% than non-vaccinated piglets. The survivability of the vaccinated group (87.5%), although not statistically significant ($p>0.05$), was higher than the non-vaccinated group (75%). Therefore, the *EdemaVac*™ was proven successful in increasing the body weight of vaccinated pigs.

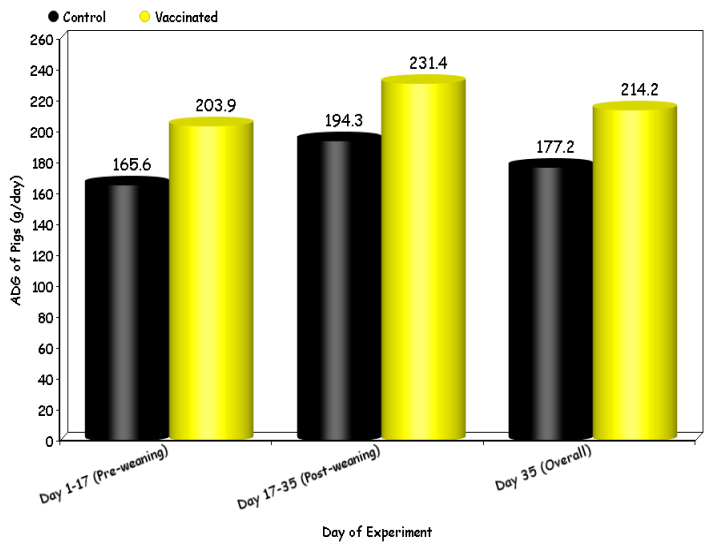

Figure 1: Average daily body weight gain of *EdemaVac*™-vaccinated piglets

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ANTIBACTERIAL EFFECT OF LIME (*CITRUS AURANTIFOLIA*) AGAINST OPPORTUNISTIC BACTERIAL ISOLATES CAUSING OTITIS EXTERNA IN DOGS

Leow Yuan Ting, [1]Arifah Abdul Kadir & [2]Saleha Abdul Aziz
[1]Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Pathology and Microbiology
Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

ABSTRACT

Otitis externa is a regular problem in dogs and usually due to many factors, one of which is bacterial infection. Lime (*Citrus aurantifolia*) is believed to have antibacterial effect. This study was carried out to identify the opportunistic bacteria in the external ear canal of dogs and to study the antibacterial effect of lime (*Citrus aurantifolia*) against the isolates, as well as to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Sixty (60) ear swab samples were collected from both ears of thirty (30) dogs. Coagulase-positive *Staphylococcus* sp. (2 isolates), *Corynebacterium* sp. (4 isolates), and *Pseudomonas aeruginosa* (1 isolate) were isolated and identified. Inhibitory activity of lime juice at different concentrations (10, 25, 50, 100%) against the isolated bacteria was investigated. The mean zone of inhibition for lime ranged from 6.80±0.10 – 33.70±0.26 mm. MIC was mostly observed at 25% concentration of lime juice, which is against *Corynebacterium* sp. (2 of 4 isolates), and coagulase-positive *Staphylococcus hyicus* (2 of 2 isolates). Ten percent concentration of lime juice was determined as the MBC for *Pseudomonas aeruginosa*, *Corynebacterium* sp. (2 of 4 isolates), and coagulase-positive *Staphylococcus hyicus*. The results showed that the antibacterial effect of lime juice and the effect of different concentration of lime juice differed among species of bacteria.

Keywords: dog, otitis externa, lime (*Citrus aurantifolia*), minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Otitis externa is inflammation of the externa ear and it affects 10% to 20% of dogs. The commonly isolated bacteria from dogs with otitis externa are coagulase positive *Staphylococcus* spp., β-haemolytic *Streptococcus* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia* coli (Petrov *et al.*, 2013).

Numerous classes and types of antibiotics commonly selected to treat otitix externa, however, resistance to these drugs by microorganisms have increased. In such cases, traditional medicine serve as an important role to overcome this problem and lime (*Citrus* aurantifolia) is one of the common plants used in traditional healing.
The undiluted lime juice has shown to have effect on *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp., though its effect on gram positive organisms was found more than that of the gram negative organism (Onyeagba *et al.*, 2004). To date, there is lack of published information on the antibacterial effect of different concentrations of lime juice (minimum inhibitory concentration (MIC)) and minimum bactericidal concentration (MBC) against opportunist bacteria isolated from external ear canal of dogs.
Therefore, the ojectives of this preliminary study are to identify the opportunistic bacteria isolated from external ear canal of dogs; to evaluate the antibacterial effects of lime juice, as well as to determine the MIC and MBC of lime juice against the opportunistic bacterial isolates from otitis externa of dogs.

MATERIALS AND METHODS

Ear swab sampling
Sixty (60) samples were taken from both external ear canals of 30 healthy dogs.

Isolation and identification of bacteria
Bacterial isolation and identification was conducted at the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Samples were streaked and grown on blood agar supplemented with 5% horse blood and MacConkey Agar. The colonies were then sub-cultured onto blood agar. Gram staining was done to identify bacteria morphology. Based on the morphology, biochemical tests were carried out according to standard bacteriology procedures.

Preparation of extract
The lime fruit was washed with distilled water and cut in half with a sterile knife on a sterile chopping board. Lime juice was pressed out into a sterile beaker. Filtration was done using layers of sterile gauze and 0.22µm microfilter. The extract was stored at 4ºC until analyses. Four different concentrations of lime juice which is 100%, 50%, 25% and 10% were prepared by diluting lime juice stock with sterile distilled water.

Susceptibility test
Agar well diffusion method was used for susceptibility test. Each bacterial suspension was made in sterile distilled water and adjusted to 0.5 McFarland standard. Each Mueller Hinton (MH) agar plate was uniformly seeded with the test organism by dipping sterile swab in standardised test suspension and streaked onto the agar plate (Owhe-Ureghe *et al.*, 2010). Wells were punched in the agar media

using the end of glass pasteur pipette. Various concentrations of extract and sterile distilled water (as negative control) were pipetted into each well to fullness. For positive control, 10µg gentamicin disc was placed in the middle of the plate. The setup was allowed to stabilise for 3 h before incubated at 37ºC for 24 h (Owhe-Ureghe *et al.*, 2010). The mean zone of inhibition was measured using vernier calliper.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) was determined by broth dilution method. A hundred µL of each standardised bacterial inoculum was mixed with crude extract of 100%, 50%, 25% and 10% concentrations in 96 well plate. The plate was then incubated at 37ºC for 24 h. The lowest concentration of the extract which showed no turbidity was recorded as the MIC (Unnisa *et al.*, 2012). After incubation for determining the MIC, inoculum from each wells (of 96 well plate) was streaked onto Mueller Hinton (MH) agar and incubated at 37ºC for 24 h. The lowest concentration that prevented bacterial growth was recorded as the MBC (Aibinu *et al.*, 2007).

RESULTS

Bacterial isolation and identification was conducted on 60 ear swab samples and opportunistic bacteria were selected. The results are shown in Table 1. *Staphylococcus hyicus* isolate 1 and *Staphylococcus hyicus* isolate 2 were named to differentiate between two isolates of *Staphylococcus hyicus* from different samples. Also, *Corynebacterium* sp. isolate 1, *Corynebacterium* sp. isolate 2 and *Corynebacterium* sp. isolate 3 were named to differentiate the three isolates of *Corynebacterium* sp. which were of different colony morphology but could not be identified based on Bacteria Identification Manual (2008).

Table 1: Opportunistic bacteria isolated from external ear canal of dogs

Bacteria sp.	Gram stain characteristics	Number of isolates
Coagulase positive *Staphylococcus hyicus*	Gram positive, small cocci in pairs	2 isolates
Corynebacterium auriscanis	Gram positive, short rods in 'V' or 'Y' configuration	1 isolate
Corynebacterium sp.		3 isolates
Pseudomonas aeruginosa	Gram negative, rod in single	1 isolate

Antibacterial activity of different concentrations of lime juice was studied and agar well diffusion method was adopted to determine the antibacterial potency. The mean diameter of inhibition zone produced by different concentrations of lime juice

against the opportunistic bacteria isolates were measured in millimetres (mm) and recorded.

For antibacterial effect of undiluted lime juice, the largest zone of inhibition was produced by *Corynebacterium auriscanis*, which was 33.70mm. On the other hand, the smallest zone of inhibition, which was 12.10mm, was produced by *Corynebacterium* sp. isolate 3. Based on Kruskal–Wallis analysis, the mean zones of inhibition were significantly different ($p<0.05$) for different bacterial isolates, which suggested that lime at different concentrations inhibited different species of bacteria. Regardless the species of bacteria, undiluted lime (100% concentration) gave the highest mean zone of inhibition as compared to other concentrations.

Quantitative evaluation of antimicrobial activity was carried out via broth dilution method using 96 well plate and minimum inhibitory concentration (MIC) was determined. The inoculums from the wells were then cultured on MH agar plate to determine the minimum bactericidal concentration (MBC). Four (57%) bacteria showed MIC values at 25% lime concentration whereas for MBC, 71.4% (n=5) bacteria isolates were killed at 10% lime concentration.

DISCUSSION

Coagulase positive *Staphylococcus hyicus*, *Pseudomonas aeruginosa* and *Corynebacterium* spp. that were isolated are in agreement with Petrov *et al.* (2013) that these bacteria are the normal flora commonly isolated from external ear canal of dogs. In a case control study done by Aalbæk *et al.* (2010), *C. auriscanis* and other coryneform bacteria may be regarded as opportunistic bacteria since their occurrence in ears of dogs with otitis externa is higher than that of healthy ears.

For the susceptibility test, it was observed that different bacteria had different measurement of inhibitory zones. This concluded that lime at different concentrations inhibited different species of bacteria. Based on the inhibitory activity of bacteria at 100% lime concentration, *C. auriscanis* has the highest zone of inhibition, thus, it is said to be most susceptible to the undiluted lime juice as compared to other bacterial isolates. When compared to the mean zone of inhibition produced by different concentrations of lime juice disregarding the bacterial species, 100% (undiluted lime juice) showed the highest inhibitory zone. Hence, it is suggested that the undiluted lime juice has the highest or strongest antibacterial effect as compared to other concentrations (50%, 25% and 10%). Onyeagba *et al.* (2004) also studied the antimicrobial effect of undiluted lime juice and it was also proven that all the tested microorganisms (*Staphylococcus aureus*, *Bacillus* spp., *Escherichia coli* and *Salmonella* spp.) were susceptible to undiluted lime juice.

Flavonoid is one of the phytochemicals that is synthesised in response to microbial infection of the lime plant, which was proven to show antimicrobial effect (Bansode and Chavan, 2012). This study showed that undiluted lime juice has the highest antimicrobial activity compared to other concentrations which were diluted

with sterile distilled water. This might be due to the different concentrations of flavonoid that was present in different concentrations of lime juice.

The MIC value showed that the value was at the 25% concentration of lime juice for *Corynebacterium* sp. isolate 1, *Corynebacterium* sp. isolate 3, *Staphylococcus hyicus* isolate 1 and *Staphylococcus hyicus* isolate 2. This explains that at 25% lime concentration, this is the minimum lime concentration that can exhibit inhibitory activity against the bacterial isolates. On the other hand, MBC value was observed at 10% concentration of lime juice for *Pseudomonas aeruginosa*, *Corynebacterium* sp. isolate 1, *Corynebacterium* sp. isolate 2, *Staphylococcus hyicus* isolate 1 and *Staphylococcus hyicus* isolate 2. This suggests that 10% concentration is shown to be the minimum concentration that can kill these bacteria. According to Perilla *et al.* (2002), turbidity of the wells can be affected by the presence of sediments and inoculum that is not precisely standardised to 0.5 McFarland's standard. As for the MBC determination, the growth of bacterial isolates on the agar can be affected by the quality of the culture medium and also the incubation condition in the incubator.

Future studies should be directed to determine the effective concentration of lime juice and to conduct toxicity tests.

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CANCER CHEMOTHERAPY IN DOGS AND CATS IN MALAYSIAN SMALL ANIMAL PRACTICE

Loh Choy Yean, [1]Rasedee Abdullah, [2]Gayathri Thevi Selvarajah & [3]Mohamed Ariff Omar

[1]Department of Veterinary Laboratory Diagnosis
[1]Department of Veterinary Clinical Studies
[3]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Veterinary practitioners are regularly using chemotherapeutic agents in cancer treatment. Exposure to these cytotoxic agents during preparation, administration, and after care is a potential health risk to veterinary practitioners and patients. Currently there is little information on the use of cytotoxic agents in Malaysian veterinary practices. Thus, the objectives of this study were to determine the type and frequency of cytotoxic drugs use in cancers treatment of cats and dogs and to determine the safety measures and availability of facilities in handling of cytotoxic drugs in Malaysian small animal veterinary practices. The study was conducted by questionnaire. Consent to participate in the study was obtained from small animal veterinary practices by telephone. The questionnaires were sent to 233 practices via email or post. The survey data was subjected to statistical analysis using SPSS version 20.0. Chi-square or Fisher's exact test and Mann-Whitney-U test were performed where necessary. P-value<0.05 was considered statistically significant at 95% confidence interval. Fourty-two (47%) of the practices, which provide cancer chemotherapy, returned the questionnaires, however 70 practices responded not handling chemotherapy at the premises via telephone conversation. The most frequent cancer treated with chemotherapy was canine transmissible venereal tumour. Parenteral cytotoxic drugs were prescribed in all the practices (n=42) and six of them prescribed oral cytotoxic drugs during the preceding 12 months. Median frequency for the use of parenteral cytotoxic drugs was once every 3 months and oral cytotoxic drugs was at least once a day. Practices administering chemotherapy used vincristine to treat cancers in dogs and cats. Most of the practices are not well-equipped to handle and administer parenteral cytotoxic drugs. The majority of practices used indwelling catheter to administer parenteral drugs while a minority used butterfly catheters and needles. The study reflected the poor safety measures and lack of facility in handling cytotoxic drugs in the Malaysian small animal practices.

Keywords: chemotherapy, questionairre, Malaysia, vincristine, transmissible venereal tumour

INTRODUCTION

Cancer chemotherapeutic agents commonly known as cytotoxic, anticancer, antitumour or antineoplastic drugs have been used to treat cancer in people and companion animals particularly dogs and cats (Cave *et al.* 2007). Exposures to chemotherapeutic drugs are potential occupational health hazards especially to the veterinarian and the staff handling these chemotherapeutic agents (OSHA, 1999). Since the drugs can become increasingly hazardous to animal handlers with frequency of use, there is need to regulate their uses in veterinary practices. Among precautions that should be taken are proper drug storage, handling and delivery that adhere to strictly safety protocols. This would reduce health risk to the veterinarians and staff from unnecessary exposures (Crump and Douglas, 2011).

Currently in Malaysia, information on the use of chemotherapeutic agents among veterinary practitioner lacking, particularly on the types and frequency of chemotherapeutic agents used, knowledge of users on the properties of chemotherapeutic agents and application of safety measures and availability of facilities for handling, storage, delivery and disposal of chemotherapeutic agents. Thus the objective of this study was to determine the status of chemotherapeutic agent usage in the Malaysian small animal clinics. This study should reflect the awareness of veterinary practitioners on the risk of cytotoxic agent exposure.

MATERIALS AND METHODS

Subject

A list of registered veterinary surgeons under Malaysian Veterinary Council was obtained with the approval of registrar and secretary of this council. The data was fully updated in 2013 and the survey was conducted based on this data. The data obtained include the name of the veterinary surgeons, veterinary practices' names, phone numbers and address. The data was rearranged according to the small animal practices in each state in Malaysia by using Microsoft Excel spreadsheet and they are total of 233 veterinary practices in the list.

Questionnaire

A questionnaire was developed with modifications to the originally compiled and first used by Cave *et al*. (2004) to evaluate cytotoxic drug use in treatment of dogs and cats with cancer by UK veterinary practices. The questionnaire were organised into five sections: (1) Information on the veterinary practice; (2) use of oral anti-cancer drugs during the last 12 months; (3) use of parenteral anti-cancer drugs during the last 12 months; (4) treatment recommendations for cancer in dogs and cats and (5) personal beliefs in chemotherapy.

Procedure

The study was conducted between 8 January and 8 February 2014. Consent to participate in the study was obtained from small animal veterinary practices by telephone. The reasons for not practicing cancer chemotherapy were obtained through telephone survey. Final validated questionnaires were sent to respondents that practice chemotherapy through e-mail.

Data analysis

Overall veterinary practices information obtained included total number of veterinary surgeons in the practices, total number of veterinary surgeons attended courses or training specifically on chemotherapy and total caseload of the veterinary practices. Frequency of chemotherapy provided to dogs or cats is calculated. Association of caseload of cat with the chemotherapy offered to cat was obtained using the Mann-Whitney U tests when the variables were not normally distributed.

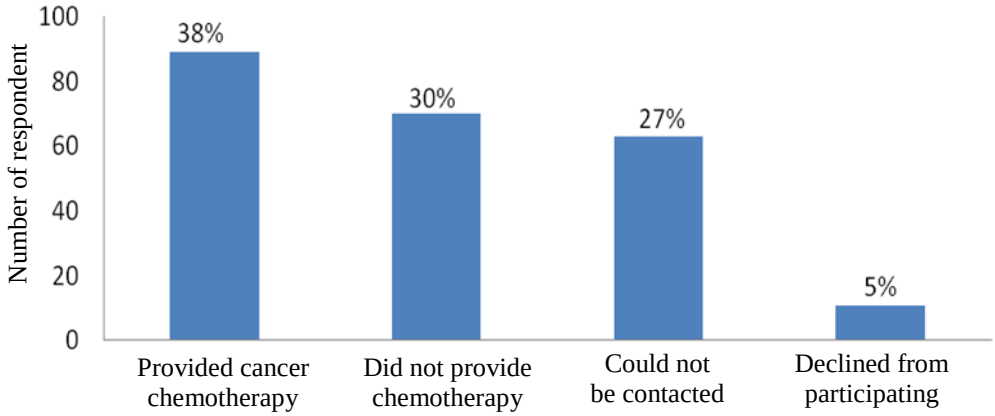
Percentage, median and frequency the practices prescribing oral and parenteral cytotoxic drugs are identified and proportions of type of cytotoxic drugs compared. Frequency of each safety measures that was taken were categorised as ordinal as the responses were according to "never, sometimes, always" and also some of the responses were according to "yes and no". Chi-square test was employed to identify the significant different between the responses of each measures. Method of storage of cytotoxic drugs and the method of disposal of excreta from cats and dogs were also identified. Cancer of dogs and cats that are commonly treated by chemotherapy were identified. Personal belief and attitude of veterinary surgeons in chemotherapy section including adequacy of the precautionary measures taken in the practices, use of anti-inflammatory drugs in cancer treatment, interest in workshop related to safety precaution for cytotoxic drugs used in chemotherapy, plan on chemotherapy facilities improvement and also their familiarity on rescue therapy in chemotherapy treatment were obtained.

RESULTS

Out of 233 veterinary practices, 38% (n=89) of practices provided cancer chemotherapy 30% (n=70) did not while 5% (n=11) refused to participate and the remaining 27% (n=63) could not be contacted (Figure 1).

Chemotherapy

All practices provided cancer chemotherapy to dogs while 13 provided chemotherapy to cats. Only 6 small animal practices practicing chemotherapy had prescribed oral cytotoxic drugs during the study period. The median frequency for prescription of oral cytotoxic drugs of these practices during the study period was at least once a day, reflecting high exposure to oral cytotoxic drugs. Three practices prescribed chlorambucil, 2 prescribed mephalan, 1 each prescribed cyclosphosphamide and isotretinoin. During handling oral cytotoxic drugs, 50% of clinics used gloves, 33.3% while the other 16.7% used glove occasionally.



In the storage of oral cytotoxic drugs, 50% of the respondents showed the drugs were stored in the refrigerator while the other 50% stored the drugs in closed cabinets.

Parenteral cytotoxic drugs were prescribed in all clinics (n=42) during the preceding 12 months. The median frequency for the use of parenteral cytotoxic drugs was once every 3 months. All the veterinary practices prescribed vincristine (n=42), followed by vinblastine (n=5), cyclophosphamide (n=5), carboplatin (n=3), doxorubicin (n=3), epirubicin (n=1), L-asparaginase (n=1), 5-fluorouracil (n=1), and cystosine arabinase (n=1).

Most (47.6%) of the practices providing chemotherapy used indwelling catheter in administration of parenteral cytotoxic drug.

To dispose faeces from dogs and cats receiving chemotherapy, most of the practices (37.3%, n=19) washed the faeces down the drain, 37.3% (n=19) dispose into normal dustbin, 9.8% (n=5) dispose in clinical waste bin and 7.8% (n=4) practices flushed the faeces down to the toilet. Another 7.8% (n=4) of the respondents did not know how was the faeces was disposed.

Fifty-five percent of the respondents believed that precautionary measures taken at the practice were adequate for chemotherapy. However, the majority respondents 67% (n=28) were interested to attend workshop on safe use of cytotoxic drugs and plan to improve chemotherapy facilities in their practices.

Cancer cases

Cancers of dogs commonly treated by chemotherapy were transmissible venereal tumour (59%), followed by lymphoma (16%), mast cell tumour (13%), mammary gland tumour (6%), squamous cell carcinoma (4%), osteosarcoma (1%) and leukemia (1%). Cats most commonly treated cancer by chemotherapy was lymphoma (46%), followed by mammary gland tumour (15%), squamous cell carcinoma (11.5%), leukemia (11.5%), mast cell tumour (8%) and hemangiosarcoma (8%).

There were 40.5% (n=12) of the respondents not using any anti-inflammatory drugs as palliative treatment in cancer treatment. 30.9% (n=17) respondents were using ant-inflammatory drugs alone for cancer treatment and 28.6% (n=13) respondents use anti-inflammatory drugs in combination with cytotoxic drugs.

In addition, 55% (n=23) of the respondents were unaware of rescue therapy, 31 % (n=13) of the respondents not quite familiar with the rescue therapy and 14% (n=6) of the respondents were familiar with rescue therapy.

DISCUSSION

The study showed the oral cytotoxic drug was not commonly used in Malaysian small animal veterinary practices. These may due to several factors including preference of veterinary surgeons in Malaysian veterinary practices which may cause by prejudice against the effectiveness and reliability of anticancer oral therapy. Other reasons for not practicing cancer chemotherapy are inadequate facilities or lack of diagnostic tools for cancer diagnosis and inadequate or unskilled personnel. Another reason for the lack of provision of chemotherapy by the practices is low cancer case-load.

The most common type of parenteral cytotoxic drug that used by Malaysian small animal practices was vincristine. Most drug handlers in practices were not equipped with appropriate mask to protect them from cytotoxic drug exposure. In general, proper personal protective equipment including non-permeable gown, goggles or other eye protection, proper mask were not available in these practices. This put the drug the handlers in jeopardy to cytotoxic drugs exposure through inhalation, ingestion and direct contact. Although most of the handlers wear gloves, it was not protective enough against exposure to cytotoxic drugs. Thus the findings suggest that Malaysian small animal practices are poorly equipped to deal with cytotoxic drugs in cancer chemotherapy.

In the administration of cytotoxic drugs, although the choice of catheters is dependent on the volume of drugs, butterfly catheters seem not to be an ideal choices for vesicant drugs since movement and temperament of the animals are always unpredictable. Furthermore, doxorubicin which can cause severe tissue reaction is recommended to be given by indwelling catheters only.

Transmissible venereal tumour was most commonly treated cancer by chemotherapy in the practices, which is also a reflection of widespread use of vincristine in these practices. This disease is commonly treated by chemotherapy because it responds well to treatment (Withrow *et al*., 2013).

Lymphoma is one of the most common cancer seen in cats and there was evidence that feline immunodeficiency virus infection increases incidence of lymphoma. In general, cats tolerate lymphoma chemotherapy well. The study also showed that most clients are generally happy with their choice for chemotherapy because the quality of life of their animals generally improved following treatment (Withrow *et al.*, 2013).

Although most small animal practitioners are of the opinion that their practices have taken adequate safety measures in dealing with cytotoxic drugs, the survey showed that this is not true.

CONCLUSION

The most common cytotoxic drug used in cancer chemotherapy was vincristine. The median frequency of parenteral cytotoxic drugs used was once every three months. The most common cancer that was treated by chemotherapy was transmissible venereal tumour. Poor safety measures and lack of chemotherapy facilities in veterinary small animal practices in Malaysia were reflected in this study.

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SEROPREVALENCE AND MOLECULAR DETECTION OF CANINE LEPTOSPIROSIS IN KLANG VALLEY, MALAYSIA

Low Kae Neng, [1, 3]Abdul Rani Bahaman, [2]Khor Kuan Hua, & [2]Lau Seng Fong

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Clinical Studies
[3]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

A seroprevalence and molecular detection study on canine leptospirosis was conducted among the dog population (n=57) in selected areas of Klang Valley, Malaysia. Blood samples from stray dogs (n=31) were obtained from two local animal shelters whilst pet dogs (n=26) were randomly selected from nine local private veterinary clinics. Seroprevalence was determined by microscopic agglutination test (MAT) on the serum samples, which also tested against ten *Leptospira* serovars, while polymerase chain reaction (PCR) assay was performed on the plasma samples of pet dogs using primer targeting the 531 bp pathogenic-specific *Leptospira* gene. Three of 57 dogs (5.3%) were tested positive for *Leptospira canicola* and one (1.7%, n=1/57) was positive for *L. icterohaemorrhagiae* based on MAT at the titer of 1:80. The seroprevalence of eight other Leptospiral serovars was not evident in this study. Besides, 3.9% (n=1/26) of the pet dogs was tested positive for pathogenic-specific *Leptospira* by the PCR assay. In conclusion, pet dogs in this study have a higher serovar *L. canicola* seroprevalence than the stray dogs. Microscopic agglutination test showed higher detection of canine leptospirosis in pet dogs with overall 11.5% (3/26) positive results than PCR assay with 3.8% (1/26) positive results.

Keywords: canine leptospirosis, seroprevalence, molecular detection, MAT, PCR, serovar

INTRODUCTION

Leptospirosis, also known as rat urine disease, is an infectious disease caused by the motile spirochetal bacterium of the genus *Leptospira* (Gitton *et al*., 1994; Bharti *et al*., 2003). Leptospirosis is a worldwide zoonosis (Pappas *et al*., 2008), which has been recognised as one of the important zoonoses and animal diseases in Malaysia, affecting domestic animals and wildlife as well as humans, causing substantial

production losses and posing great threats to public health (Bahaman and Ibrahim, 1988). Nowadays, dogs have become popular pets that are becoming one of the risk factors for human leptospiral infection (Chandrasekaran and Pankajalakshmi, 1995). Investigation on leptospirosis in dog population in Malaysia is still limited as dogs are regarded as pets and assumed to have low prevalence for leptospirosis (Bahaman and Ibrahim, 1988). Most pet dogs are now vaccinated against serovar icterohaemorrhagiae and serovar canicola because they are the major causes of canine leptopspirosis throughout the world (Michna and Campbell, 1970; Sullivan, 1974). Vaccination may reduce clinical cases of leptospirosis, however it cannot fully protect the animals from infection. To date, there is no research done to demonstrate cross-protection offered by vaccines against various serovars that may be more prevalent in the dog population. Therefore, the objectives of this study were to determine the seroprevalence of canine leptospirosis among stray and pet dogs in Klang Valley, Malaysia and detect canine leptospirosis in the pet dogs by serological and molecular methods.

MATERIALS AND METHODS

Dogs and Sample Collection

Thirty-one dogs were randomly selected from the Society for the Prevention of Cruelty to Animals (SPCA) Selangor and Paws Animal Welfare Society (PAWS) to represent the stray dog population. Another 26 dogs were randomly selected from 10 private veterinary clinics located in Klang Valley, Malaysia to represent the pet dog population. The dogs were restrained and 2 mL of blood collected from the cephalic vein and placed in EDTA (Ethylenediaminetetraacetic acid)-anticoagulant and plain tubes. Samples were immediately sent to the Bacteriology Laboratory at the Faculty of Veterinary Medicine, Universiti Putra Malaysia and then centrifuged at 250 × g for 10 minutes. Serum and plasma were stored at -30°C until further use.

Microscopic Agglutination Test

Leptospires from 10 different serovars were grown in EMJH medium and incubated at 30°C for 5 to 7 days served as live antigens for the antigen-antibody agglutination test. Microtiter plate wells were filled with 50 µL of PBS. Ten µL of serum sample were added to the wells from the second row to the second last row and serially diluted. Ten µL of positive control serum were added to the wells of the last row. Finally 50 µL of leptospira culture were added to each well and mixed thoroughly on a microshaker and incubated overnight at 30°C. The wells of the first column are the negative controls while the wells of the last row the positive controls. A drop of mixture from each well was dropped on a microscope slide and examined under the dark-field microscope for evidence of microscopic agglutination. The end-point antibody titer for each sample was then recorded.

Polymerase Chain Reaction Assay and Agarose Gel Electrophoresis

DNA extraction and isolation of genomic DNA from plasma were carried out using Wizard® Genomic DNA Purification Kit (Promega, USA), using the recommended protocols, for the DNA templates. The preparation of PCR product from the DNA templates was done with Top Taq Master Mix cocktail solution together with pathogenic specific leptospira primers for gel electrophoresis. Agarose gel was prepared by mixing 1.5 g of HyAgarose™ powder into 100 mL of 0.5% TBE solution and allowed to be solidified at room temperature. Electrophoresis tank was filled with 0.5% TBE solution flooding over the solidified agarose gel. Two microlitres of loading dye was then mixed with 5 µL of PCR product and carefully loaded into the wells of the agarose gel. Electrophoresis was then run at 100 V 50 mA for 90 minutes. The agarose gel was stained in ethidium bromide solution and viewed under UV light for evidence of leptospiral DNA bands.

RESULTS AND DISCUSSION

Serum samples from both stray and pet dogs were tested serologically against 10 leptospiral serovars, which are *Leptospira canicola, L. Pomona, L. icterohaemorrhagiae, L. australis, L. andaman, L. bataviae, L. hebdomadis, L. tarassovi, L. grippothyphosa and L. shermani*. From the study, the seroprevalence for *L. canicola* was 5.3% (n=3/57) and for *L. icterohaemorrhagiae* it was 1.8% (1/57). Three samples from the pet dog group showed positive results towards *L. canicola* and one sample tested positive against *L. incterohaemorrhagiae* using MAT at the titer of 1:80 (Figure 1). However, the animals were tested negative to the other eight serovars.

Plasma samples from the pet dogs (n=26) were subjected to PCR assay to detect pathogenic leptospira using specific primer targeting the 531 bp of the pathogenic leptospira gene. From the study, one dog (3.9%, 1/26) was tested positive for pathogenic *Leptospira*.

Differences in prevalence *leptospira* infection in this study and that reported by others may to be due to several factors. The first factor is the difference in sample size used in these studies. In order to more accurately calculate the required sample size in a descriptive study, several important criteria such as level of precision and confidence and degree of variability must be taken into account. The larger the sample size, the greater the precision and thus, greater power for a given study design to provide accurate results (Kasiulevičius *et al*., 2006). Our study involved 57 dogs, which may not be adequate to represent the entire dog population in the area under study. The second factor could be the different MAT cut-off titer references used the various studies. Our study used a cut-off titre of 1:80 as the significant criteria. One diagnostic titre in this study however, may not represent the true prevalence of the disease. The third factor is the environment. The environmental factors cover a wide range of risk factors that play major roles in the transmission and prevalence of canine leptospirosis. According to Lau *et al*. (2010), the combination of climate change, flooding, population growth and urbanisation

will almost certainly lead to drastic escalations in leptospirosis. Certain areas like urban slum and low-lying areas have higher risks for leptospira infection. Since, dedicated non-government organizations (NGOs) and government sectors such as PAWs, SPCA and Dewan Bandaraya Kuala Lumpur (DBKL) began to control the stray population, the spread of potential infectious diseases among the dog populations in Klang Valley, Malaysia had reduced and been prevented.

CONCLUSION

In conclusion, the seroprevalence of canine leptospirosis in the Klang Valley, Malaysia is 7.0% (n=57). The major leptospiral serovars found were *L. canicola* and *L. icterohaemorrhagiae*. Microscopic agglutination test and PCR assays have detected canine leptospirosis of different clinical stages in this study.

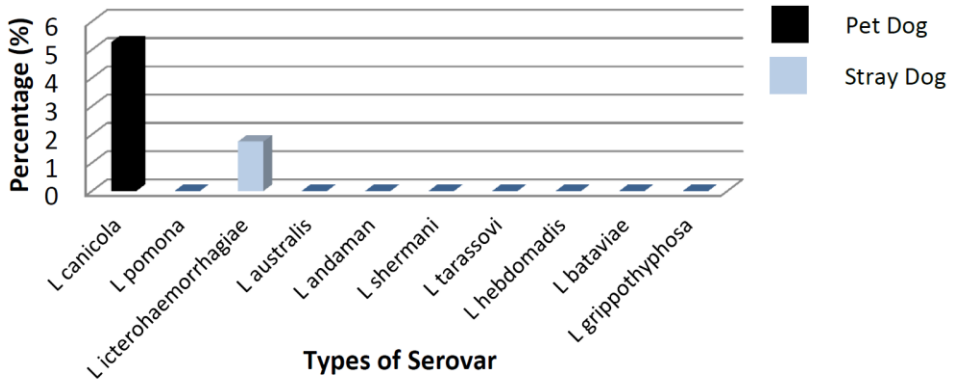

Figure 1: Seroprevalence of leptospirosis tested in stray and pet dogs

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EFFECT OF SLAUGHTER TECHNIQUES ON CHICKEN MEAT QUALITY

Maslinda Elliana Kamaruddin, [1]Noordin Mohamed Mustapha [1,3]Saleha Abdul Aziz, [2]Mohamed Ariff Omar, [4]Awis Qurni Sazili & [5]Muhmad Kamarulzaman Muhmad Sarif

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Preclinical Sciences
[3]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
[4]Department of Animal Science
Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[5]Kerabat Processing House Sdn Bhd, Pedas, Negeri Sembilan, Malaysia

ABSTRACT

An experiment was conducted based on assumption that increasing concentration of residual blood from imperfectly halal slaughtering technique would lead to lower wholesomeness of the meat. Bleeding time, death time, blood volume lost, meat pH, meat colour, spoilage microbe count and odour score were determined immediately at post-slaughter. Twelve commercial broilers weighing 1.8 to 2.0 kg of the same breed, age and farm management were used. The birds were electrically stunned at 0.28 mA and subjected to two slaughtering methods; perfectly halal slaughtering technique (H) and the imperfectly halal slaughtering technique by leaving the carotid arteries intact (NH). The different slaughtering techniques did not significantly affect the bleeding time or pH, colour, and odour of meat. However, microbe spoilage counts and death time of the NH group were significantly ($p<0.05$) higher than that of the H group. Blood volume lost during slaughtering was found to be significantly ($p<0.05$) lower in the NH group. Significant ($p<0.05$) correlations existed between bleeding time and blood lost with microbe spoilage count, pH and colour. These data suggested that imperfectly halal slaughtering technique caused a higher residual blood, which supports more spoilage microbe growth that led to a shorter shelf-life and lower wholesomeness of the carcass.

Keywords: halal slaughter, residual blood, broiler breast muscle, meat quality, shelf-life

INTRODUCTION

Broilers (*Gallus gallus domesticus*) are chickens bred and raised particularly for meat production and traditionally they are Cornish bird and White Plymouth Rock crosses. Proper killing through the halal slaughtering technique will allow large

evacuation of blood following exsanguination. This will result in minimal residual blood in the muscle that improves meat wholesomeness. However, an improperly done technique may lead to retention of high residual blood in the muscle causing poor meat quality. The objectives of this study were to assess effect of different slaughter techniques on meat colour, pH changes during storage, spoilage count, odour development during storage, bleeding time, death time and blood volume at slaughter. This issue is timely as it encompassed both human consumption (food safety) and animal welfare. This study may pave the way for trouble shooting the possible root of errors during the halal slaughtering procedure in large-scale plants.

MATERIALS AND METHODS

Sample collection

Twelve live commercial broilers weighing between 1.8 and 2.0 kg of the same breed, origin and farm management were used. The chickens were allocated into two groups of six chickens. At the slaughter house, all chicken were stunned using 0.26 mA electricity. Each group of 6 chickens were subjected to two slaughter techniques, perfectly halal slaughtering technique (H) where the jugular veins, carotid arteries, oesophagus and trachea were severed and imperfectly halal slaughtering technique (NH) where the only the carotid arteries were left intact. Bleeding time, death time and blood volume at slaughter were recorded.

Meat quality analysis

Approximately 30 g of the breast muscles from each chicken were used in the assessment of meat colour (l = lightness, a = redness and b = yellowness) using the ColourFlex EZ spectrophotometer. Indirect meat pH was determined using 3 g of breast muscles from each chicken. After snap-freezing and storage at -80°C, the meat samples were crushed into smaller particles in liquid nitrogen. Then, 27 mL of distilled water were added to the crushed meat and homogenised. The pH (Mettler Toledos) of homogenised meat samples was recorded. Approximately 25 g of breast muscle were procured and sampled at two-day intervals that is at days 0, 2, 4, 6 and 8 of storage. Then, 225 mL of steriled peptone water were added to 25 g meat sample and homogenised for 60 seconds. Psychrotrophic plate counts (PPC) were conducted using Petrifilm aerobic count plates and incubated at 7°C for 10 days. Subjective odour evaluations were conducted by a panel of 10 randomly selected individuals. Odour scores were determined by opening a sample bag, sniffing the meat sample, and recording score according to the following: 1 = fresh chicken odour, 2 = no odour, 3=slight odour development but still acceptable, 4=definite off-odour indicative of spoiled chicken, 5=very strong off-odour related to spoiled chicken.

Statistical Analyses

Meat colour, pH and microbe spoilage count data were analysed using an independent t-test while the subjective odour evaluation data was analysed using

Mann-Whitney U test. Correlation coefficients for bleeding time, death time, blood volume during slaughter, meat colour, pH and odour and microbe spoilage count were generated using Pearson's correlation coefficient. All analysis was performed by using IBM SPSS statistics 20 software.

RESULTS AND DISCUSSION

Bleeding time

There was no significant difference between slaughter methods, which is in contrast to earlier findings of Stevenson (1993). However despite being comparable, this study reported that cutting both carotid arteries and jugular veins are important for a much shorter bleeding time and thus death.

Blood lost

The blood volume loss of the NH group was significantly ($p<0.05$) lesser than that of the H group which resembled results documented by Latimer and Pederson (1923). The severance of both vessels has expedited loss of blood leading to a much shorter time of death. In a bird weighing 1.1 kg, severing the jugular veins only and both the jugular and carotid veins will, in 3 minutes, lead to a loss of 3.8 and 4.4%, respectively (Davis and Coe, 1953).

Death time

The death time of chickens from the NH group was significantly ($p<0.05$) longer compared to that in the H group. This finding is supported by Gregory and Wotton (1986) that failure to cut both carotids can add 2 minutes to the time taken for brain failure to occur in birds. Severing of only one jugular vein will cause the bird to retain consciousness while in severe pain for as long as 8 minutes. In the present study, the time taken for chickens from the NH group to die following exsanguination was approximately 2 minutes longer than that of the chickens in the H group.

Meat pH

The difference in slaughter methods did not yield any significant difference in meat pH, although much higher pH value and darker meat colour in the NH group was expected (Bourbab and Idaomar, 2012; Cornforth, 1994). This is probably due to the smaller sample size and lack of employment of snap-freezing in our study, which could have accounted for the comparable meat pH between both groups.

Subjective odour development

Similarly, there was no significant difference between subjective odour development in meat from different slaughter methods. However, overall ordour score procured by the NH group was significantly ($p<0.05$) higher. This is likely due to increase in number of spoilage microbe that occurred immediately after slaughter. Therefore, it is suggested that the odour development was due to the

ongoing spoilage in the meat that could eventually be detected. Cox *et al*., (1975) and Thronley *et al*. (1960) observed that spoilage of poultry as indicated by development of putrid and ammonia-like odours usually occur when spoilage bacterial populations reach 10^6 to 10^7 cells/cm^2.

Meat colour measurement

There was no significant difference in meat colour between slaughter methods. These findings were unexpected since the H group meats is supposedly to have greater haemoglobin concentration leading to a higher meat colour parameter values. Meat colour is largely dependent on the amount of meat pigments such as myoglobin, haemoglobin and heme (Barbut, 2001). Bourbab and Idaomar (2012) showed that the residual blood of the poultry is highly responsible for high meat colour parameter values, which deteriorate the quality of meat. The discrepancy between our results and those from earlier reports could to be attributed to the small sample size, thinne meat samples and inadequate exposure to air to allow for oxygenation before measurement.

Spoilage microbs count

The correlation analysis showed a significant ($r>0.9$; $p<0.05$) correlation between blood lost and microbe spoilage count on meat samples. The amount of spoilage microbe count between slaughtered techniques was significantly ($p<0.05$) different, where those in the NH group had higher counts at days, 2 and 4 of sampling. This finding suggests a significant correlation between volume of blood lost and microbe spoilage count. Greater amount of residual blood in the meat of NH group after slaughter will stimulate growth of spoilage microbe in the samples. In fact, it was earlier shown that excessive haemorrhages during NH slaughter can increase muscle haemoglobin content that decreases the shelf-life of meat (Alvarado *et al*., 2007). Thus, the higher volume of blood lost at slaughter minimises growth of spoilage microbe in meat samples.

CONCLUSION

The result from this study indicated that different slaughtering techniques do not directly affect bleeding time, meat pH, colour, or odour. However, spoilage microbe counts, blood lost and death time were influenced by the different slaughtering techniques. There was an also significant correlation between bleeding time and blood lost on microbe spoilage count, pH and colour of meat. These data suggest that imperfectly halal slaughtering technique, causing accumulation of greater amount of residual blood, causes greater growth of spoilage microbe that leads to shorter shelf-life and lower wholesomeness of chicken meat.

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EFFICACY OF KILLED PORCINE CIRCOVIRUS VACCINE (*FOSTERA*™) IN REDUCING VIRAEMIA IN PIGS

Moo Kar Shen, [1]Siti Suri Arshad & [2]Peck Toung Ooi
[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Post-weaning multisystemic wasting syndrome (PMWS) in pig is caused by porcine circovirus type 2 (PCV 2). In 2007, sporadic cases of PMWS had major economic in Malaysia swine industry. Field trial on *Fostera*™ PCV2 vaccine was performed in order to investigate the efficacy of the vaccine in decreasing the PCV2 viraemia in pigs. Thirty pigs aged three-weeks from sow of 3 different parities were equally divided into two groups of 15 pigs. They were vaccinated intramuscularly with 2 mL PCV2 vaccine. Body weight and blood sample were collected on days 0 (pre-vaccination), 5 and 50 post-vaccination. Samples were tested using quantitative PCR (qPCR) assay to detect virus load in the blood. On day 25 post-vaccination, vaccinated pigs showed lower PCV2 viral DNA copies number (4.46 $\log_{10}$ copies/µL) compared to control pigs (5.31 $\log_{10}$ copies/µL). Post-mortem examination on selected pigs (n=2) at day 50 revealed that there are no significant findings in the inguinal lymph nodes in both vaccinated and control pigs. The average body weight shows no significant between vaccinated pigs and control pigs. This preliminary result suggested that *Fostera*™ PCV2 vaccine able to reduce PCV2 viraemia in vaccinated pigs.

Keywords: post-weaning multisystemic wasting syndrome (PMWS), porcine circovirus 2 (PCV2), Malaysia, *Fostera*™ PCV2 vaccine, quantitative PCR (qPCR)

INTRODUCTION

Porcine circovirus type 2 (PCV2) is one of the most economically important swine pathogens world widely and causing post-weaning multisystemic wasting syndrome (PMWS) in pigs aged 5 to 12 weeks. This multifactorial syndrome induces wasting, respiratory signs, increased post-weaning mortality, enlargement of lymph nodes and occasionally pallor, jaundice and diarrhoea (Chae, 2012). Secondary pathogens, such as *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza also have an impact on the development of clinical signs (Allan and Ellis, 2000). The PCV2 is ubiquitous and

is frequently present in both Post-weaning multisystemic wasting syndrome (PMWS)-affected and unaffected pigs (Opriessnig *et al*., 2007). Moreover, PMWS has been shown to cause morbidity rates of 4 to 30% and increased mortality rates from 4 to 20% (Segalés and Domingo, 2002). In Malaysia the prevalence of PCV2 infection was 88.1% (Seetha et al., 2011).

Several studies suggested that a value of more than 10^7 PCV2 genomes/mL of serum corresponds to a pig developing clinical signs of PMWS (Olvera *et al*., 2004). Opriessnig *et al*. (2009) demonstrated that pigs receiving one dose of PCV2 vaccine had reduction in viraemia up to 78% of the population and therefore reduce risk of getting PMWS.

While the vaccine has been used in many pig farms in Malaysia, the efficacy of the vaccine is yet to be determined. This preliminary study is to examine the efficacy of the vaccine in reducing the PCV2 viraemia status in vaccinated pigs.

MATERIAL AND METHODS

Vaccine

Chimaeric PCV1/2 vaccine, Fostera™ is a killed vaccine formulation given as a single-dose.

Animals

This study was conducted at a commercial pig farm located in Jawi, Penang, Malaysia. Thirty 3-week-old piglets from 5 farrowed sows were selected and divided into 2 groups designated the vaccination and control group. One dose of 2 mL killed vaccine was given intramuscularly in the piglets of treatment group, while placebo group received 2 mL of sterile water only. All the piglets were fed *ad libitum* with commercial pig feed.

Body weight and blood sampling were taken at day 0, before the vaccination, followed by days 25 and 50 post-vaccination. For piglets weighing less than 20kg and pigs more than 20 kg, blood was collected via anterior vena cava and jugular vein respectively. Serum samples were processed for DNA extraction.

Extraction of viral DNA

DNA from serum was extracted using DNeasy Blood and Tissue Kit (Qiagen, USA) according to the manufacturer's instructions.

Quantitative Polymerase Chain Reaction (qPCR

The DNA samples were analysed using SensiFAST™ SYBR No-ROX Kit (Bioline, USA) in a Bio-Rad CFX 96^{TM} Real time PCR system (Bio-Rad, USA).

Post-mortem and histopathology

Post mortem and histopathological examinations were performed on day 50 post-vaccination on selected pig for assessment of the lymph nodes.

RESULTS AND DISCUSSION

The efficacy of PCV2 Fostera™ was evaluated and the results demonstrated that the vaccine could successfully reduce PCV2 viraemia. The finding support previous reports that PCV2 Fostera™ vaccine tested on specific pathogen free pigs showed significant reduction in viraemia (Fenaux *et al*., 2004; Opriessnig *et al*., 2008). Comparing between the vaccinated and control pigs, the virus load in the serum of vaccinated pigs are consistently lower (4.46 $\log_{10}$ copies/µL) than control pigs (5.31 $\log_{10}$ copies/µL) and they are significantly ($p<0.05$) different (Table 1). However, there was no significant ($p>0.05$) different in their body weight gain throughout the experiment (Table 2). There was also no abnormal finding either grossly or microscopically on the inguinal lymph nodes of the animals. The use of PCV2 vaccine in this farm for more than 2 years could have reduced the virus challenge in the farm, which could be the reason for the low viraemia (less than (7 $\log_{10}$ copies/µL) in unvaccinated pigs without showing clinical sign or lesion of PMWS. In conclusion, PCV2 vaccine used this study was able to reduce PCV2 viraemia in vaccinated pigs. This study suggests that *Fostera*™ PCV2 vaccine has an effect in reducing the risk of developing PMWS in pigs.

Table 1: Mean PCV2 copies number in treatment and control group of pigs as detected by quantitative PCR assay

Groups	Viral DNA copies number (log 10)		
	Day 0	Day 25	Day 50
Treatment	4.70±0.40	4.64±0.73	4.81±0.69
Control	4.40±1.23	5.31*±0.52	4.96±0.36

Value expressed as mean±SD; * significantly different ($p<0.05$).

Table 2: Mean body weight gain of pigs in the treatment and control group following vaccination with PCV2 vaccine

Groups	Mean body weight gain (kg)		
	Day 0	Day 25	Day 50
Treatment	7.31±0.29	10.31±0.48	23.62±1.07
Control	6.94±0.26	10.72±0.47	23.70±0.96

Value expressed as mean±SD.

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CHANGES IN SERUM TESTOSTERONE AND ORGANS OF MALE MICE AFTER ORAL INOCULATION WITH *BRUCELLA MELITENSIS* AND ITS LIPOPOLYSACCHARIDES

Muhammad Firdaus Ab Rahman, [1,3]Abdul Aziz Saharee, [1,3]Faez Firdaus Jesse Abdullah, [2,3]Mohd Zamri Saad & [1,3]Abdul Wahid Haron

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
[3]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM, Selangor, Malaysia.

ABSTRACT

Brucella melitensis is facultative intracellular gram-negative coccobacilli which can cause brucellosis in goats and sheep, leading to abortion in females. The infection can compromise reproduction performance, resulting in economic loss. There is a lack of knowledge on male reproductive cell and hormone response towards *B. melitensis* and its lipopolysaccharides. In this experiment, 24 experimental male mice were divided into three groups (8 mice each). Group 1 was inoculated orally with 0.4 mL phosphate-buffered saline (PBS), Group 2 was inoculated orally with 0.4 mL of 1×10^9 cfu *B. melitensis* and Group 3 was inoculated orally with 0.4 mL lipopolysaccharides from 1×10^9 cfu *B. melitensis*. After 10 days, blood samples were collected from the moribound or surviving mice by cardiac puncture to determine serum testosterone concentration. Histopathological lesions in pituitary glands and testes from euthanised mice were examined for cellular changes. Histopathology result revealed that there was a significant difference ($p < 0.05$) in necrotic and degenerative lesions between the groups. Group 2 give *B. melitensis* alone showed increased in testosterone concentration by 76% while Group 3 showed testosterone concentration decreased by 55%. Therefore, oral inoculation of *B. melitensis* and its lipopolysaccharides can cause cellular changes in male reproductive organs and hormone.

Keywords: *Brucella melitensis*, brucellosis, lipopolysaccharides, histopathological lesion, testosterone.

INTRODUCTION

Brucella melitensis is a coccobacillus, aerobic, non-motile, lack capsule, falcutative, intracellular, gram-negative bacteria that cause brucellosis in goats, sheep and even humans (Bauman, 2009). Among *Brucella* species, B. *melitensis* is the most virulent

species and considered the highest zoonotic risk. The infection manifests as undulating fever and is also known as Malta fever in humans (Xavier *et al.,* 2010). Transmission of the pathogen can occur through inhalation, contact or ingestion of birth fluid or foetus, placenta or abortion secretion and milk or colostrum of infected female. Main clinical manifestations of disease in female are abortion and stillbirth while in the males, epididymitis and orchitis (Banai, 2007; Blasco, 2011).

B. melitensis lipopolysaccharides are smooth-type lipopolysaccharides. The lipopolysaccharides structure gives endotoxic characteristic to the gram-negative bacteria. *B. melitensis* has a cell wall that consists of peptidoglycan layer associated with the outer membrane (Diaz *et al*., 1968).

A previous study by Jesse et al. (2013) has shown that orally inoculation with *B. melitensis* and its lipopolysaccharides cause changes in the male reproductive of mice. However, there is a lack of knowledge on male hormone changes in association with the *B. melitensis* and its lipopolysaccharides. Thus, this study was conducted to determine the effect of experimental *B. melitensis* infection on testosterone level in male mice.

MATERIALS AND METHODS

Brucella selective agar (BSA) was prepared to reculture the *B. melitensis* isolates. Sterile distilled water was mixed with the bacteria colonies on the plate. The mixture was diluted to MacFarland Standard to obtain concentration of 1×10^9cfu.

An extraction kit was used to prepare lipopolysaccharides from 1×10^9 cfu *B. melitensis*.

Twenty-four mice from Institute Cancer Research (ICR) were divided into three groups (8 mice each). Group 1 was orally inoculated with 0.4 mL phosphate-buffered saline (PBS), Group 2 orally inoculated with 0.4 mL of 1×10^9 cfu *B. melitensis* while Group 3 orally inoculated with 0.4 mL lipopolysaccharides from 1×10^9 cfu *B. melitensis*. The clinical signs and mortality were observed for 10 days.

After the day 10, 1 mL blood samples were collected from moribound and surviving mice by cardiac puncture. Serum testosterone concentration was measured by radioimmunoassay. The mice were humanely euthanised by cervical dislocation and post-mortem was done on the mice. The brain pituitary gland and testes were taken for histopathology examination. Lesion scoring was done and data were analyzed using SPSS version 20.

RESULTS AND DISCUSSION

Kruskal Wallis test revealed that there was a significant ($p< 0.05$) difference among treatment groups with respect to necrotic and degenerative of cellular changes (Table 1).

Table 1: Kruskal-Wallis test on data obtained from mice treated with phosphate-buffered saline, *B. melitensis* organism and *B. melitensis* lipopolysaccharide

Parameter	Inflammatory Cells	Necrosis and degeneration	Oedema	Haemorrhage
Chi square	12.945	2.000	1.625	3.675
Df	2	2	2	2
Asymptomatic significance	*0.002	0.368	0.444	0.159

Df = degree of freedom; *Significant difference at $p<0.05$.

The necrotic and degenerative lesions of Group 2 were mild in the testes and pituitary gland, with mean score of 0.86 and 0.40, respectively. Mean lesion score of the testes and pituitary gland of mice in Group 1 was 0, which was considered normal while mean score for Group 3 was 0.25 for testes and 0.15 for pituitary gland, suggesting mild pathological change.

The presence of inflammatory cells showed mean scores of 0.02 and 0.04 for the testes and pituitary gland, respectively in Group 2 mice. The lesions were mild. Mean lesion score of pituitary gland in Group 3 was 0.02, which was normal to mild, while testes showed a normal mean score of 0. Group 1also showed anormal mean score of 0 in both the testes and pituitary gland.

Necrotic and degenerative lesions and presence of inflammatory lesion in testes and pituitary gland were slightly higher in Group 2 compared to Group 1 (Figures 1 and 2). Cellular necrosis and degeneration occurred as a result of the endotoxic effect of *B. melitensis*. According to Gorvel and Moreno (2002), *Brucella* species have the ability to invade host cells without activating innate immune defense system. This could be the reason for a scarcity of inflammatory cells in the organs of mice treated with *B. melitensis* and bacteria LPS.

Necrosis and degeneration were more prominence in the testes than pituitary gland. In male, localisation of *B. melitensis* in testes is common in acute brucellosis (Scientific Committee on Animal health and Animal Welfare, 2001) which accounted for the prominence of localised lesion in this organ.

Oedema of the testes occurred in Group 2 mice with mean score of 0.02 which was considered normal to mild lesion. In the pituitary gland in the same Group there was no oedema. Mean score in Groups 1 and 3 was 0 both for the testes and pituitary gland (Figures 3 and 4).

None of the organs of treated mice showed haemorrhagic lesion in the testes or pituitary gland.

Testosterone concentration in Group 2 was highest with mean of 2.34 ng/mLfollowed by Group 1 with 1.33 ng/mL and Group 3 with 0.6 ng/mL. In comparison with the control Group, Group 2 mice showed higher hormone concentration by 1.01 ng/mL or 76%, while Group 3 was higher by 0.73 ng/L or 55%. Testosterone concentration of Group 3 mice was 55% lower than the control. A study by O'Bryan *et al*. (2000) showed that the introduction of *Escherichia coli* lipopolysaccharides to adult male rats induced either mild or severe inflammation,

which caused a decrease in Leydig cell testosterone production and gonadotropin responsiveness.

Brucellosis is a chronic disease with variable incubation period from weeks to months; the effect of the infection in the reduction of testosterone production can take time. In this study, the necrosis and degeneration of the testis were mild, producing insufficient damage to leydig cells to cause significant disruption to testosterone production.

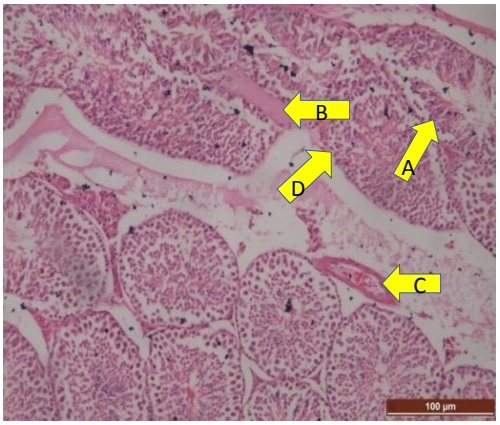

Figure 1: Testis of a mouse orally inoculated with *B.melitensis*. Presence of necrosis and degeneration (A), oedema (B), congestion (C) and inflammatory cell (D) in seminiferous tubules.

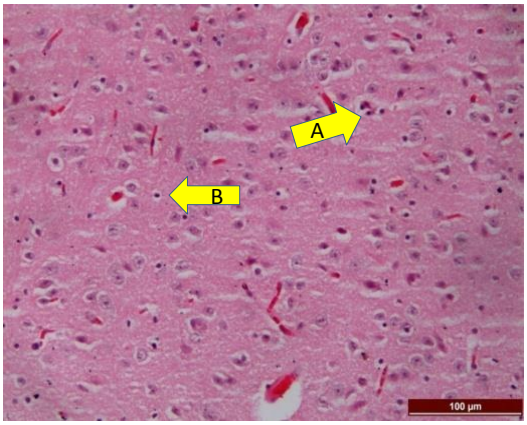

Figure 2: Pituitary gland (anterior part) of a mouse orally inoculated with *B.melitensis*. Presence of necrosis and degeneration (A) and Inflammatory cell (B).

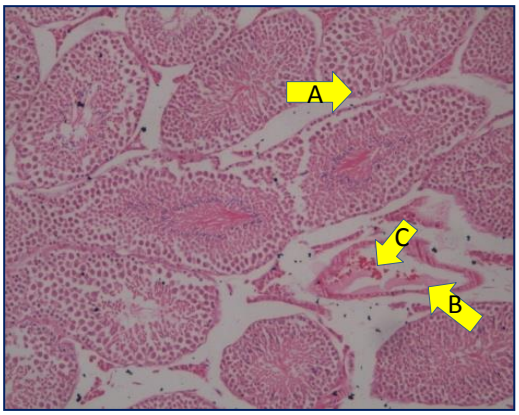

Figure 3: Testis of a mouse orally inoculated with *B.melitensis* lipopolysaccharides. Presence of necrosis and degeneration (A), oedema (B) and haemorrhage (C) in seminiferous tubules.

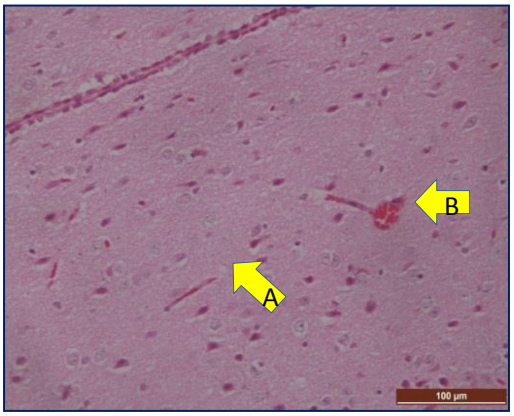

Figure 4: Pituitary gland (anterior part) of a mouse orally inoculated with *B. melitensis* lipopolysaccharides. Presence of necrosis and degeneration (A) and congestion (B).

In conclusion, *B. melitensis* and its lipopolysaccharides can produce pathological changes in male reproductive organ and changes in the serum testosterone concentration in mice.

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CHANGES IN SERUM TESTOSTERONE AND ORGANS OF MALE MICE AFTER ORAL INOCULATION WITH *PASTEURELLA MULTOCIDA* TYPE B AND ITS LIPOPOLYSACCHARIDES

Muhammad Jamil Sabu, [1,3]Abdul Aziz Saharee, [1,3]Faez Firdaus Jesse, [1,3]Abd. Wahid Haron and [2,3]Mohd Zamri Saad

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
[3]Ruminant diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Haemorrhagic septicaemia is the disease caused by *Pasteurella multocida* type B. There is still lack of knowledge about changes of male reproductive hormone and organ after oral inoculation of *P. multocida* type B. Therefore, this study was designed to examine the histopathological change in the reproductive organ and pituitary gland and to estimate reproductive hormone concentration in male mice after oral inoculation with *P. multocida* type B and its endotoxin. In this experiment, 24 male mice were divided into three equal groups of 8 mice each. Group 1 mice were inoculated orally with 0.4 mL sterile phosphate-buffered saline, pH 7. Group 2 were inoculate with 0.4 mL of 1×10^9 colony forming unit (cfu) of *P. multocida* type B, while the mice from group 3 were inoculated with 0.4 mL of lipopolysaccharides (LPS) from 1×10^9 *P. multocida* type B. Blood was collected via cardiac venepuncture from mice showing severe sign and surviving after 10 days before they were euthanised. Post-mortem examination showed significant ($p<0.05$) difference lesions, such as necrosis and degeneration, oedema, haemorrhage and inflammation among groups. Mean serum testosterone concentration of *P. multocida* type B-treated group showed two fold higher values than the PBS- and LPS-treated group. In conclusion, oral inoculations of *P. multocida* type B and its endotoxin produced lesions in the reproductive organs and increased testosterone concentrations in male mice.

Keywords: haemorrhagic septicaemia, *Pasteurella multocida* type B, mice, testosterone, histopathological lesion

INTRODUCTION

Haemorrhagic septicemia is an acute, highly fatal septicaemic disease caused by the bacteria *Pasteurella multocida* type B:2 or E. Haemorrhagic septicemia characterised by a rapid course, high fever, loud and stertorous breathing due to oedematous swelling, petechial haemorrhages in the throat and brisket region,

profuse salivation, severe depression and death within 24 hours. *P. multocida* is a small, non-motile, pleomorphic, nonspore-forming, bipolar Gram-negative cocco-bacillus or short rod measuring (De Alwis, 1999).

MATERIALS AND METHODS

Mice

24 healthy ICR male mice aged 3 weeks old were used in this study. All the mice were obtained from Institute of Cancer Research (ICR).

Inoculums

The wild type *P. multocida* type B used in this study was obtained from stock culture from the Veterinary Research Institute Ipoh Perak, Malaysia. Identification of *P. multocida* was made using the Gram-staining method and biochemical characterisation using oxidase, urea broth, sulphur indole motility (SIM), triple sugar iron (TSI) and citrate test. The isolate was confirmed to be *P. multocida* type B. Pure stock culture that was stored on nutrient agar slants were subcultured on 5% horse blood agar and incubated at 37˚C for 18 hours. A single colony of the *P. multocida* was selected to grow on brain heart infusion (BHI) broth, incubated in shaker incubator at 37˚C for 24 hours before the concentration was determined using McFarland Nephelometer Barium Sulfate standards.

Study designs

The mice were divided into 3 groups of 8 mice. Group 1 were inoculated orally with 0.4 mL phosphate-buffered saline (PBS), group 2 mice were inoculated orally with 0.4 mL of 1×10^9 colony forming unit (cfu) *P. multocida* type B and group 3 mice with 0.4 mL of LPS from 1×10^9 of colony *P. multocida*. After inoculation, all groups were observed for 10 days. Mice that showed severe clinical signs, such as laboured breathing, reduced in responsiveness and closed eyes were humanely euthanised before blood samples were collected via cardiac venipuncture for determination of serum testosterone concentration using a radioimmunoassay technique. Post-mortem was done on the mice. The brain and testis were collected and placed in 10% formalin for histopathological examination and determination of lesion scores. The data were analysed using SPSS 20 to compare the severity of responses among groups.

Radioimmunoassay (RIA) testosterone technique

Determination of serum testosterone concentration using radioimmunoassay was done on serum samples. Firstly, an addition step was performed where 50 µL of calibrator were mixed with 500 µL of tracer and added to antibody-coated tubes. The tubes were covered and incubated for 1 hour at 37°C in a water bath. The radioactive emission was estimated using the Wallac Wizard Gamma Counter model 1470.

Histopathology lesions scoring

The lesion were classified as follows; 0 = normal, 1 = mild, 2 = moderate and 3 = severe lesions. The lesions was considered as normal if < 30% of the field were affected, mild if only 30% field affected, moderate if 60% field affected and severe if >60% of field affected. The lesions were scored were based on necrosis and degeneration, oedema, haemorrhage and presence of inflammatory cells.

RESULT

Histopathological findings

The testis and pituitary gland were examined for histopathogical changes. The results upon analysis by SPSS version 20 using the non-parametric test, Kruskal-Wallis Test showed that there were significant ($p<0.05$) difference in lesions scores among groups (Table I). Mann-Whitney Test showed that there were significant difference ($p<0.05$) between the treatment and control groups (Table 2).

Table 1. Kruskal-Wallis test of the lesions between groups

	Necrosis and degeneration	Oedema	Haemorrhage	Inflammatory cells
Chi-square	38.509	32.532	21.423	30.604
df	2	2	2	2
Asymptomatic Significance	.000	.000	.000	.000

df = degree of freedom.

Table 2. Mann-Whitney test of the lesion between groups

Group	Necrosis and degeneration	Oedema	Haemorrhage	Inflammatory cells
Group 1	8.5^{a}	8.5^{a}	12.00ac	10.50^{ac}
Group 2	24.5^{b}	24.5^{b}	21.00^{b}	22.50^{b}
Group 3	8.75^{c}	10.10^{c}	12.00c	10.50^{c}

^{a,b,c} Means within column with different superscript are significantly ($p<0.05$) different.

DISCUSSION

This study for the first time reports the effect of *P. multocida* type B on reproductive hormone concentration and histopathological change in the reproductive organs of a mouse model.

Oral inoculation with *P. multocida* type B into mice produced histopathological lesions in testis, which is similar to that reported earlier (Jesse *et al*., 2013). The

pituitary gland of the treated mice showed significant histopathological changes after oral inoculation of the bacteria. This observation is new finding.

The testosterone concentration was two-fold higher in the *P. Multocida*-treated than the PBS- or LPS-treated groups. Although increased serum testosterone concentration can stimulate reproductive behaviour in humans, the effect on mice in this study is not clear.

P. multocida-treatment had produced abnormal histopathological changes in reproductive organs of mice, which is expected to affect hormone production. However, this was not true in the current study, because the hormone concentration increased with treatment. Therefore, this study describes a new finding on the effect of *P. multocida* type B on reproductive hormone concentration and histopathological changes in the reproductive organs of mice.

CONCLUSION

The result showed that there were significant ($P<0.05$) necrosis and degeneration, oedema, haemorrhage and inflammatory reponse in *P. multocida*-treated mice. The mean testosterone concentration in *P. multocida* type B-treated was two-fold higher than in the PBS- and LPS-treated mice. Thus, oral inoculation *P. multocida* type B and its endotoxin produce cellular changes in male reproductive organs and in serum testosterone concentration. Future studies should be conducted to determine the correlation between male serum reproductive hormone concentration and the histopathology of hormone-producing organs upon treatment with *P. multocida* type B.

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PHYSICAL MEAT QUALITY OF PEKIN DUCK REARED IN OPEN-HOUSE FREE-RANGEAND CLOSE-HOUSE SYSTEMS

Muhammad Tariq Shamsuddin & [1]Lokman Hakim Idris
[1]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Carcasses from Pekin duck (*Anas platyrhynchos domestica*) of the Cherry Valley strain duck aged 52 days were subjected to assessment of physical meat quality, which included carcass composition, pH, colour, water holding capacity and meat tenderness. The carcass composition determination was conducted according to dissecting methods using the whole carcass. The breast muscle was used to determine pH, colour, water holding capacity and muscle tenderness. The pH was measured according to an indirect method. The colour was analysed using the ColorFlex machine according to the CIElab method. The water holding capacity was determined according to the cooking loss method. Finally, the tenderness analysis was performed using the Stable Micro System Texture Analyzer (Model TA-XT plus, UK) equipped with a flat Volodkovich shear blade. Twenty Pekin duck carcasses from Perak Duck Food Industry Sdn Bhd, Trong, Perak, Malaysia were obtained for the study. Ten broiler duck carcasses each were from open free range and close housing systems. The samples were collected from the slaughter house after de-feathering and rapid freezing at -25ºC. There was a significantly ($p<0.05$) lower pH in the free-range open house compared to the close house system duck meat. The open house system duck meat had lower pH than those from closed house system. The meat relative weights were significantly ($p<0.05$) higher for the closed house system ducks than the open house system. There were no significant ($p>0.05$) difference in the colour, tenderness and water holding capacity between the two housing systems.

Keyword: carcass composition, color, water holding capacity, Pekin duck (*Anas platyrhynchos domestica*), Cherry Valley

INTRODUCTION

Duck farming in Malaysia began as a backyard activity practiced by Chinese farmers. The duck industry has not experienced rapid expansion in the market like the chicken industry. However, the demand for duck meat is increasingly growing because of improved genetic selection and husbandry management.

The broiler chicken production in 2013 was 720 million while broiler duck production in 2013 is only 24.43 million. In Asia, China is becoming a major player contributing 2.2 million tons of duck meat to the market, representing 81% the production for the region. Malaysia is one of the top three duck meat producers in South East Asia, contributing 51000 tons per year (Baeza, 2006). Currently, Peninsular Malaysia has 9 commercial duck breeding companies that actively produce ducklings with 20 farms in operation in the states of Selangor, Perak, Penang and Johore. The hot and humid climate of Malaysia is suitable for duck rearing, regardless of the housing management. The breeds currently used in the Malaysian duck industry are the Cherry Valley, Pekin, Muscovy and local crosses. There are four systems that are currently being practiced which include the intensive, semi-intensive, integrated and free-range systems. However, the intensive system is more expensive in comparison to other systems due to the cost of housing and feeding system.

There is little information in the meat quality of ducks reared under different housing systems. Thus, the aim of this study was to determine and compare the meat quality of the Pekin duck in the open house, free range and closed house system, by determining the carcass composition and determining the pH, colour, water holding capacity and tenderness.

MATERIALS AND METHODS

Animals

Twenty Pekin duck (*Anas platyrhynchos domestica)* carcasses of Cherry Valley 2000 (CV2000) strain from open house free range and closed systems were obtained from a slaughter house of Perak Duck Food Industry Sdn Bhd, Trong, Perak, Malaysia. Each type of housing systems contributed ten carcasses for this study and the sampling was done by random sampling method where sex of the duck was not included in the selection criteria. All ducks, regardless of their housing systems, were fed commercial poultry feed: the starter up to the 10^{th} day, the grower from 11^{th} to 28^{th} day and the finisher for the remaining time of production and drinking water *ad libitum* throughout the production line. Prior to the experiment, all ducks received similar pre-slaughter conditioning whereby they were relaxed and fasted for 24 hours with water provided. All the ducks were halal-slaughtered on the 52^{th} day.

Carcass composition measurement

The live weight of each duck was obtained prior to slaughtering. Then, the carcasses were dissected and the meat, fat bone was separated and weighed using a digital weighing scale.

pH
pH analysis was conducted according to an indirect method using a homogenising technique. Three grams of breast meat were used to determine the pH. The homogenised sample was analysed by using Mettler Toledo (USA) pH meter.

Colour
The whole breast meat was used for colour analysis using the ColorFlex from HunterLab. The analysis conformed to the method using the coordinates L* (luminosity), a* (redness-greenness) and b* (yellowness-blueness) according the method of the Commission Internacionalede L'Eclairage Laboratory (CIELAB).

Water holding capacity
The breast meat, which was stored at -25°C, was thawed 4°C for 24 hours. The water holding capacity was determined according to the cooking loss method using the following formula (Honikel, 1998).

$$\text{Cooking loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

W1 = Initial weight; W2 = final weight

Tenderness
The breast meat from the cooking loss was cut into pieces of 1 × 1 × 2 cm with the long axis accurately aligned with the muscle fibre direction. The tenderness analysis was performed using the Stable Micro System Texture Analyzer (Model TA-XT plus, UK) equipped with a flat Volodkovich shear blade.

RESULTS AND DISCUSSION

pH
The pH of meat from the two housing systems was acidic, which was due to accumulation of lactic acids in the muscle after death (Khan, 1970). The pH value of the breast meat from the open house free range system was significantly ($p<0.05$) higher than that from the closed house system, agreeing with an earlier report (Alvarado *et al.,* 2005), which stated that outdoor access birds had higher use of muscles resulting in higher muscle pH. However, this is in contrast with other reports that showed otherwise (Culioli *et al.,* 1990; Castellini *et al.,* 2002a).

Water holding capacity
There were no significant differences in the meat from different housing system but the meat under closed house system had a higher cooking loss percentage, which suggested lower water holding capacity. Theoretically, the low pH will cause higher protein denaturation and water binding ability of many proteins as reported (Fujii *et*

al., 1991). However, results of the present study contradicted this theory whereby the higher pH value of meat under closed house system resulted in a higher cooking loss percentage. In this study the influence of other factors such as amount of collagen and method of cooking may be the cause of lower water binding ability of the duck meat (Lokman, 2012).

Meat Colour

The colour analysis revealed no significant ($p>0.05$) difference between meat from the two housing systems. However, the meat under the open house free range system has a higher lightness (L*), redness (a*) and yellowness (b*). In the present study, the higher lightness in meat under the open house system was due to low pH. However, the higher redness of meat under open house system could be due to a high number of Type 1 muscle fibre which is oxidative and containing higher number of blood capillaries that resulted in higher haemoglobin pigment in the meat. Furthermore, since species with highly motor activity have intensely pigmented muscles, the meat from ducks under open house system with greater motor activity would show more redness that those from closed house system.

Tenderness

There were no significant differences in the meat between the open house free range system and closed house system. However, the open house free range system had a higher peak force which indicated low tenderness. The lower tenderness of meat form ducks in the open house free range the closed house system is also related to the higher motor activity of the former (Forrest *et al.*, 1975). In fact, high activity muscle tends to have coarser meat texture than the infrequently used muscle. Coarse texture of meat will result in a higher peak force.

Carcass composition

There was no significant ($p>0.05$) difference in the bone, fat and dressing percentage between ducks from the two housing system used in this study. However there was significant ($p>0.05$) difference in the meat relative percentage. The carcass of ducks under open house free range system had a lower meat yield in comparison to the carcass under close house system. Ducks from open house free range system also produced low meat yield, which could be the result of high stress level from high ambient temperature of the housing environment. The ambient temperature under an open house free range system was 32°C while that of the close house system was 26°C.

It can be concluded that although given the same diet, housing systems can contribute to duck meat weight not to other physical characteristics. Ducks raised in close house system gave higher meat yield than those in the open house free range system.

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FINANCIAL IMPACT OF FOOT AND MOUTH DISEASE ON SELECTED CATTLE AND BUFFALO FARMS IN SELANGOR AND NEGERI SEMBILAN, MALAYSIA

Nadia Natasha Nordin & [1]Siti Zubaidah Ramanoon
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Foot and Mouth disease (FMD) remains a threat to the livestock industry in Malaysia due to its impact on animal health, production, and deaths. The aims of this study were to assess farmer's knowledge and recognition of FMD, to determine the financial impact of the disease in a herd, and to get opinion from farmers on importance of FMD vaccination as a preventive measure. Ten farmers were selected and data were collected through a questionnaire survey. The findings showed that all ten farmers were aware and recognised FMD. Based on the last FMD outbreak, the average losses reported by five farmers were RM5,680 due to treatment cost, RM18,500 from deaths, and RM17,775 from milk production. Nine farmers believed that vaccination against FMD is vital for their livestock. In conclusion, there was a great financial impact on the farmers following FMD outbreaks and they also have realised that vaccination is important to prevent outbreaks.

Keywords: foot and mouth disease, cattle, buffalo, questionnaire, financial losses

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly transmissible and economically overwhelming infection of cloven-footed animals (Forman et *al.*, 2009). Because of its impacts on production, global markets and effect on the economy, FMD is one of the most significant problems to the livestock industry (James and Rushton, 2002).

Foot and Mouth Disease caused by an *Aphthovirus* (family *Picornaviridae*) which consists of seven immunologically discrete main serotypes: A, O, C, and Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1 (Radostits *et al.*, 2006). There is no cross immunity among the different serotypes. The impact of FMD has always been underestimated due to low mortality rate in adult cattle and buffaloes (James and Rushton, 2002). However, the level of awareness about the disease and its financial impact in the community including the farmers is

questionable thus a need for a study. The goals of the present study were to assess farmer's knowledge and recognition of FMD, to determine the financial impact of the disease in a herd, and to get opinion from farmers on importance of FMD vaccination as a preventive measure.

MATERIALS AND METHOD

Farms

This study conducted from 13 January to 16 February 2014 and involved 10 cattle and buffalo farms in Selangor and Negeri Sembilan. Few farmers have enrolled in the *Ladang Angkat* programme of the University Veterinary Hospital, Universiti Putra Malaysia. The vaccination information of the animals in the farm such as the recent vaccination dates, animals vaccinated and the susceptible animals on the day of vaccination were gathered from the Department of Veterinary Services (DVS) of Negeri Sembilan and Selangor. The price of FMD vaccine was obtained from the DVS Headquarters of Putrajaya.

Questionnaire survey

All 10 owners with complete information contacted either by telephone and direct face-to-face interview to get their consent to take part in this study. The questionnaire designed to collect information about the management, vaccination status, earlier FMD outbreaks, farmer's opinion on vaccination, the farmer's knowledge about the clinical signs of FMD and the treatment and control of the disease, estimation of losses resulting from the particular outbreaks including reduced in milk production, death of the calves, culling and cost of treatment. Data obtained managed in Microsoft Excel 2010. Descriptive analysis computed by using frequency tables and charts. Results were presented in mean and standard deviation (Mean + SD).

RESULTS AND DISCUSSION

From the data, all 10 farmers (100%) knew about FMD, whether or not their farms had a history of FMD. In addition, all farmers also agreed that FMD can affect their business activity. Additionally, all farmers concerned that FMD can affect their business activity as they acknowledged that FMD may cause death, milk losses and loss of body weight. Besides, farmers recognised the main clinical signs of FMD as: ulcer (100%), vesicle (90%), hypersalivation (80%); lameness, (80%) death in calves (70%), teat lesion (30%) and fever (30%).The total cost experienced by the five farmers which experienced FMD outbreaks presented in Table 1. The total cost included mortality, treatment, milk loss to dairy farmers and weight loss for beef farmer.

Table 1. Estimated cost of the 2014 FMD outbreak to farms

Farm	Cost (RM)				
	Mortality	Treatment	Milk loss	Weight loss	Total cost
1	16,000	10,000	14,400	NA	40,400
2	10,000	3,200	7,500	NA	20,700
3	50,000	7,200	31,200	NA	88,400
4	7,500	1,000	NA	7,500	16,000
5	9,000	7,000	18,000	NA	34,000

NA=Information not available

Based on the recent FMD outbreak, the average losses reported by five farmers were RM5,680 due to cost of treatment, RM18,500 from deaths, and RM17,775 from milk production. It was obvious from this study that FMD outbreaks had caused losses directly to the farmers in the form of sick and dead animals, cost of treatment, milk loss and/or weight loss. Previous study conducted in Southern Cambodia reported similar findings. They reported that the farmers with severe financial impact on FMD outbreaks due to loss of body weight (RM618.80), dead cattle (RM247.90), treatment and management cost (RM46.60) (Young *et al.*, 2013). In our study, free vaccine provided by DVS (personal communication). Otherwise, the price is RM4.20 per dose.

Ninety percent of the farmers agreed that it is vital to practice FMD vaccination on their herd to prevent future outbreak. Meanwhile, one of them did not practice FMD vaccination. Consequently, all farmers (100%) were in the opinion that vaccination can protect their animals from FMD.

This study revealed that most farmers agreed that FMD vaccination is important as a preventive measure in their herd, thus they adopted vaccination programme. One farmer that did not practice FMD vaccination thought that hygiene and adequate nutrition will prevent FMD in his animals. Not all farmers in the current study would vaccinate their herds in future. The reason for refusal to take part in the vaccination programme could be that they perceived it as a waste of time and troublesome as they need to gather and restraint their animals. The study found that all selected farmers were willing to spend on vaccination.

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IN VITRO GAS PRODUCTION AND RUMINAL FERMENTATION PATTERN OF AGRICULTURAL BY-PRODUCTS

Noor Adilah Redzuan, [1]Yusof Hamali Ahmad & [1]Mahdi Ebrahimi
[1]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Methane is the primary greenhouse gas produced from ruminant production, accounting for 37% total arthropogenic methane (CH_4) emissions. Methane production is essential for effective degradation of organic matter, but also represents an energy loss of 2 to 12% gross energy intake. Consequently, information on methane and fermentation pattern of feedstuffs is important which can be used to mitigate CH_4 production emissions for the benefit of the environment and animal. This study evaluated the *in vitro* emissions and ruminal fermentation patterns of guinea grass (GG), rice straw (RS), sugarcane bagasse (SCB), palm kernel cake (PKC), oil palm fronds (OPF), and oil palm leaflets (OPL) using *in vitro* gas production technique of goat rumen liqour over a period of 48 hours. Methane gas production (MGP), rumen pH and volatile fatty acid (VFA) for each treatment were recorded at 6, 12, 24 and 48 hours. Mean total VFA production from SCB was significantly higher compared to other treatments. Palm kernel cake recorded the higher ruminal concentration of acetate of 17.85 mmol/mL than guinea grass or rice straw. The propionate for SCB treatment recorded the highest concentration among treatments ($p < 0.05$). Mean total MGP for GG, RS, SCB, PKC, OPF and OPL were 3.65, 3.66, 3.23, 3.25, 3.53 and 3.51 ppm/mL, respectively and did not show significant difference among treatments. The study indicates that feeding ruminants with these agricultural products increases methane emission into the atmosphere.

Keywords: *in vitro*, fermentation, methane, VFA

INTRODUCTION

Currently, climate change is a subject of global environmental concern and methane is the largest contributor to the global warming, with 25 times more global warming potential than carbon dioxide. Ruminants, such as cattle, sheep, and goats have been identified as one of the main sources of global anthropogenic methane emissions as they produce 86 million metric tonnes of methane per year (Hook *et al*., 2010). Ninety-five percent of methane production in ruminants comes from enteric fermentation by microrganisms called methanogens, whereas the remaining 5% is

produced by manure. There are a few recognised factors that influence ruminant methane production, with changes in the rumen fermentation pattern resulting from altered diets being the most important. The ruminant industry in Malaysia depends primarily on locally available feedstuffs, primarily agriculture by-products such as rice straw, palm kernel cake (PKC), and oil palm fronds. However, agricultural by-products as main source of feed serve some disadvantages, such as high in lignocellulosic materials, low in crude protein content, high in crude fibre and low digestibility. Thus, this study evaluates the methane gas production of various agriculture by-products in order to address the effect of the methane gas emissions towards the environment. In addition, this study also aims to evaluate the fermentation patterns of these agriculture by-products.

MATERIALS AND METHODS

Three Katjang crossbred fistulated goats were used as rumen liquor donors that were fed with standard ration of 50% alfalfa and 50% concentrate twice daily. Six different feed samples were used. They were guinea grass and 5 agriculture by-products feedstuffs, which were rice straw, sugar cane bagasse, palm kernel cake (PKC), oil palm fronds (OPF) and oil palm leaflets. These feed samples were oven dried at 60°C for 3 days. Then, they were ground into fine particles and passed through 1 mm sieve.

Rumen fluid was used to compare and evaluate the effects of guinea grass and agriculture by-products feedstuff on *in vitro* gas production, methane gas production, rumen fluid pH and VFA profiles. The rumen fluid collected was pooled and sieved into a thermal jar and transported immediately to the Physiology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Rumen fluid was filtered with 4 layers of cheese cloth to remove feed particles and coarse materials and the rumen pH determined. The fluid was then flushed with carbon dioxide (CO_2). Rumen fluid and buffer suspension were prepared and mixed at 1:4 and constantly stirred and kept warm on a hot plate at 40°C. Twenty-five milliliter of rumen fluid/buffer suspension was added into each syringe by connecting to a plastic tube connected to a glass funnel.

All syringes were then incubated at oven temperature of 39°C. For incubation, 3 replicates were prepared for each group including blanks in 3 separate runs. These samples were incubated to determine cumulative gas production at 0, 2, 4, 6, 8, 10, 12, 24 and 48 hours. *In vitro* gas production was measured as described by Menke and Steingass (1988). Rumen pH, methane gas production, and total VFA production were recorded at four time intervals of 6, 12, 24 and 48 hours.

RESULTS AND DISCUSSION

Mean total cumulative gas production and methane gas concentration after 48 hours are presented in Table 1. The *in vitro* gas production technique has been frequently

used to assess biological values of feed based on their pattern of accumulated gas when incubated with rumen fluid under anaerobic conditions (Tedeschi *et al*., 2008). Cumulative gas production reflects digestibility of feed. Sugar cane bagasse (SCB) recorded the highest ($p<0.05$) mean total cumulative gas production among all treatments, except PKC. Thus, it can be concluded that among all treatments, SCB is more digestable. However, there were no significant ($p>0.05$) differences in methane gas production between treatments. Guinea grass, rice straw, sugar cane bagasse, PKC, OPF and OPL produced similar rate of methane from the *in vitro* ruminal fermentation. High levels of neutral detergent fiber (NDF) yield a higher CH_4 production (Rasmussen and Harrison, 2011).

Table 1: Cumulative gas and methane gas production of agricultural by-products

	Cumulative Gas Production (mL/ 0.25g)	Methane Gas Concentration (ppm/mL)
Guinea Grass	11.07^{a}±4.83	3.65^{a}±2.01
Rice Straw	9.31^{a}±4.27	3.66^{a}±1.62
Sugar Cane Bagasse	20.38^{b}±7.25	3.23^{a}±2.78
Palm Kernel Cake	$18.97^{b,c}$±6.99	3.25^{a}±1.15
OPF	16.88^{c}±7.07	3.53^{a}±2.08
OPL	16.74^{c}±4.90	3.51^{a}±2.12

^{a,b,c,d} Means with different superscripts within columns have significant difference ($P<0.05$). OPF=palm oil frond; OPL=palm oil leaf.

Table 2: Volatile fatty acid production of Guinea grass and agricultural by-products

Treatment	Acetate (mM/mL)	Propionate (mM/mL)	Butyrate (mM/mL)	Total (mM/mL)
Guinea Grass	13.68^{a}± 5.42	$7.05^{a,b}$±2.48	$4.76^{a,b}$±1.68	$25.07^{a,b}$±9.62
Rice Straw	$12.13^{a,b}$±5.04	6.49^{a}±2.51	4.34^{a}±1.79	22.96^{a}±15.58
Sugar Cane Bagasse	$17.27^{b,c}$±6.40	16.26^{d}±6.59	7.04^{c}±2.72	40.57^{d}±15.17
PKC	17.85^{c}± 6.97	10.62^{c}±3.78	$6.05^{b,c}$±2.23	$33.5^{c,d}$±3.84
OPF	$17.64^{b,c}$±8.20	$8.99^{a,b,c}$±3.60^{c}	5.83^{b}±2.46	$32.46^{b,c}$±4.07
OPL	17.80^{c}±6.25^{c}	9.09^{c}± 3.08	$5.11^{a,b}$± 1.80	$31.99^{b,c}$±0.90

^{a,b,c,d} Means with different superscripts within columns have significant difference ($P<0.05$). PKC=palm kernel cake; OPF=oil palm frond; OPL=oil palm leaf.

Ruminal fermentation pattern can be evaluated from the volatile fatty acid (VFA) profiles and presented in Table 2. The microbial fermentation yielded the highest amount of VFA in SCB, followed by PKC, OPF, OPL, guinea grass and rice

straw. Palm kernel cake recorded the highest ruminal concentration of acetate compared to guinea grass and rice straw ($p < 0.05$), whereas the propionate for SCB treatment recorded higher ($p<0.05$) concentration than other treatments. The highest ruminal butyric acid concentration was also recorded by SCB, which is significantly ($p<0.05$) higher than guinea grass and rice straw. It can be concluded that all feed treatments produced similar fermentation patterns (Figure 1). When methane gas and total VFA production within treatments are compared, it showed that when one particular feed produced higher VFA, the methane gas production was lower.

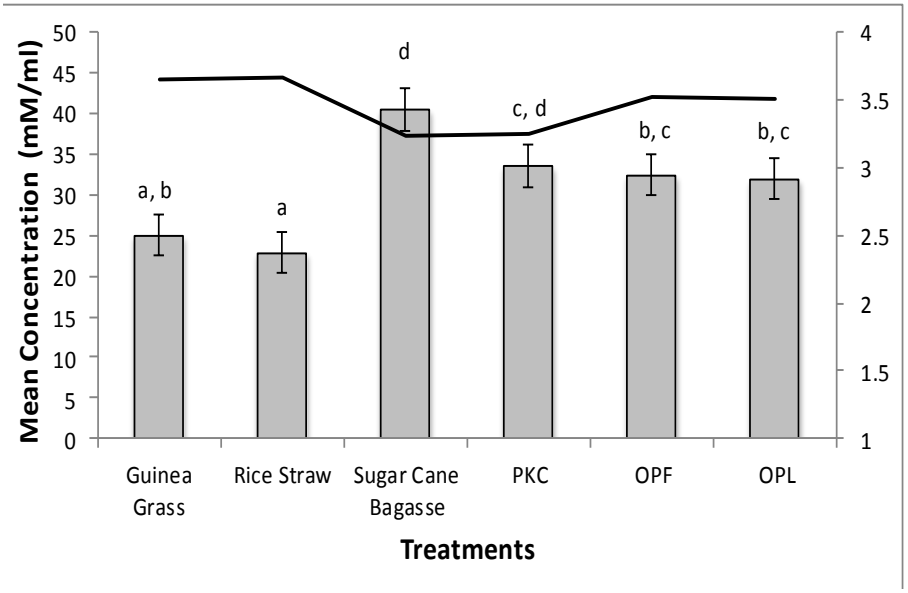

Figure 1: Rumen fluid volatile fatty acid and methane of treated goats. PKC=palm kernel cake; OPF=oil palm frond; OPL=oil palm leaf.

CONCLUSION

In conclusion, this preliminary study showed that local agriculture by-products have high potential of producing high levels of methane gas, which is harmful to the environment. Under these circumstances, developing systems for reducing methane emissions seems an appropriate and logical problem to address.

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ANTIBACTERIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM GOAT'S AND COW'S MILK

Noor Akmayati Abu Bakar, [1]Latiffah Hassan & [2,3]Saleha Abdul Aziz
[1]Department of Veterinary Laboratory Diagnosis
[2]Department of Veterinary Pathology and Microbiology
[3]Centre of Excellence on Swiflet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Milk from goats is gaining popularity due to their various nutritional and medicinal properties. The milk contained lactic acid bacteria that have antagonistic effect on common pathogens. The antagonistic effect is mainly related to the production of lactic acid, acetic acids, propionic acids, sorbic acid, benzoic acids, diacetyl, ethanol, phenolic, hydrogen peroxide, proteinaceous and bacteriocin by bacteria itself. Five raw bulk goats and 5 raw bulk cow's milk samples from different *Ladang Angkat*, Faculty of Veterinary Medicine, Universiti Putra Malaysia were collected. Milk were serially diluted in MRS broth and surface plated on MRS agar. Seven types of lactic acid bacteria were isolated from the goat's milk and nine from cow's milk. The mean count of lactic acid bacteria from cow's milk was 1.1×10^2 cfu/mL compared to goat's milk of 5×10^5 cfu/mL. This difference was found to be statistically ($p<0.05$) significant. The antimicrobial properties of lactic acid bacteria against eight common spoilage and pathogenic bacteria were measured using disc diffusion. The comparison of the inhibition zone of three genus of lactic acid bacteria between cow's and goat's milk were analysed statistically using Mann-Whitney U test. The analysis showed no significant ($p>0.05$) difference between the three genus of bacteria, which were *Enterococcus, Lactobacillus,* and *Leuonostoc spp*. In conclusion, the total amount of lactic bacteria in goat's and cow's milk differed significantly ($p<0.05$) but inhibition of bacteria used in the study was similar.

Keywords: goats milk, lactic acid bacteria, antimicrobial activity

INTRODUCTION

Failures of antibiotic treatment on diseases caused by bacteria have led to the increased in studies on antimicrobial originating from natural products. In the past two decade, various studies have focused on antimicrobial activities of probiotic and prebiotic products from lactic acid bacteria. Various species of lactic acid bacteria can grow under different environmental conditions, and they are

widespread in nature. Lactic acid bacteria which are Gram-positive, catalase- and oxidase-negative, nonspore-forming, rod bacilli or cocci shape, in chain or in pairs, has been extensively manipulated as probiotic. They are also a major industrial key player in the production of prebiotic products applied in the food industry and also as bio-preservatives. Lactic acid bacteria are commonly found in the gastrointestinal tract of various animals, in milk and dairy products, seafood products (Mauguin and Novel, 1994), and on some plant surface. They are generally used in the production and preservation of food products like cheese, sauerkraut, meat, yoghurt and silage (McKay and Baldwin, 1990). Milk from goats is gaining popularity due to the various nutritional and medicinal properties. The milk has been used as a prescription in various traditional therapies and as supplementary to cure various ailments. This study was designed to examine the availability of various lactic acid bacteria in goat's milk in comparison with cows' milk and determine the antimicrobial activities of against common pathogens.

MATERIALS AND METHODS

Sample collection

Bulk milk samples from 5 goat and 5 cow dairy farms samples were collected. Fifty millilitre of raw milk were obtained from 10 *Ladang Angkat* of Faculty of Veterinary Medicine, Universiti Putra Malaysia and Negeri Sembilan. All laboratory analyses were done at the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Enumeration of bacteria

The milk samples were serially diluted using the MRS (deMann, Sharpe, Rogosa) broth for isolation procedure and were surface plated on MRS agar. The MRS agar plate which contained samples was incubated anaerobically for 72 hours at 37°C. Plates containing 30 to 300 colonies were selected for enumeration.

Bacterial isolation and identification

Bacterial colonies grown on the plates were picked randomly and streaked on fresh MRS agar then were incubated anaerobically for 72 hours at 37°C. The genus was assigned to taxonomic groups using the schemes proposed by Nikita and Hemangi (2012).

Disc diffusion method

The 72 hours cultures of lactic acid bacteria were adjusted at concentrations of McFarland 0.5. Twenty microliter of lactic acid bacteria were added to blank paper disc and were incubated anaerobically for 24 hours at 37°C. The indicator bacteria to be tested were adjusted at concentrations of McFarland 0.5 and 500 µL of the suspension were mixed with 7 mL BHI soft agar (80% agar). This mixture was overlayed on the plate containing 24-hour incubated lactic acid bacteria cultures. After incubation for 24 hours at 37°C the inhibition zone was measured. Data were

analysed using Mann Whitney U test SPSS IBM 20 with significance level set at $p<0.05$.

Table 1: Indicator bacteria from lactic acid bacteria from milk

Gram-positive bacteria	**Gram-negative bacteria**
Bacillus cereus	*Eschericia coli*
Bacillus subtilis	*Pseudomonas aeruginosa*
Enterococcus faecium	*Salmonella sp.*
Enterococcus faecalis	*Enterobacter aerogenes*

RESULTS AND DISCUSSION

Lactic acid bacteria enumeration

The amount of lactic acid bacteria present in milk from both species was significantly ($p<0.05$) different. The isolation rate of lactic acid bacteria from goat's milk was higher than cow's milk. This study suggests significantly more lactic acid bacteria were present in goats than cow's milk. This finding supports the claim that goat's milk are more beneficial for health, which is consistent with the use of the milk in traditional therapy (Ray *et al*., 2008).

Lactic acid bacteria identification

The sugar tests identified four genuses in goat's milk which were *Enterococcus sp., Bifidobacterium sp., Lactobacillus sp.* and *Leuconostoc sp.* In cow's milk there were five genus namely *Enterococcus sp., Lactobacillus sp., Leuconostoc sp., Pediococcus sp.* and *Lactococcus sp.* The type of lactic acid bacteria present was more in cow's than in goat's milk. The differences in the composition of milk from different animals favour growth of different types of lactic acid bacteria (Ray *et al*., 2008).

Table 1: Antibacterial activity of lactic acid bacteria from goat's milk against indicator bacteria

Lactic acid Bacteria	**Inhibition zone (mm)**							
	Gram +ve indicator bacteria				**Gram –ve indicator bacteria**			
	B.cereus	*B.subtilis*	*E.faecalis*	*E.faecium*	*E.Coli*	*Salmonella*	*P.aeruginosa*	*E.aerogenes*
Enterococcus sp 1	NA	NA	NA	NA	NA	NA	NA	7.63
Enterococcus sp 2	NA	NA	10.6	8.2	13	14.5	NA	17.9
Bifidobacterium sp	NA	NA	NA	NA	NA	NA	NA	NA
Lactobacillus sp 1	9.9	12.1	16.5	16.2	25	19.7	NA	29.9
Lactobacillus sp 4	9.5	12.8	14.7	6.7	11	10.4	21.7	13.6
Leuconostoc sp 1	NA	9.6	NA	NA	NA	NA	NA	7.1
Leuconostoc sp 2	15.3	7.7	13.9	11.9	26.8	16.7	12.4	26.6

+ve = positive; -ve = negative

Table 2: Antibacterial activity of lactic acid bacteria from cow's milk against indicator bacteria

Lactic acid Bacteria	**Inhibition zone (mm)**							
	Gram +ve indicator bacteria				**Gram –ve indicator bacteria**			
	B.cereus	*B.subtilis*	*E.faecalis*	*E.faecium*	*E.Coli*	*Salmonella*	*P. aeruginosa*	*E.aerogenes*
Enterococcus sp 1	NA	NA	NA	NA	NA	NA	NA	NA
Lactobacillus sp 2	22	21.6	9.1	13.6	13.6	15.2	7.3	14.5
Lactobacillus sp 3	32	28.9	9.2	22.1	22.1	14.2	9.9	25.2
Lactobacillus sp 4	7.8	8.4	8.0	12.9	12.9	11.9	8.4	8.9
Leuconostoc sp 1	NA	10.4	NA	NA	NA	NA	NA	NA
Pediococcus sp 1	9.3	15.7	12.1	8.8	8.8	16.1	10.4	NA
Pediococcus sp 2	NA	NA	NA	NA	NA	NA	NA	NA

+ve = positive; -ve = negative; NA=not available

The activity three genuses of lactic acid bacteria in cow's and goat's milk was compared using the Mann-Whitney U test. There was no significant ($p>0.05$) difference in activity of the three bacteria, namely *Enterococcus., Lactobacillus,* and *Leuonostoc spp*.

Antibacterial activity of lactic acid bacteria

The zone of inhibition against the bacteria (Table 1) indicates that there was antimicrobial activity. This was determined in this study and presented in Tables 2 and 3. Lactic acid bacteria inhibit the growth of *Bacillus subtilis, Escherichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa*. By the values of zone of inhibition, these bacteria were sensitive towards *Lactobacillus* sp. (Bilge and Sumru, 2005). However, the antimicrobial activity of lactic acid bacteria in cow's and goat's milk did not differ significantly ($p>0.05$).

In conclusion, the study showed that although the total amount of lactic bacteria in goat's and cow's milk differed significantly ($p<0.05$), the inhibition of bacteria used in the study was similar.

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ULTRASTRUCTURE OF SWIFTLET DIGESTIVE TRACT

Nor Azreen Mohd Nasir & [1,2]Tengku Azmi Tengku Ibrahim
[1]Department of Veterinary Preclinical studies
[2]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The digestive tract of the swiflet is part of the body system responsible for the production of highly priced edible bird nest (EBN). The study aimed was to examine the ultrastructure of the digestive tract of the swiftlets. Swiftlets forage" on insects while flying over tree canopies of forests, plantations and fruit orchards. To relate the production of EBN and the nature of its feed and feeding behavior, it is hypothesised that the swiftlet has a simple but very efficient digestive system. True to the hypothesis, grossly the digestive tract of the swiftlet comprised of only the esophagus, gizzard and intestine. Compared to its ground dwelling counterpart, notably the chicken, the crop and caecum were absent in the swiftlet while there was no clear gross distinction between the large and small intestines. An interesting and distinct observation under scanning electron microscopy (SEM) was the presence of villi lining the esophagus. The villi in the esophagus could indicate a means to improve absorption of nutrients from insects, on which the swiftlet forages. This inference was made from an observation under the SEM that there was a whole, intact insect trapped in-between the finger-like projections of the esophagus. Another important finding in the study of the digestive system of the swiftlet was the unusually predominant mitochondria in the supranuclear and infranuclear cytoplasm of the intestinal epithelial cell. Mitochondria are known to be the energy storing house for cells with the prominent array of microvilli at the apical epithelial intestinal mucosa further lends support to the fact that the swiftlet has a simple yet a very efficient digestive system.

Keywords: swiftlet, digestive tract, ultrastructure, villi, mitochondria.

INTRODUCTION

Swiftlets belong to family of Apodidae, a small-sized swift found abundantly in the South-East Asian region. The *Aerodramus fuciphagus,* the species that produce the white edible bird's nest are semi-intensively ranched in modified bird houses equipped with bird calling systems to attract birds, suitable bird house temperature, lighting and humidity control systems. Swiftlets are insectivorous and they eat of forage for insects while flying over various tree canopies, forest, plantation and fruit

orchards.

The anatomy of the avian digestive tract markedly influences the utilisation of feed. This study reports on the ultrastructure of the digestive tract of the swiftlets focusing on the mucosa especially on the epithelium of the digestive tract. This study provides a better understanding of the digestive physiology and nutrition of the swiftlets.

MATERIALS AND METHODS

Three swiftlets (*Aerodramus fuciphagus*) were used in this study. They were caught early in the morning in Serdang, Selangor, Malaysia and immediately brought to the Microscopic Unit, Institute of Bioscience, Universiti Putra Malaysia. Following cervical dislocation, the abdominal cavity of the swiftlets was opened and the digestive tract removed for examination of gross structure. Samples of the esophagus, gizzard, small anterior and posterior parts of the intestines were collected and fixed in 4% glutaraldehyde. After thrice washing with 0.1 M Sodium cacodylate buffer for 10 minutes at each change, the samples were post-fixed in 1% Osmium tetroxide. The tissues were washed again with 0.1M Sodium cacodylate buffer. Sample dehydration was done using ascending concentrations of acetone (35, 50, 75 and 95%). Samples were finally dehydrated thrice in 100% Acetone with 10 minutes each change.

Scanning Electron Microscopy

Following final dehydration the samples were critical-point dried and mounted onto specimen stubs before sputter coated with gold palladium. The samples were examined under scanning electron microscope operating at 15 mm of working distance and 15 kV.

Transmission Electron Microscopy

Following final dehydration the samples were infiltrated with an equal volume of acetone: resin mixture. The samples were embedded in a flat embedding mould filled with the resin mixture and polymerised in an oven at 60°C for 48 hours. Semithin section (1µm thick) was obtained using an ultramicrotome. The semithin sections were stained with toulidine blue stain and viewed under the light microscope to select the area of interest prior to the ultrathin sectioning. Ultrathin sections of 1Å thick were obtained and mounted on the 200-mesh-cooper grids and dried using filter paper. The sections were stained with uranyl acetate and washed with double distilled water, stained with lead citrate and washed with double distilled water. The stained sections were examined under a transmission electron microscope operating at 80 kV.

RESULTS AND DISCUSSION

The digestive tract of swiftlet was made up only of the esophagus, gizzard and intestine. In the absence of the crop, proventriculus, caecum and no clear distinction on different parts of the intestine showed that the swiftlets are different from domestic fowl such as the chicken. This swiftlet has a simple but efficient digestive tract that is adapted to its feeding behavior of eating insect while flying (Lim and Cranbrook, 2002).

Grossly, the gizzard has a very thick muscular wall. No microvilli were observed on the mucosal surface of the gizzard. The presence of a part of an insect in the gizzard and the absence of villi and or microvilli in the mucosa of the gizzard could indicate that there is no absorption in the gizzard. It is probable that the gizzard is involved in the breakdown of hard chitinous insects.

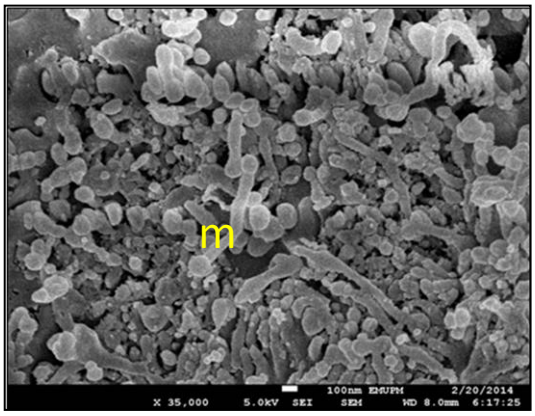

Figure 1: Scanning electron micrograph of microvilli-like (Mv) structure on the mucosal surface of esophagus. Magnification ×35,000.

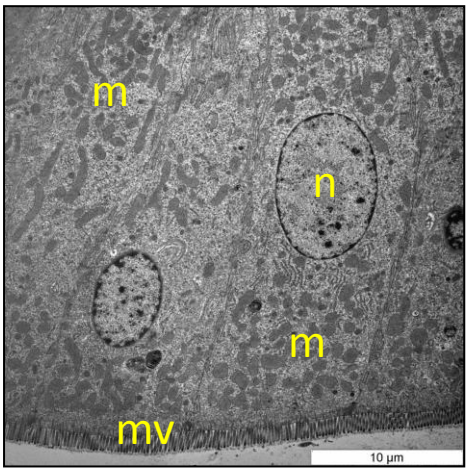

Figure 2: Transmission electron micrograph of simple tall columnar epithelium cells lined with villi. Numerous microvilli (mv) covered the epithelium with numerous mitochondria (m) in the apical and basal cytoplasm of epithelial cells. n=nucleus. Magnification ×5,000.

With the presence of the finger-like projections on the mucosa of the esophagus and an insect trapped amongst the projections, it is very tempting to speculate that the finger-like projections could functionally be involved in the absorption of nutrients directly from the insect. Microvilli-like structure on the surface of the epithelial cell of the esophagus (Figure 1) and a large number of mucus granules within the epithelial cells indicate that apart from providing mucus to protect the mucosal surface, the epithelium of the esophagus may also function in absorption of

nutrients.

The small intestine is the normal site of absorption of nutrients. The surface of the tall columnar cells is provided with numerous microvilli to increase the surface area of the absorptive tall columnar cells. Predominance of mitochondria and the presence of microvilli are indicative of highly efficient absorptive columnar cells of the intestinal mucosa (Figure 2).

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PATHOGENICITY OF MALAYSIAN *PASTURELLA MULTOCIDA* ISOLATE IN COMMERCIAL BROILER CHICKENS

Nor Hidayah Zailani, [1]Mohd Hair Bejo, [1]Siti Khairani Bejo & [1,2]Zunita Zakaria

[1]Department of Veterinary Pathology and Microbiology
[2]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Univeri Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT

Pasturella multocida is a causative agent of fowl cholera in chickens causing economic losses to the poultry industry due to respiratory and musculoskeletal problems. The treatment of this disease can be achieved through proper vaccination programmes and antibiotic therapy. It is vital to determine the pathogenicity of *P. multocida* isolate for future development of fowl cholera vaccine. The objectives of this study were to isolate and identify *P. multocida* from a field outbreak of fowl cholera in commercial broiler breeder chickens and to determine the pathogenicity of the isolate based on different routes of infection. Fifty-four day-old commercial broiler chicks were divided into 6 groups namely the sacrificed groups A1, B1 and C1 and mortality groups A2, B2 and C2. Groups A1 and A2, and Groups B1 and B2 were inoculated with 0.1 mL *P. multocida* containing 10^8 organisms through intra-nasal and intra-muscular route, respectively. The groups C1 and C2 remained uninoculated and served as the control groups. All chicks were observed for clinical signs and other abnormalities throughout the trial. Three chicks from the control group were sacrificed on day 0 post-inoculation (pi), whilst three chicks from each group (Groups A1, B1 and C1) were sacrificed at days 1, 4, 7 and 14 pi. The body weight of the sacrificed and dead chicks were recorded and samples which consisted of cloaca swab, blood, liver, trachea, lung, egg yolk and spleen were taken for bacterial isolation and identification. The study showed 100 and 20% mortality of chicks from groups B2 and A2, respectively No mortality was recorded in groups C1 and C2. A chick in group A1 showed mild diarrhoea with faecal stain at the cloaca area on day 4 pi. All chicks in group B1 died on day 1 pi. At necropsy, pin-point necrosis of the liver, hepatomegaly, congestion of the lung and spleen, haemorrhages on the serosa of the gizzard and heart were observed. At post-mortem there was no gross lesion in live chicks of group A1 on day 4, 7 and 14 pi. Bacterial isolation from group A1 dead chicks at days 1 and 2 pi revealed *P. multocida* in the liver (100% for both days), spleen (100 and 66%, respectively), lung (100 and 33%, respectively), trachea (50 and 66%, respectively) and egg yolk (50 and 0% respectively). *P. multocida* was isolated from the liver (58%), spleen (42%), lung (58%), trachea (33%), egg yolk (33%) and cloaca swab (8%) of Group B1 dead chicks. In the sacrificed group A1, *P. multocida* was only isolated from the liver

(100%), spleen (63%) and egg yolk (63%) on day 1 pi. No *P. multocida* was isolated from the control group or at days 4, 7 and 14 pi. *P. multocida* was isolated from the trachea and lungs of the dead chicks, not from live chickens. Histopathology examination revealed focal necrosis of the liver, pneumonia and congestion of the lungs and heterophil infiltration of trachea of dead chicks. Focal massive heterophil infiltration in liver and trachea as well haemorhages of the lung for live chicks were also observed. In conclusion, *P. multocida* isolate is highly pathogenic to chicks when administered given via intra-muscular route.

Keywords: pathogenicity, *Pasturella multocida*, commercial broiler chickens, intra-muscular and intra-nasal routes.

INTRODUCTION

Fowl cholera is cause by *Pasturella multocida* and is usually associated with septicaemic disease in chickens. *P. multocida* in avian have 5 types of capsular strains namely A, B, D and F. However *P. multocida* type A is recognised as the primary cause of fowl cholera in chickens (Glisson and Cheng, 1991).

P. multocida is a gram-negative bacteria, non-motile and non-spore forming rod occurring singly in pairs and occasionally as chains or filament and can grow either aerobically and anaerobically. In the fresh organ, isolated *P. multocida* stains bipolar. The bacteria may grow on blood agar but not on MacConkey agar.

There are two forms of fowl cholera, namely acute and chronic forms. In acute form, fever, anorexia, ruffled feather, mucoid discharge from mouth, diarrhoea and increased respiratory rate can occur, while chronic infection is usually associated with localised infection especially at the wattles, sinuses, leg or wing joints, foot pads and sternal bursae, which get swollen. In the acute form of the disease, at necropsy, the gross lesions that can be observed are petechial and echymotic haemorhages, hepatomegaly and focal necrosis of the liver. In chronic form, infections are characterised by localised infections that become suppurative and widely distributed in the body. Histological findings in acute form of infection are coagulative necrosis and heterophilic infiltration, while fibrinoheterophilic meningitis can occur in acute form of infection (Fadly *et al*., 2008).

The objectives of this study were to isolate and identify *P. multocida* from field outbreak of fowl cholera in commercial broiler chickens, to determine the gross and histological findings in chicken infected with the isolate and to determine the pathogenicity of the isolate based on the different routes of infection.

MATERIALS AND METHODS

Pasteurella multocida

Liver samples were collected from commercial broiler breeder chickens with typical lesions of fowl cholera and the samples were cultured on the blood agar. The

features of colony was described as medium, mucoid, cream to grey colour, round shape with size 1×1 mm. The organisms appeared gram-negative, non-spore forming cocci, occurring singly or in pairs under the microscope (100 × magnification). Bacteria in bipolar form under wright-stained isolates from fresh organ were suggestive of *P. multocida*. Biochemical tests confirmed the isolates as *P. multocida*.

Inoculum preparation

To prepare 1×10^9cfu/mL of inoculum, 8 mL of phosphate-buffered saline was transferred into the test tube and 9 µL mixture of antigen added to the tube and comparison made against 0.5 McFarland standard. The mixture was transferred into a sterile Falcon tube using a pipette and the inoculum was ready to be used.

Experimental design

Fifty-four day-old commercial broiler chicken were divided into 6 groups namely the sacrificed groups A1, B1 and C1 and mortality groups A2, B2 and C2. Group A1 and A2, and group B1 and B2 were inoculated with 0.1 mL *P. multocida* containing 10^8 organisms through intra-nasal and intra-muscular routes, respectively. Group C1 and C2 remained uninoculated. Three chicks from each group A1, B1 and C1 were sacrificed on days 1, 4, 7, and 14 days post-inoculation (pi). Liver, trachea, lung, spleen, egg yolk, cloaca swab and blood samples were collected for bacterial identification and isolation. The liver, trachea and lung were taken for histological examination.

Bacteria isolation and identification

Samples were streaked on blood agar and as direct smears on glass slides to examine the characteristic features and bipolar form respectively. Biochemical tests using SIM (+ve), TSI (+ve), urease (-ve), citrate (-ve), oxidase (+ve), catalase (+ve), trehalose (-ve), sorbitol (+ve), mannitol (+ve), dulcitol (-ve), and ODC (+ve) for *P. multocida* identification were done for confirmation (Charitha *et al*., 2012).

RESULTS AND DISCUSSION

Two and 3 chicks from Group A1 died on days 1 and 2 pi, respectively. Only one chick showed mild diarrhoea with faecal stain at the cloaca area on day 4 pi. However all chicks in group B1 died on day 1. Meanwhile, chicks from group C1 were healthy throughout the trials. Group A2 showed only 20% mortality rate while 100% mortality was observed in group B2 and no death was observed in group C1 throughout the trial. Bacterial isolation and identification revealed that *P. multocida* was isolated from group A1 from all samples collected from the dead chicks on days 1 and 2. However *P. multocida* was not isolated from the samples of live chicks at days 4, 7 and 14 pi. This was probably due to the role of IgA in eliminating the organisms from 3 days onward after re-infection (Tamura and

Kurata, 2004). *P. multocida* was isolated from all samples from dead chicks except the blood from Group B1 (Pabs-Garnon and Soltys, 1971; Tsuji and Matsumoto, 1989)

The overall results of this study indicated that *P. multocida* isolate was highly pathogenic when given in chicks via intra-muscular route compared to intra-nasal route with 100% and 20% mortality, respectively. It is interesting to note that damages to the respiratory organs such as trachea and lung were the main cause of death to the infected chicks. It was suggested that the cause of death of chicks was due to respiratory failure as a result of pneumonia and congestion of the lungs from the infection (Matsumoto and Strain, 1993). However, the high mortality rate in chicks inoculated with the bacteria through intra-muscular route could be due to two possibilities; first as a result of respiratory failure due pneumonia and congestion of the lung and second, as a result of massive bacteraemia and endotoxic shock also caused by *P. multocida* (Rimler and Rhoades, 1987). The study suggested that systemic invasion rate of *P. multocida* occurred during the 24 hour period post-inoculation.

Gross lesions such as pin-point haemorrhages on the liver with hepatomegaly, congestion of the lung and spleen with splenomegaly, ecchymotic hemorrhages at the apex of the heart were observed in the dead chicks. No significant gross lesion was observed in the live chicks. It is suggested that the systemic spread and toxic effects of the organisms had caused development of the lesions prior to severe bacteraemia before death of the chicks (Kyaw, 1944).

In conclusion, systemic spread of *P. multocida* to vital organs and production of toxin are suggested to be the cause of death of the chicks inoculated with the organism. The organism is especially pathogenic to chicks when administered via the intra-muscular route.

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ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY OF HORSE NASOPHARYNX BACTERIAL FLORA

Nor Jawahir Abdullah, [1,3]Zunita Zakaria, [2]Nurul Hayah Khairuddin & [4]John Tito Sapalo

&

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Large and Exotic Animal Medicine and Surgery
[3]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Skuadron Istiadat Berkuda
Kem Sungai Buluh, 47000 Sungai Buluh, Selangor, Malaysia

ABSTRACT

Data on normal bacteria flora profile on upper respiratory tract of the horses in tropical countries is lacking. A preliminary study was undertaken to identify the bacteria flora in the nasopharyx of horses and to determine their antibiotic sensitivity pattern. Fifty-six bacteria strains isolated from nasopharyngeal lavage of 13 apparently healthy horses were subjected to antibiotic sensitivity test using the Kirby Bauer method. They were *Klebsiella pneumoniae* (19.6 %), *Pseudomonas aeruginosa* (16.1%), *Staphylococcus aureus* (14.3%), *Staphylococcus intermedius* (12.5%), *Acinetobacter baumannii* (10.7%), *Actinomyces* spp. (8.9%), *Streptococcus zooepidemicus* (7.1 %), *Chryseobacterium indologens* (3.5%), *Escherichia coli* (3.5%), *Pasteurella haemolytica* (1.8%) *and Enterococcus faecium* (1.8%). *Pseudomonas aeruginosa*, *Chryseobacterium indologens* and *Acinetobacter baumannii* were multidrug resistant.

Keywords: horse, respiratory tract, bacterial flora, antibiotic resistance

INTRODUCTION

Horses are obligate nasal air breathers where complete separation is normal between the nasopharynx and oropharynx except when they are swallowing or coughing. Upper respiratory tract is very crucial in a sense that any changes in the airways will affect the exercise capacity, performance as well as health of horses. Nasopharynx is a site that is naturally colonised by a variety of normal bacteria flora, which is also opportunistic and under certain circumstances alter the local immunity of the nasopharynx causing diseases. In recent years, there are evidences showing increase of occurrence of multiple antibiotic resistant bacteria in humans and animals

(Fletcher *et al.*, 2004; Fraser and Jorgensen, 1997). The trend of bacteria resistance towards selected antibiotics commonly used in equine medicine in Malaysia needs to be determined. It is also important to determine the most effective antibiotics for treatment of bacterial infections. This preliminary study was conducted to isolate and identify the bacterial flora population in the nasopharynx of horses and to determine their antibiotic sensitivity pattern.

MATERIALS AND METHODS

Sampling of nasopharynx lavage

Thirteen apparently healthy horses were randomly sampled from two stable located in Selangor, Malaysia, where 2 horses were from Stable 1 and 11 from Stable 2. Nasopharynx lavage was performed while the horse was restrained in a crush with a muzzle twitch applied. Sedation was achieved with combination of Acepromazine and Xylaxine administered intravenously. Nasopharynx lavage was obtained using a sterile extension tube of 1 m length attached to a flexible endoscope cable. The endoscope was then introduced into one of the nostrils through the nasal meatus to reach the nasopharynx region. Approximately 100 mL sterile saline was flushed, using a 20 mL syringe, through the extension tube to irrigate the mucosa of the nasopharynx. The lavaged fluid was then aspirated, placed in a sterile 30 mL glass bottle and transported to the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia where all samples were centrifuged at 400 × *g* for 10 minutes. The supernatant was discarded leaving the sediment.

Isolation procedures

A drop of sediment was then streaked onto blood agar and McConkey agar and incubated at 37ºC for 24 to 48 hours. Gram staining was done on the colonies and pure culture was made from the primary cultures on blood agar.

Bacteria identification

The colonies from pure cultures were grouped according to gram staining and colony morphology characteristics. Representatives from each group were subjected to biochemical tests.

Antibiotic Sensitivity Test

A bacteria colony from respective pure culture plate were picked using an inoculating loop and transferred to 2 mL sterile saline. The tubes were vortexed to achieve a homogenous suspension. Using Wickerham's card, the turbidity of the bacteria suspension was compared with the turbidity of McFarland 0.5 standard suspension. By using this standard, visual comparison of bacterial density was adjusted to be the same to bacterial suspension containing between 1×10^8 and 2×10^8 cfu/mL of *E. coli.* Bacterial suspension on sterile swab was streaked onto Mueller Hinton Agar (MHA) plate surface using three overlapping directions. Using

a dispenser**,** antibiotics discs were placed onto the MHA. Antibiotic discs used were Gentamycin (10 µg), Enrofloxacin (5 µg), Ampicillin (10 µg), Streptomycin (10 µg), Trimethoprim – sulphamethoxazole (25 µg) and Penicillin (10 µg). The plates were then incubated at 37ºC for 24 hours. Each zone of inhibition around antibiotics was measured using a caliper to determine the susceptibility of bacteria. By referring to CLSI 2012, the zone of diameter measurements was used to classify the bacteria as susceptible, intermediate or resistant.

RESULT AND DISCUSSION

Based on Table 1, 5 most frequently isolated bacteria were *Klebsiella pneumoniae* with (19.6%), *Pseudomonas aeruginosa* with (16.1%), *Staphylococcus aureus* comprises (14.3%), *S. intermedius* (12.5%) and *Acinetobacter baumannii* (10.7%) of total number of isolates. Three bacteria isolates obtained in the study that are not commonly found in temperate climate include *Enterococcus faecium* (1.8%), *Chryseobacterium indologens* (3.5%) and *Acinetobacter baumannii* (10.7%). *Enterococcus faecium* is a normal flora of the intestinal tract of mammals and can be found in faeces and waste-water. The organism may be accidently sniffed from the air in the stables contaminated with faecal material. *Chryseobacterium indologens* is actually not the normal flora of vertebrate mammals; they only can be found in the environment, especially soil. Many *Chryseobacterium* and *Elizabethkingia* strains are present in the soil, freshwater, and marine environments. *Acinetobacter baumannii* is an opportunistic bacterium responsible for nosocomial infection in immunocompromised animals (Endimiani *et al*., 2011).

Table 1: Bacteria isolates from nasal larvage of horses

Bacteria	No. of samples with isolates	%
Streptococcus equi subsp. zooepidemicus	4	7.2
Enterococcus faecium	1	1.8
Actinomyces spp.	5	8.9
Staphylococcus aureus	8	14.3
Staphylococcus intermedius	7	12.5
Acinetobacter baumannii	6	10.7
Pseudomonas aeruginosa	9	16.2
Chryseobacterium indologens	2	3.5
Klebsiella pneumoniae	11	19.6
Pasteurella haemolytica	1	1.8
Escherichia coli	2	3.5
Total	56	100

Multidrug resistant can be defined as resistance of bacteria to three or more antimicrobial agents. Based on that definition, out of the isolates in this study, three

bacteria species, *Pseudomonas aeruginosa*, *Chryseobacterium indologens* and *Acinetobacter baumannii* were multidrug resistant. All bacteria isolates were sensitive to Fluoroquinolones and highly sensitive to Trimethoprim-Sulphadiaxolone (Table 2). However, 50% of the isolates showed resistance towards ß-lactams antibiotics. As the first line antibiotics, the ß-lactams may have been over-used or misused in treating infections. Gram-negative bacteria are highly resistance to this antibiotic class and this potentially herald the end of treatment using ß-lactam drugs (Nordmann *et al.*, 2011).

Table 2: Response of bacteria to antibiotics

Antibiotic	Sensitivity (%)	Intermediate (%)	Resistance (%)
Enrofloxacin	100	0	0
Trimethoprim- Sulfamethoxazole	91.7	0	8.3
Penicillin G	41.7	8.3	50
Ampicillin	41.7	8.3	50
Gentamycin	50	33.3	16.7
Streptomycin	41.7	16.7	41.7

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PATHOGENIC MICROORGANISMS IN WILD RATS AND SHREWS

Nur Ain Shafiqah Mohd Said, [1]Mohd Azmi Mohd Lila, [1,3]Shaik Mohamed Amin Babjee, [1,3]Abdul Rahim Mutalib, [2]Abdul Rani Bahaman & [1]Zeenathul Nazariah Allaudin

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Laboratory Diagnosis
[3]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Rats are the most widespread pest species and well-known to transmit zoonotic disease agents and cause destruction to the environment. In this study, twenty rodents were trapped by using wire traps baited with various baits in Serdang, Sepang, Seremban and Rembau, Malaysia. The locations represented the urban and suburban habitats and distribution of rodents. Three rat species and one shrew species were trapped from these habitats. There was no significant ($p>0.05$) difference in bait preference by the rats from either habitat. No *Salmonella* was detected in the rodents. Seventy-five percent of the rodents were found to be infested with at least one of nine different types of ectoparasites identified. All rodents (100%) were infested with at least one type of endoparasites. There was significant ($p<0.05$) difference in parasitic distribution between habitats, with urban rats more infested with ectoparasites. Among the parasites of potential medical importance identified were *Polyplax spinulosa*, *Hoplopleura pacifica* and *Ornithonyssus bacoti*. In conclusion, the species parasites of rats were partly determined by the nature of their habitats. Also, the rodents live in close proximity to human especially in urban areas and they carry organisms that may be detrimental to the health of humans and other animals. Thus, appropriate measures are needed to control the rodent population to prevent the spread of diseases to humans and other animals.

Keywords: rat infestation, bait preference, S*almonella*, ectoparasites, endoparasites, public health

INTRODUCTION

Rats are the most common urban pest species that has been a major problem all around the world. The rodent pests have caused extensive structural damages, foodstuff spoilage and are carriers of various disease agents, such as *Leptospira* sp.,

Seoul hantavirus, murine typhus, *Yersina pestis* and *Coxiella burnetti* (the cause Q fever in humans). Rats also harbour various types of ecto- and endo-parasites such as *Taenia taeniformis, Hymenolepsis diminuta, Capillaria hepatica*, *Toxocara cati*, which causes toxocariasis in humans, *Nippostrongylus brasiliensis, Heterakis* spp and *Toxoplasma gondii*. Some of these ecto- and endo-parasites are important vectors of pathogenic microorganisms and parasitic zoonoses such as babesiosis and plague (Paramasvaran *et al*., 2009). Since the rodents live in the close proximity to humans, they are in constant contact with humans and increasing the risk of zoonotic disease transmission.

This study was conducted to investigate the habitat and the potential role of rats in transmitting disease agents.

MATERIALS AND METHODS

Trapping and Identification of Rodents

Trapping of rodents was conducted in Serdang and Sepang and Rembau and Seremban, Malaysia. All rodents were trapped alive using ten wire traps of various sizes. Oil palm fruit, banana, baked coconut, dried fish, bread and leftover food were used to determine bait preferences of the rodents. Trapped rodents were euthanised by placing the rodents in a container with cotton wool soaked with chloroform. Identification of rodent species was by morphological measurements and physical appearances of the rodents using the keys and illustrations described by Brooks and Rowe (1987) and Herbreteau *et al.*, (2011).

Collection and Processing of Samples

Upon euthanasia, the fur of the rodents was combed vigorously with a flea comb to dislodge ectoparasites onto a tray layered with a piece of white paper. Fine forceps was used to extract ticks that did not dislodged by combing. Skin scrapping and hair pluck were taken around skin lesions. All recovered ectoparasites were preserved in a collection bottle containing 75% alcohol, and mounted with Hoyer's medium before they were examined under the microscope for identification. The rodents were dissected and the gastrointestinal tracts were examined for adult worms. Colon content was taken for faecal floatation technique to look for the presence of ova and *Coccidia*. All adult worms were put in the 75% alcohol for preservation. Cestodes were stained with aceto-alum-carmine to view of the scolex and mature proglottid for more accurate identification.

To isolate *Salmonella*, swab samples of colon content were incubated in Rappaport-Vassiliadis broth as selective-enrichment broth for *Salmonella* and incubated at temperature of 37^{o}C for 48 hours. Then loopfuls of the cultured broth were spread onto XLD and BGA agar and incubated at 37^{o}C for 24 hours. If the typical *Salmonella* colonies appeared on BGA (as pink/red colonies) and on XLD (as blackish colonies), subculture was done on blood agar to purify before dentification using biochemical tests, which included SIM, TSI, citrate, urea, ONPG

and LDC. If the results of the biochemical tests showed typical characteristics of *Salmonella*, serotyping with Polyvalent O was conducted.

All findings were analysed using chi-square test.

RESULTS AND DISCUSSION

Species Distribution

Seventeen rats and 3 shrews were caught during the study. *Rattus tiomanicus* was the most dominant population of rat species caught. This could be due to the fact that most of the suburban sampling locations were located near oil palm plantations and *R. tiomanicus* are usually found in secondary forests and oil-palm plantations.

Bait Preference

Chi-square test showed that there was no significant difference between types of bait preferred by the trapped animals. However, oil-palm fruit was observed to be much more preferred by rodents in sub-urban areas where most of the *R. tiomanicus* were caught. According to Brooks and Rowe (1987), rodents tend to select a nutritional balanced diet if given the choice, and oil-palm fruits are known to have high nutrient value. The urban rats appeared not to be uninterested in oil-palm fruit, most possibly because the fruit is not a familiar food material for urban rodents. The fruit was probably considered by the rodents as something new and need to be avoided. This phenomenon may be associated with the 'neophobia', which is fear of eating new and unfamiliar foods (Inglis *et al.*, 1996). Neophobia may also explain why in this study it was difficult to capture the rats, especially in urban habitats, which are commonly inhabited by *Rattus norvegicus.* In this study, the rats seemed to be avoiding the traps.

Isolation of Salmonella

No *Salmonella* was isolated from the colon content in this study. *Salmonella* is excreted intermittently in the faeces of the rats, and only a small percentage (20%) of infected rats became carriers and shed the bacteria in their faeces (Bartram *et al*., 1940). Dunlap *et al.* (1991) showed that *Salmonella* tends to reside in the spleen and liver, by localising in the cells of those organs as a means to escape the host immune system or from antibiotic treatment.

Ectoparasite

Almost all rats were found to harbour at least one species of ectoparasites. Chi-square test showed that there is significant ($p<0.05$) difference between number of ectoparasite-infested rats and habitats with the urban being more infested with ectoparasites than suburban rats.

Rats from both habitats harboured almost the same species of ectoparasites. Among ectoparasites species that were collected were mites: *Ornithonyssus bacoti, Laelaps echidninus, Notoedres muris,* and Trombiculid mites; lice: *Polyplax spinulosa, Polyplax serrata,* and *Hoplopleura pacifica;* ticks: *Ixodes;* and fleas:

Xenopsylla cheopis. The finding of the study is similar that of an earlier study (Paramasvaran *et al.,* 2009).

Endoparasites

All rats and shrews were found to be infested with more than one endoparasites. There was no significant ($p>0.05$) difference between number of infested rats and habitats. Also there was no significant ($p>0.05$) difference between type of endoparasites between habitats.

Using the faecal floatation technique, it was found that very few rats and shrews had *Coccidia* (Urban: 25%, Sub-urban: 33.3%). The rodents were also found to habour *Capillaria*, *Ascarididae*, Strongyles, and *Hymenolepididae.* Examination of the intestinal contents recovered mostly tapeworms and they were of the genus *Hymenolepididae*. One acantocephalan was also found in one of the rats from urban habitats. Very few adult worms were collected from direct examination of intestinal contents. This suggests that these rats were mildly infested, thus the number of the adult worms were too low to be detected by examination of the intestines.

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EFFECT OF SHORT-TERM DIETARY OMEGA-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION ON COGNITIVE FUNCTION DEVELOPMENT IN MICE

Nur Asmiza Mustaffa Kamal, [1]Wan Mastura Shaik Mohamed Mossadeq & [1]Hafandi Ahmad

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

ABSTRACT

Dietary omega-3 polyunsaturated fatty acids are associated with improvement in learning and memory function in animals. The best dietary sources of omega-3 fatty acids are fish from the ocean and fish oils. The improvement of learning and memory function is associated with the duration of dietary omega-3 polyunsaturated fatty acid (PUFA) supplementation. There is little information on the short-term effect omega-3 fatty acid on learning and memory in animals. Therefore, this study was performed to assess the effect of short-term dietary omega-3 PUFA supplementation on cognitive function development in mice. Seven week old male BALB/c mice were allocated equally into four treatment groups. The mice were fed either normal pellets with added 6.66% fish oil (Group 1, n=6), normal pellets with added 6.66% soybean oil (Group 2, n=6), normal pellets with added 10% butter (Group 3, n=6) or normal pellets only (control group, Group 4, n=6). After 3 weeks on the treatment diets, all animals were tested for learning and memory function using the Y-maze test. In this test performance is based on the number of entries and time spent in the novel arm than other arms of the Y-maze. The results showed that animals fed with dietary omega-3 PUFA supplementation showed an increased cognitive performance with a significantly ($p<0.05$) higher total arm entries and time spent in the novel arm. Thus, the study showed that short-term dietary omega-3 PUFA supplementation can improve cognitive function of mice.

Keywords: omega-3 polyunsaturated fatty acids supplementation, Y-maze test, cognitive function, learning and memory function

INTRODUCTION

Learning can be defined as a process that involves changes in behavioural or neuronal responses that are related to the external stimulation and metabolic state of organisms (Sokolov, 1977). Memory is developed by modification of intrinsic brain mechanisms that caused changes in the efficiency of the subsequent responses,

identification of the previously given signal, or reproduction of the learned response.

Several factors can influence learning and memory functions. For example, dietary supplementation with sardine oil that is rich in omega-3 fatty acids improved learning and memory performances in the Morris water maze tasks (Chung *et al*., 2008; Joshi *et al*., 2004; Jensen *et al*., 1996). Also, nutritional status may influence the mental ability performance of rats (Whalley *et al*., 2004). The improvement of learning and memory functions is also associated with the duration of dietary omega-3 fatty acid supplementation (Lerch *et al.,* 2011; Tang *et al.,* 2012) and this dietary supplementation is important for metabolic changes in the animal body.

A number of short- and long-term studies were performed to assess the effect of polyunsaturated fatty acid (PUFA) in the diet. Long-term feeding of triglyceride, ethyl ester, free fatty acid, docosahexanoic acid bound phospholipid and eicosapentaenoic acid at high dosages have been shown to affect omega-3 PUFA absorption on the lipid membrane in mice (Tanaka *et al*., 2003). A short-term study of a seven day duration performed by Tang and colleagues in (2012) showed that mice fed with different formulations of omega-3 fatty acids in high fat diets showed increase in lipid metabolism in the brain, the consequence of which improved learning and memory functions.

There is limited information on the effect of short term omega-3 PUFA feeding on cognitive function. Therefore, the objective of this study was to determine the short-term effect of dietary omega-3 PUFA supplementation on cognitive function development in mice.

MATERIALS AND METHODS

Experimental design

The study was conducted to determine the effects of omega-3 PUFA supplemented diet on the cognitive function of BALB/c strain male mice. Cod liver fish oil, soybean oil and butter were incorporated as supplemental diets. Mice were divided into four groups and fed according to their designated supplemental diet. Each group consists of six mice. The treatment groups were as follows: mice fed with normal pellets with added 6.66% fish oil and 3.33% soybean oil (Group 1, n=6), normal pellets with added 6.66% soybean oil and 3.33% fish oil (Group 2, n=6), normal pellets with added 10% butter (Group 3, n=6) and normal pellets only (control group, Group 4, n=6). All mice were fed treatment diets for three weeks and tested for learning and memory functions using the Y-maze test.

Animals

A total of 24 BALB/c male mice were kept in the Animal House, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The mice housed in polycarbonate cages (45 × 30 ×20 cm), with stainless steel mesh cover, sawdust and 2 plies of paper towel as the bedding material. The animal room was air-conditioned and

regulated at an ambient temperature of 23±2°C with a relative humidity of 50±10%. Room lighting was 12 hours light and 12 hours dark. This project was approved by the Institutional Animal Care and Use Committees (IACUC) FPV/FYP/2013/087.

Diet

As shown in Table 1, the four dietary groups were formulated by adding specific oil mixtures. Mice were fed at 2.5 to 3% day dry matter body weight (DMBW) or 15 g/day, and body weight changes were recorded once a week. Clean drinking water was provided *ad libitum* throughout the entire experiment. The diets were prepared twice a week and stored at -20°C.

Table 1: Recommended ratio of n-3 and n-6 in diet according to American Dietetic Association.

Composition	Composition of diet (% dry weight)			
	G1	G2	G3	Control
Fish oil	6.66	3.34	-	-
Soybean oil	3.34	6.66	-	-
Butter	-	-	10.0	-
Crude protein	18.8	18.8	18.8	18.8
Crude fibre	5.0	5.0	5.0	5.0
Crude fat	3.0	3.0	3.0	3.0
Ash	8.0	8.0	8.0	8.0
Calcium	1.0	1.0	1.0	1.0
Phosphorus	0.8	0.8	0.8	0.8
Non-protein nitrogen	53.4	53.4	53.4	63.4
Moisture (% of wet weight)	13.0	13.0	13.0	13.0

Fish oil (source of omega-3), soybean oil (source of omega-6) and butter (source of saturated fatty acids) were purchased from the local market. The dietary supplements were prepared by measuring and mixing with the mice pellet. For Group 1, fish oil and soybean oil were added to the pellet at a ratio of 3:1 (3 mL fish oil and 1 mL soybean oil). Group 2 was given a 3:1 ratio of soybean oil to fish oil diet, Group 3 was given pellet mixed with 4 mL butter and Group 4 was given pellet only and served the control.

Y-maze behavioral test

To determine cognitive function in the treated mice, the Y-maze test was used, because it induces minimal stress to the mice (Wang *et al*., 2009). The Y-maze test was conducted in a lighted room and by placing the mice, one at a time, in the start arm of the maze. The mice were allowed to acclimatise in the start arm for 10 minutes by blocking the novel arm blocked. After an hour, mice were again placed

in the start arm, this time with all three arms opened to allow the mice to explore the maze. The response of the mice was based on the willingness of mice to explore, as mice usually prefer to investigate new arms of the maze rather than returning to those previously visited. The frequency entries and time spent exploring the novel arm was recorded and evaluated. An entry is deemed to have occurred when all four limbs of the mice are within the arm. The floor of the device was cleaned between introductions of mice to prevent bias.

RESULTS AND DISCUSSION

The data obtained were analyzed and shown in Table 2 and Table 3 below. In Table 2, the mean time spent in the novel arm by the mice treated with high ratio of fish oil was significantly higher compared to the control group ($P<0.05$). The different mean times spent by mice given different diets suggests that there were differences in the level of learning and memory function between each treatment diet.

Table 2: Time mice spent in novel arm of Y-maze.

Treatment group	Time (seconds)
Fish oil	75.9±19.9
Soybean oil	61.4±8.2
Butter	72.0±9.4
Control	52.9±5.9

Values are mean ± SEM (n=6)

Table 3 showed the frequency of mice entering to the novel arm. The mean frequency of mice treated with fish oil entering the novel arm was also significantly ($p<0.05$) different from the control group. The different frequencies of mice entry into the novel arm of the Y-maze indicate that there was difference in the level of learning and memory functions between each treatment diet.

Table 3: Frequency of mice entering novel arm of Y-maze.

Treatment group	Time (seconds)
Fish oil	6.8±1.4
Soybean oil	4.8±0.4
Butter	5.4±0.4
Control	4.9± 0.2

Values are mean ± SEM (n=6)

Dietary supplementation with omega-3 fatty acids improved the cognitive function as shown in the Y-maze test results. The Y-maze was mainly used to

determine the behavioural performance of mice related to learning and memory function, especially after the intake of treatment diet with omega-3 supplementation.

Mice that were treated with diet with a higher ratio of fish oil showed a significant increase with the time spent and frequency of entering the novel arm than the control group. This suggested that dietary supplementation of high ratio of omega-3 for three weeks resulted in an improvement of cognitive function. Meanwhile, mice treated with high ratio of omega-6 also showed increased effect on cognitive function than the control group.

Omega-3 fatty acids can enhance the function of the brain derived neurotrophic factor and increased the population of neuron cells (Ahmad *et al.,* 2002). The authors reported that diets with omega-3 fatty acid supplementation will increase the size and population of neurons in the hippocampus area. It was also suggested that the branches of dendrites increased and influenced the neuron messenger in cognitive function. This factor is responsible for maintaining the molecular processes underlying cognitive function in terms of memory and learning (Wu *et al*., 2004).

The Y-maze was chosen in this study because Y-maze is believed to have less stressful due to the exploration of the maze is voluntary without external stressful stimuli (Van der Borght *et al*., 2007). Conversely, the Morris water maze test may induce stress based on the duration time allocated before and during the experiment (Wu *et al*., 2004). Thus, the Y-maze was safe, easy to handle and clean, produces less stress and appropriate to measure the cognitive function in terms of learning and memory function in mice.

In conclusion, three weeks of omega-3 PUFA supplementation can improve cognitive function of learning and memory in the mice.

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INVESTIGATION ON THE PRESENCE OF SANGUINICOLASIS IN COMMONLY CULTURED FISH IN PENINSULAR MALAYSIA

Nur Fadhilah Hasnawi, [1] Bee Lee Ong & [1]Mohamed Shariff Mohamed Din
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Sanguinicolid is a digenean trematode that inhabits vascular system of both freshwater and brackishwater fishes and contributes to high morbidity and mortality in infected fish. However, reports on prevalence of sangunicoliasis in various fish species in Peninsular Malaysia are limited. A study was conducted in January to February 2014 on thirty juvenile fish consisting of five freshwater fish species and two brackishwater fish species reared in earthen ponds located in the states of Melaka, Johor, and Selangor, Malaysia. The heart samples collected from all freshwater and brackish water fish species did not reveal blood fluke nor did any tissue samples collected for histopathology showed the presence of Sangunicolid triangulated eggs. This study may indicate that the prevalence of Sanguinicolid in Malaysian freshwater and brackishwater is very low.

Keywords: sanguinicolid, preliminary survey, cultured freshwater and brackish water fishes, Malaysia

INTRODUCTION

Aquaculture in Malaysia is contributing significantly towards the economy of the country and provides protein food source for human consumption. There was an increase in the aquaculture industry in terms of production value from 2009 to 2010 with a total of 362,155 mt compared to 333,450 mt in the previous year. The production value also increased to RM 2.522 million compared to RM 2.295 million in 2009. This suggests the importance of aquaculture in Malaysia, thus more research and development is needed to enhance the industry.

Both freshwater and brackishwater fish can be infected with blood flukes due to presence of intermediate hosts and importation of infected fish from other countries. Hence, this study was conducted to determine the presence of Sanguinicolid infection in selected fish species in Peninsular Malaysia.

MATERIALS AND METHODS

In this study, 30 freshwater and brackishwater fingerlings that were cultured in earthen ponds were collected from various hatcheries in Melaka, Johor, and Selangor, Malaysia. The fingerlings were chosen as they are still naïve and had not developed immune response towards the pathogens.

Four freshwater species, namely red tilapia (*Oreochromis* sp.), catfish (*Claria gariepinus*), walking perch (*Anabas testudineus*), and freshwater Pomfret (*Colosoma macropomum*) were obtained from the Brother Ng Fish Farm, Durian Tunggal Pond, Malacca, Malaysia. Asian seabass or locally known as Siakap (*Lates calcarifer*) was obtained from Kong Kong Hatchery Farm in Masai, Johor. The red tilapia was obtained from My Fish Aquaculture, Semenyih, Selangor. Forty grass carp (*Ctenopharyngodon idella*) fingerlings nurtured in earthen pond were purchased from Three Ocean Fish Pond and Trading in Rawang, Selangor. Mixed breed tiger (*Epinephelus fuscoguttatus*) with giant grouper (*Epinephelus lanceolatus*) was purchased from Kee Aquaculture Sdn Bhd located in Banting, Selangor.

The fishes were kept in glass aquarium containing aerated, de-chlorinated water. For brackishwater fishes, seawater was used and the salinity was adjusted according to the water of source farm. Water was changed daily by siphoning out the dirty water and replacing with seasoned tap water. Water was analysed daily using a portable water meter to ensure good quality. The fingerlings were fed commercial pelleted food twice daily and 10 to 15 fingerlings were removed daily for dissection and recovery of blood flukes.

The fish were dissected after decapitation with new and sharp scalpel blade and standard length measurement were taken for each individual fish. The heart, together with bulbous arteriosus and ventral aorta were excised and removed and placed in individual petri dishes containing 0.9% saline. A pair of fine needles was used to tease the heart and vessels thoroughly under the stereomicroscopy to obtain Sangunicolids. If blood flukes were present, they were pipetted into cavity block containing 0.9% saline.

The gills, kidney, stomach, liver, and spleen were collected from the newly dead fish for histopathology examination. Selection of tissue samples was made according to convenience of sampling.

RESULTS

No blood fluke was obtained from the samples examined. Tissue sections did not reveal Sangunicolid eggs. Some of the fish samples with unknown digenea cysts in the primary lamella of the gills showed thick fibrous reaction under high magnification (1000×).

DISCUSSION

In this study, due to limited time and resources only 30 fingerlings per species from five freshwater and two brackishwater fishes were collected. Thus the findings form this small sample size is not representative of the actual Sangunicolid prevalence in Malaysian fishes.

Ideally, in this kind of study, the fingerlings must be exposed in earthen pond for 1.5 to 2 months to give sufficient time for infection to set in because the minimum period for a complete *Sangunicola armata* life cycle is 40 to 43 days. Some fingerlings in this study were only exposed for only 25 to 35 days in the earth pond. None of the farms that provided the fingerlings reported morbidity and mortality. This complicated the possibility of finding sanguinicolid in the study. Some farms did have aquatic snails, which are essential for the infection to occur. The hybrid groupers were raised in fibre glass tank. However, the fries were purchased from Bali, Indonesia and they may be infected with Sangunicolid. Moreover, the water used for brackishwater fishes originated from the nearby sea and it may contain polychaete, which is an intermediate host.

The various tissues collected did this not revealed any Sangunicolid eggs upon histopathological examination, which is consistent with negative finding of flukes in the heart. However, it is common to find in the gills, one or two eggs belonging to digenea or monogenea species in every two or three samples per species. This finding is expected especially for samples from earthen pond. However, most of other organs were negative for Sangunicolid.

Differential diagnosis for Sangunicolid infection should be made in farms facing high morbidity and mortality with clinical signs of respiratory insufficiency. In future studies, collaboration with aquatic health unit will be beneficial for the detection these flukes, while polymerase chain reaction can also be used to facilitate detection of Sanguinicolid in the fishes.

CONCLUSION

In conclusion, this preliminary survey for Sanguinocolid in Peninsular Malaysian fishes by means of recovery of live blood flukes and tissue histopathology did not show presence of Sanguinicolid in the fish species examined between January to February 2014. Thus no pathological lesions associated with presence of Sanguinocolid could be observed in the fish tissues. However, the observation may still mean that the prevalence of Sanguinicolid in Malaysian fishes is low.

PREVALENCE OF PREDOMINANT BOVINE MASTITIS IN DAIRY CATTLE OF *LADANG ANGKAT*, FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA AND ITS RESISTANCE TOWARDS COMMON ANTIBIOTICS

Nurul Shuhada Abdul Razak, [1]Rozaihan Mansor & [2]Siti Zubaidah Ramanoon
[1]Department of Farm and Exotic Animal Medicine and Surgery
[2]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Mastitis is a common disease in dairy cattle that caused by many pathogens with subclinical mastitis serves as more important disease economically due to higher prevalence and reduced in milk production. A study was conducted to estimate prevalence of mastitis in dairy cattle, to identify the associated bacterial pathogens, and *in vitro* antimicrobial susceptibility. In total, 32 composite/quarter milk samples collected at five different dairy farms. Milk samples subjected for bacterial isolation and identification and tested for antimicrobial susceptibility. Prevalence of subclinical mastitis was 63.6% and clinical mastitis was 9.1%. The most bacterial group found in clinical mastitis cases in this study was *Streptococcus sp*. (27.3%) followed by *Staphylococcus aureus* (18.2%). In contrast, coagulase negative Staphylococcus (CNS) (27.5%) was the common bacterial group causing subclinical mastitis followed by *Streptococcus sp*. (11.8%). The most predominant pathogens tested for antibiotic sensitivity test was found sensitive toward all antibiotics except for Streptococcus *sp*. which was resistant to streptomycin, enrofloxacin and oxytetracycline. These findings are useful in preventing and controlling mastitis in future.

Keywords: mastitis, dairy, CMT, bacteria

INTRODUCTION

Mastitis is an injury of any internal tissues of mammary glands that cause inflammation. Mastitis is distinguishable by physical, chemical, bacteriological, cytological changes in milk and pathological changes of the gland. Mastitis classification is either contagious mastitis or environmental mastitis. The most important contagious pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus Dysgalactiae* (Scott *et al*., 2011). These pathogens easily spread from infected quarters, primarily during milking. Bacteria, such as *Strep. uberis, Strep. dysgalactiae,* coliforms and others present in the environment

and transmitted at any time of cow's life either during milking, dry period, especially at first calving or heifers (Radostits *et al*., 2007). Three characterised groups under contagious mastitis are (a) clinical mastitis characterised by the gross inflammation signs such as swelling, heat redness and pain; (b) subclinical mastitis described by changes in milk composition but no signs of gross inflammation or milk abnormalities detected on routine diagnostic tests, and (c) chronic mastitis defined as an inflammatory process existed for months, and may continue from one lactation to another (Hamadani *et al*., 2013).

Therefore, aim of this study are to determine prevalence of mastitis, name common and main mastitis bacterial pathogens and their antibiotic sensitivity patterns in a dairy cattle at *Ladang Angkat*, UPM.

MATERIALS AND METHODS

Farm and animals

This study was on 44 lactating cows from five farms of Ladang Angkat UPM located in Selangor. Lactating cows were randomly sampled leading to about 10% of lactating cows in each farm included and examined in this study. Milk from 176 quarters of cows tested using California Mastitis Test (CMT). Milking was done twice a day in all farms; morning and evening. Farm owner provided records on number of lactation per cow.

Pre-Milking Udder Preparation

Cleaning and drying of the udders and especially teats done before sample collection. Cleaning done using water and each teat wiped dry with paper towel and finally 70% alcohol sprayed to each teat before milk collection.

Physical examination of udder

Clinical mastitis identifiable based on clinical examination such as hard and swollen quarter, pain, heat udder and abnormal milk appearance such as clot, watery appearance or flakes.

California Mastitis Test (CMT)

Foremilk discarded and small amount of milk obtained from each teat into corresponding well of the paddle with four separated wells. About 3mL of CMT reagent added into each well of the paddle. The paddle rotated gently in a circular motion so that the reagent and milk thoroughly. The scoring was based on colour changes or formation of viscous gel. The CMT score is; 0= healthy mammary gland, +1=mixed mild infection (positive), +2=distinct infection (positive), +3=heavy infection (positive).

Collection of milk sample
Aseptic technique during milk sampling is important to avoid any contamination or microorganisms present on the skin of cow`s flanks, udder and teats, on the hands of the sampler, and in the barn of environment. Composite milk sample stored in sterile tubes based on positive CMT results. The collecting tube positioned as horizontal as possible and by turning the teat to a near horizontal place where 2ml of composite milk stored in each tube. Sample for bacterial culture placed in ice box during transportation and then immediately refrigerated at 4ºC for further procedure.

Post-dipping
Post-dipping was not carried out after milking in all the farms selected for sampling.

Bacterial culture and isolation
Each milk sample was cultured on blood agar. The inoculated plate incubated aerobically at 37ºC for 24 to 48 h and colony morphology examined. Colony size, shape, color, haemolytic characteristics, gram staining and catalase production was further evaluated. Single bacterial colonies were isolated and sub-cultured on blood agar. Gram staining differentiated between gram negative and gram positive bacteria. Biochemical tests were done to identify the specific bacterial isolates.

Antibiotic sensitivity test
The antimicrobial resistant pattern was determined using Kirby Bauer disk diffusion method. A pure colony mixed into 1mL of distilled water. Turbidity of mixture determined by comparison done against sodium chloride, which was 0.5 Mc farland standard. Mueller Hinton agar used as the plating medium and antibiotic impregnated discs placed on the surface of inoculated plates. The plates incubated aerobically at 37ºC for 24 hours. The inhibition zone measured using caliper and translated into susceptible, intermediate, and resistant. In this study, the antibiotic tested were; penicillin, oxytetracycline, enrofloxacin, streptomycin and gentamycin.

RESULTS AND DISCUSSION

Study was conducted on five dairy farms of *Ladang Angkat* UPM located in Selangor. A total of 44 lactating cows were included in this study.

Prevalence of Mastitis
Cows with clinical mastitis were identified based on presence of swelling, redness, or pain of the affected quarter, milk contained clots, flakes or become watery or systemic signs such as fever, depression or anorexia (Hamadani *et al*.,2013). As for subclinical mastitis, no visible signs of udder or milk except for increase somatic cell count (SCC) and reduced in milk production. In this study, California Mastitis Test (CMT) was used to detect subclinical mastitis. A total of 44 lactating cows tested using CMT. Prevalence of clinical mastitis of Farm A was 14.3% whereas

Farm D was 30.0%. Prevalence of subclinical mastitis was low as 57.1% up to 83.3%.

Bacteriology

A total of 32 composite milk samples from clinical mastitis and another 28 composite milk samples from subclinical mastitis included for bacteriological isolation and identification. The different isolate were classified according to National Mastitis Council (1999) as presented in Table 1.

Table 1: Prevalence of predominant pathogens causing clinical mastitis

Bacterial species	Groups	Prevalence n (%)
Staphylococcus aureus	Contagious	2 (18.2)
Streptococcus sp.	Environmental	3(27.3)
Staphylococcus saprohyticus	Environmental	1 (9.0)
Coagulase negative staphylococci	Normal flora	2 (18.2)
Staphylococcus hyicus ss hyicus	Normal flora	2 (18.2)
Staphylococcus hyicus	Normal flora	1(9.0)
	Total	11 (99.9)

Prevalence of main subclinical mastitis pathogens based on bacterial culture summarised in Table 2.

Based on this study, it is found that prevalence of bovine mastitis in five farms had the highest percentage of subclinical mastitis prevalence ranging from 57.1% to 83.3% whereas incidence of clinical mastitis was only identified in two farms with prevalence of clinical mastitis ranged from 14.3% to 30%. Thus, variation in these results shows that there was a difference in management of the farms. Good management practices, practical experience of mastitis control, ventilation, milking machine, proper milking technique and hygiene of the udder are the key factors of controlled environment to prevent and control the mastitis occurrence (Elbably *et al.*, 2013).

This study found that 9.1% prevalence of clinical mastitis and 63.6% of subclinical mastitis. Prevalence of subclinical mastitis was high compared to clinical mastitis because the efforts have concentrated on treating clinical mastitis.

Of these 54 isolates found from subclinical mastitis cases, coagulase negative Staphylococcus (CNS) was the most predominant in 18 isolates (33.3%), followed by *Streptococcus sp*. 8(14.8%), *Corynebacterium sp.* 7(13%), *Staphylococcus hyicus* 6(11.1%), *Staphylococcus aureus* 4(7.4%), *Staphylococcus hyicus ss hyicus* 3(5.55%), *Achromobacter* 3(5.55%), *Staphylococcus saprohyticus* 2(3.7%), *Staphylococcus intermedius* 1(1.85%), *Bacillus sp.* 1(1.85%), and *E. coli* 1(1.85%). The organisms can be isolated from the skin, teats end, and teat canal of the cows as well as from bedding and pasture. When high prevalence of CNS infections observed, improper application of teat dip or the used of an ineffective germicide after milking should be suspected (National Mastitis Council, 1999).

Table 2: Prevalence of predominant pathogens causing subclinical mastitis

Bacterial species	Groups	Prevalence (%)
Coagulase-negative staphylococcus	Normal flora	18 (33.3)
Staphylococcus hyicus ss hyicus	Normal flora	3 (5.55)
Staphylococcus hyicus	Normal flora	6 (11.1)
Streptococcus sp.	Environmental	8 (14.8)
Corynebacterium sp.	Contagious	7 (13.0)
Staphylococcus aureus	Contagious	4 (7.4)
Staphylococcus saprohyticus	Environmental	2 (3.7)
Staphylococcus intermedius	Normal flora	1 (1.85)
Bacillus sp.	Environmental	1 (1.85)
Achromobacter	Environmental	3 (5.55)
E.coli	Environmental	1 (1.85)
	Total	54 (99.95)

Corynebacterium bovis is able to spread from cow to cow during milking and known to colonise the teat canal. This study found that *Corynebacterium bovis* was the third highest major pathogens causing mastitis. According to National Mastitis Council, (1999) and Hamadani *et al*. (2013) high prevalence of *Corynebacterium bovis* in the herds associated with imperfect practice of post-milking teat disinfection using efficacious products, improper milking hygiene and dry cow therapy is not practiced.

Streptococcus sp. was the major pathogens found in clinical mastitis cases followed by *Staphylococcus aureus*, CNS, *Staphylococcus hyicus*, *Staphylococcus hyicus ss hyicus* and the least were *Staphylococcus saprohyticus*. Radostits *et al*., (2007) stated that streptococcal infections can cause clinical mastitis either mild to moderate. The exposure of cow to the contaminated environment resulted in high numbers of coliforms and streptococci (Hogan and Smith, 2012).

This study found that all tested antibiotics are effective for treatment of mastitis due to CNS, *Staphylococcus hyicus*, *Staphylococcus hyicus ss hyicus*, and *Corynebacterium sp*. except *Streptococcus sp.* which found resistant towards streptomycin, enrofloxacin, and penicillin G. Jain *et al* (2012) stated that *Streptococcus sp.* was highly resistance towards streptomycin, tetracycline, erythromycin and enrofloxacin. Thus, the usage of antibiotic should according to recommendations to avoid extensive use of antibiotics in medicine and animals husbandry which can increase antibiotic resistance among common pathogenic bacteria.

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IDENTIFICATION OF BACTERIA FROM THE GUT OF RED TILAPIA (*OREOCHROMIS* SP.) AND THEIR RESISTANCE TOWARDS COMMONLY USED ANTIBIOTICS

Nurul Husna Holal Ludin, [1]Mohamed Shariff Mohamed Din & [2]Sanjoy Banerjee
[1]Department of Veterinary Clinical studies
Faculty of Veterinary Medicine
[2]Institute of Bioscience
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

In the aquaculture industry tilapia is cultured in high stocking density which has resulted in serious disease outbreaks. In recent years antibiotic and medicated feed have been used to control fish diseases. This study was conducted to identify the bacteria from the gut of tilapia and their resistance towards commonly used antibiotics. In the present study bacteria were isolated from the gut of 10 adult tilapia purchased from a commercial farm at Hulu Langat, Selangor, Malaysia. The identification by 16s rDNA revealed 9 species of bacteria from the following families, Aeromonadaceae, Bacillaceae, Enterobacteriaceae, Moraxellaceae and Micrococcaceae. Antibiotic sensitivity test using Kirby-Bauer method revealed that 56% (6/9) of the bacteria species were resistant towards ampicillin, erythromycin 22% (3/9), tetracycline 33% (3/9), doxycycline 22% (2/9), oxolinic acid 22% (2/9) and chloramphenicol 22 % (2/9). Three of the bacteria species displayed multidrug resistance. None of the bacteria species were resistant towards Nitrofurantoin, Norfloxacin, compound sulphonamide and Trimethoprim/sulfamethoxazole.

Keywords: tilapia, antibiotic resistant bacteria

INTRODUCTION

Tilapia is commonly cultured in many parts of the world. The scientific name for Tilapia is *Oreochromis* sp. and this fish is from a large genus in the family Cichlidae (cichlids). Tilapia is freshwater fish that lives in variety of freshwater bodies like lake, river and pond. This fish also can tolerate brackish habitats (Fattah, 2006).

Asia is the largest tilapia producer in the world accounting for 79% of the production of global farmed tilapia in 2002 (Fattah, 2006). Tilapia is well known as hardy fish, however, stressful condition cause immunosuppression and making them more susceptible to diseases (Fattah, 2006).

Antibiotics are used widely to maintain the health of the fish. Antibiotic are given through medicated feed or immersion therapy and drug may be incorporated in the commercial feed. Use of antimicrobial agents in aquaculture has resulted in the emergence of antimicrobial resistant bacteria in fish, other aquatic animal and aquatic environment. Effluent from terrestrial animal and human that may end up in the aquatic environment, the reservoir in the aquatic environment like *Aeromonas* sp. may be influenced by resistant determinants and bacteria that have emerged in other environments.

MATERIAL AND METHODS

Bacteria samples were isolated from fish purchased at a commercial farm located in Hulu Langat, Selangor, Malaysia. The study involved 10 adult Tilapia of market size (550 g body weight and 29 cm long). The fish was aseptically dissected and the gut was cut into smaller pieces using sterile scissors and homogenised. Then 3 g of gut were taken and put into bijou bottle and 30 mL distilled water was added and this served as the stock culture.

The gut homogenate was centrifuged and serially diluted up to 10 folds. Each dilution was plated on nutrient agar (NA) and tryptic soy agar (TSA). Then 100 µL aliquot of each dilution was spread by spread plate method and was incubated at 30ºC for 24 to 48 hours. A replicate of each plate was made and subcultured. Identification of bacteria was carried out using 16s rDNA.

To determine antibiotic susceptibility 3 to 4 colonies from pure culture were taken using sterile wire loop, transfered to tryptic soy broth and nutrient broth and incubated at 30ºC for 24 to 48 hours. The turbidity of broth was adjusted with sterile saline to be equivalent with the 0.5 McFarland standard. The inoculums (100 µL) was spread on Muller-Hinton agar and the agar was left for 5 minutes to allow the surface of agar to dry. The antibiotic disk was placed on surface of plate using sterile forceps and the plate incubated at 30°C for 24 to 48 hours. The diameter of zone of inhibition was measured and compared with the Clinical and Laboratory Standard Institute (CLSI) chart.

RESULTS

Nine species of bacteria were isolated and identified, which were *Aeromonas hydrophila, Micrococcus luteus, Bacillus cerus, Aeromonas jandaei, Moraxella osolensis, Bacillus megaterium, Bacillus arsenicus, Bacillus thuringiensis* and *Plesiomonas shigelloides.* Five of these bacteria, namely *Bacillus cerus, Bacillus megaterium, Micrococcus luteus, Plesiomonas shigelloides* and *Moraxella osolensis* were the normal microflora of fishes. Pathogens of fish identified in the study were *Aeromonas hydrophila* and *Aeromonas jandaei. Bacillus thuringiensis* is usually incorporated into probiotics and *Bacillus arsenicus* is normally isolated from water.

The antibiotic susceptibility test revealed that 76% of the bacteria species showed susceptibility towards the tested antibiotic, 19% were resistant and 7% were intermediate. The test also revealed that 56% (6/9) of the bacteria species were resistant towards Ampicillin, Erythromycin 22% (3/9), Tetracycline 33% (3/9), Doxycycline 22% (2/9), Oxolinic acid 22% (2/9) and Chloramphenicol 22 % (2/9). None of the bacteria species were resistant to Nitrofurantoin, Norfloxacin, Trimethoprim/sulfamethoxazole and compound sulphonamide.

From this study, 6 bacteria were resistant to the tested antibiotics and they were *Bacillus cerus, Plesiomonas shigelloides, Micrococcus luteus, Bacillus arsenicus, Bacillus thuringiensis,* and *Aeromonas hyrophila.* Three of the bacteria species, *Bacillus thuringiensis, Micrococcus luteus* and *Aeromonas hyrophil* displayed multidrug resistance. Three bacteria, *Bacillus cerus, Micrococcus luteus, Plesiomonas shigelloides,* are commensal bacteria. Individuals receiving antibiotics tends to develop resistance not only in the bacteria involved in the infection but also in the commensal bacterial flora. For that reason, in this study all *Bacillus* sp., except *Bacillus megaterium* had developed resistance to ampicillin. Luna *et al.,* 2007 similarly showed that *B. cereus* and *B. thuringiensis* were resistant to Ampicilin, Amoxicilin, Oxacillin, Ceftriaxone and Penicilin.

Micrococcus luteus also show multidrug resistant to erythromycin, ampicillin, oxalinic acid and chloramphenicol, which is unlike a previous study (Liebl *et al.,* 2002) that showed the bacteria are only resistant towards erythromycin. The *Aeromonas hydrophila* was multidrug resistant towards four antibiotics, doxycyline, tetracycline, ampicillin and oxolinic acid, which is consistent with an earlier finding (Huddleston, 2006).

Plesiomonas shigelloides is an opportunist bacterium in humans and they have the potential to confer resistance to the normal microflora or pathogenic microbes. In our study, the *Plesiomonas shigelloides* was resistant to tetracycline only. In contrast, in a study done by Nadirah *et al*., (2012), *P. shigelloides* from cultured red hybrid tilapia in Terengganu River, Malaysia was resistant to ampicilin, furazolidone and oxytetracycline.

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SERUM TESTOSTERONE CONCENTRATION AND RELATIONSHIP WITH SCROTAL CIRCUMFERENCE, AGE AND BODY WEIGHT OF BOER GOATS

Nurul Syazwani Radzuan & [1]Rosnina Hj. Yusoff
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This study was conducted to evaluate the reproductive parameters in 30 male Boer goats. The main objective was directed to measure testosterone concentration (TC) and to correlate with scrotal circumference (SC), body weight and age during the four weeks of sampling. The goats were divided equally into Group A (<6 months old), Group B (6-12 months old) and Group C (>12 months old). Additionally, testicular width, height and length were also measured to calculate testicular volume and daily sperm output (DSO). Jugular blood samples were collected at weekly intervals for serum testosterone concentration analysis. Testicular measurements and scrotal circumferences were taken at the first and fourth week. Data obtained were analyzed in average values. Testosterone concentration was positively ($p<0.05$) correlated with age ($r = 0.662$), SC ($r = 0.671$) and body weight ($r = 0.665$). For daily sperm output, it was significantly ($p<0.05$) correlated with all the parameters; $r = 0.902$ (age), $r = 0.938$ (body weight), $r = 0.943$ (SC) and $r = 0.755$ (TC). The study revealed that bucks of more than 12-months-old, with body weight of at least 26 kg had higher testosterone concentration and started to produce sperm. Therefore age, body weight, scrotal circumference and testosterone concentration could be used to select bucks as sires.

Keywords: goats, testosterone concentration, scrotal circumference, testicular volume, daily sperm output.

INTRODUCTION

The main reason for raising Boer goats is meat production A study by Kaur (2010) showed that 72% of consumer respondents consumed goat meat even though the quantity and frequency of consumption was low. Nevertheless, increasing demand for high quality animal proteins in Malaysia due to increase in population followed by urbanisation has induced the society to consume more goat meat as nutritional alternative. Thus, Boer goats are chosen because this breed is known for its good body conformation, fast growth rate and good carcass quality (Lu, 2002).

Boer males can reach age of puberty as early as 3 to 4 months with a body weight of 32 kg. However, the suitable age used for breeding purpose is usually between 5 and 6 months. Previous studies have shown that age can influence scrotal circumference and semen characteristics (Toe et al., 1994) with total sperm output closely proportional related to testicular size (cited by Abd-Allah *et al*., 2007). Since age is directly related to the testicular size, which indirectly influence total sperm output. Testosterone is the most important male reproductive hormone associated with reproductive behaviour, spermatogenesis and secondary sexual characteristics (cited in Bezerra *et al*., 2009).

Although there are information about testosterone concentrations and scrotal circumference in goats, reports on the relationship of these parameters with age and body weight in Boer goats are inaccessible. Hence, this study was performed to determine the correlationship between blood testosterone levels, scrotal circumference, age and body weight and at the same time, to gauge the daily sperm output based on testicular volume in Boer goats.

MATERIALS AND METHODS

This study was conducted at Pusat Ternakan Haiwan Pondok Tanjung in Taiping, Perak, Malaysia. Thirty males goats of different ages were selected and divided equally into 3 groups (n=10 per group). They were grouped according to the following age range: Group A consisted of males less than 6 months, Group B comprised males between 6 and 12 months whereas bucks of more than 12 months constituted Group C. Age of each animal was identified from farm records. The body weights were recorded during the first and the last week of study.

Testes of the Boer goats were examined for testicular size and tonicity twice during the four weeks of study. The length (L), width (W) and height (H) of the testes were measured using a pair of vernier calipers. These values were used to calculate testicular volume using the formula; Volume = $0.5233 \times L \times W \times H$ (Pukazhenthi *et al*., 2011). From the measurement, the daily sperm output (DSO; 10^9/day) was estimated by the formula; DSO = (0.024 × testicular volume) - 1.26, in which total testicular volume represents the sum of right and left testes volume.

Approximately 3 mL jugular blood samples were collected from each animal via a venoject needle attached to a heparinised venoject tube, at weekly interval for serum testosterone concentration analysis. Then, blood samples were centrifuged at 250 × g for 15 minutes and stored at -20ºC pending analysis. The serum was analysed at the Theriogenology and Cytogenetics Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Serum testosterone concentrations (TC) were determined using the testosterone radioimmunoassay (RIA) kit (Immunotech, A Beckman Coultier Company, Prague, Czech Republic).

RESULTS AND DISCUSSION

The data showed mean values and correlation between body weight, scrotal circumference (SC), TC, and DSO for the 3 age groups (Tables 1 and 2). Among the 3 groups, body weight was significantly ($p<0.05$) different. The heavier body weight belonged to group C goats. The ages and body weights of goats showed a positive correlation with a coefficient of 0.916. However, the present finding was in contrast to that of Keith *et al*. (2009) using Kiko bucks. However, the Kiko goats were smaller than the Boer bucks of the present study. The differences could also be due to feeding types and management, seasonality and genetics of the Boer goats raised in Malaysia.

Table 1: Evaluation of reproductive parameters of Boer goats.

Parameters	Groups	Mean ± SE	Range	p-value
Body Weight (kg)	A	10.03^{a} ±0.67	7.25 - 13.75	0.00
	B	18.93^{b}±0.57	16.00 - 24.00	0.00
	C	26.38^{c}±1.56	29.00 - 33.50	0.00
Scrotal Circumference (cm)	A	9.44^{a}±0.62	6.80 - 12.20	0.00
	B	18.06^{b}±0.57	14.90 - 20.25	0.00
	C	20.02^{b}±0.66	15.70 - 22.15	0.00
Testosterone Concentration (ng/ml)	A	0.93^{a}±0.05	0.76 - 1.18	0.01
	B	1.66^{ab}±0.22	0.79 - 3.33	0.01
	C	2.33^{bc}±0.32	1.26 - 4.41	0.01
Daily Sperm Output (10^{9}/day)	A	-1.10^{a}±0.04	(-1.24) - (-0.93)	0.00
	B	-0.16^{b}±0.11	(-0.78) - 0.20	0.00
	C	0.46^{b}±0.15	(-0.34) - 1.06	0.00

For each parameter means within column with different superscript differ significantly. ($P< 0.05$) (n=30).

Scrotal circumference and DSO of group C goats were significantly higher than those of group A, but not group B. This may be that Group B is at the early part of the sexual maturity process. The present study also shows positive (r= 0.943) correlation between SC and DSO. According to Brito *et al*. (2002), measuring SC is

the key in the examination of yearling bulls, and it is highly correlated with sperm production and semen quality.

Testicular volume that represents the sperm content can be used to estimate the DSO. The mean DSO showed that the Group C goats began to produce sperm, indicating that sperm production in bucks begins at 12 months of age.

Table 2: Correlation between age, body weight, scrotal circumference, testosterone concentration and daily sperm output in Boer goats.

Parameters	Age	B.wt	SC	TC	DSO
Age (months)	1	-	-	-	-
Body weight (kg)	0.916*	1	-	-	-
Scrotal circumference (cm)	0.855*	0.902*	1	-	-
Testosterone concentration (ng/mL)	0.662*	0.680*	0.671*	1	-
Daily sperm output (10^9/day)	0.902*	0.938*	0.943*	0.755*	1

** Correlation is significant at the 0.01 level (2-tailed)
B.wt=body weight; SC=scrotal circumference; TC=testosterone concentration; DSO=daily sperm output

Testosterone concentration was highest in group C and regression analysis showed that DSO was closely related with TC at r = 0.755. There is a significant ($p<0.05$) relationship between the two variables with a regression equation of $y = 1.88 + 0.92x$, where y was the TC (ng/mL) and x was the DSO. From the equation, we can predict that for every increase of one unit of DSO, the TC will increase by 920 units. Hence, the best parameter to predict TC is DSO. This finding is supported that of Sogorescu *et al.* (2012); they showed that sperm were active when TC was high especially during the breeding season. Consequently, it can be concluded that when the testicular volume increases, the DSO also increases and increasing TC.

In conclusion, age, body weight, SC, TC and DSO were positively correlated ($p<0.01$) with each other. Therefore age, body weight, SC and TC can be used to select bucks as sires.

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EFFECT OF COCCIDIOSIS ON GROWTH AND HAEMATOLOGICAL PARAMETERS OF DORPER LAMBS

Nurzaty Ewani Abd. Hamid, [1]Mohamed Ariff Omar, [2]Rehana Abdullah Sani & [3]Rasedee Abdullah

[1]Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Pathology and Microbiology
[3]Department of Veterinary Laboratory Diagnosis
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Coccidiosis is an economically important disease as it is associated with lowered productivity and high morbidity especially in domesticated meat-producing animals. However, there is a paucity of information regarding the effect of coccidiosis effect in Dorper sheep. A study was conducted at Felda plantation Batu 8, Lepar, Pahang, Malaysia to examine the association between growth and haematological parameters and coccidia infection in Dorper lambs. Twenty purebred and 20 crossbred Dorper lambs aged 2 to 4 monts were selected for the study. Faecal samples were taken twice at 7-day interval. Blood samples were collected once for haematological profiling. The anaemia level was measured once by comparing the mucosa colour of the eyes with the FAMACHA chart. Body weight, body length, height at withers and heart girth measurements were also taken. All purebred and 90% of crossbred lamb samples were positive *for Eimeria* oocysts. Eight species of *Eimeria* identified were *E. parva* (31%), *E. marsica* (18%), *E. weybridgensis* (17%), *E. pallida* (12%), *E. bakuensis* (11%), *E. faurei* (5%), *E. ahsata* (4%) and *E. granulosa* (2%). However, no highly pathogenic *Eimeria* species, *E. crandallis* and *E. ovinoidallis* were found in the study. The mean oocyst per gram (OPG) in purebreds and crossbred Dorper lambs were similar. Similarly, there was no significant ($p>0.05$) difference between breed groups and body weight, body length, height at withers and heart girth. Body weight was significantly correlated with OPG ($r= -0.49$, $P<0.01$). The severity of coccidiosis as determined OPG count was significantly correlated with FAMACHA score ($r=0.476$, $p<0.01$). However, there were significant ($p<0.05$) differences between breed groups, with serum sodium, potassium and chloride higher in crossbreds than purebred Dorper lambs. This observation may be due to the presence of high moderately pathogenic *E. parva* in the crossbred lambs. However *E. parva* does not produce clinical signs of diarrhoea or anaemia. Finally, there was no significant ($p>0.05$) difference in growth, packed cell volume and plasma protein levels between purebred and crossbred Dorper lambs while coccidiosis appeared to affect body weight, FAMACHA scores and electrolyte levels.

Keywords: Dorper lambs, coccidiosis, growth, electrolyte parameters

INTRODUCTION

Sheep production in Malaysia is undertaken mainly by smallholder farmers in small herds. Although the sheep population decreased from 131,278 heads in 2008 to 124,787 heads in 2012 (DVS, 2013), the country aims to increase mutton production from 10.3% in 2009 to 68% in 2020 with a target sheep and goat population of 3.2 million head. To increase the small ruminant population Dorper sheep have been introduced from South Africa to supplement the local Malin sheep for mutton production. Dorper sheep are known to be hardy and have the ability to perform well in the tropical climate (Budai *et al*., 2013). It produces top quality carcass at a relatively early age (Milne, 2000). Dorper sheep have been reported to be highly resistant towards ticks and parasites and thus are easy to manage with minimal health problems. However, to date there is a paucity of information regarding many aspects of sheep health, including coccidiosis affecting Dorper sheep.

Coccidiosis is an economically important disease especially in meat producing animals as it results in lower productivity due to its associated high morbidity. Coccidiosis is caused by unicellular protozoa parasite, *Eimeria* spp., which is host-specific. *E. ovinoidallis* and *E. crandallis* were highly pathogenic species, while *E. bakuensis, E. ahsata* and *E. parva* are known to be moderately pathogenic. All ages of sheep are susceptible to *Eimeria* infection but lambs are most severely affected by clinical coccidiosis (Khan *et al*., 2011). A study was conducted to identify coccidia species, to examine the relationship among oocysts per gram, packed cell volume, FAMACHA score and electrolyte parameters as indicator variables of coccidiosis and the association between coccidia infection and body weight in in purebred and crossbred Dorper lambs raised locally.

MATERIALS AND METHODS

The study was conducted at Felda Plantation Batu 8 Lepar, Pahang, Malaysia from January to February 2014. The farm comprises mainly of purebred Dorper and Damara-Dorper crossbred sheep. The farm is managed intensively and sheep are fed with cut Napier (*Pennesitum purperum)* and Guinea (*Panicum maximum*) grasses and palm kernel expeller concentrate and mineral blocks were provided as supplements.

Forty lambs were used in this study comprising of 20 purebred Dorper and 20 crossbred Dorper lambs, divided equally by gender. The age of the lambs ranged from 2 to 4 months. The lambs included in this study were selected from an apparently healthy group of animals and confirmed free from any medication and treatment towards parasites. Faecal samples were taken twice at 7-day-interval, while growth parameters and blood samples were obtained once. The following parameters were measured: oocyst per gram (OPG) in faecal samples and

measurement of haematological parameters such as packed cell volume (PCV), plasma protein (PP), A:G, sodium, potassium and chloride concentrations.

Growth parameters included in the study were body weight, body length, height at withers and heart girth. The body length (BL) was measured from the point of shoulder to the pin bone, while height at withers (HW) was measured from the surface of the platform to the withers of the lambs. The heart girth (HG) was measured by taking the circumference of the chest. Precautions were taken during measurement body parameters to minimise error.

The *Eimeria* species were identified based on their size and morphological characteristics (Wang *et al*., 2010). The length and the width of each sporulated oocyst were measured together with the determination of morphological characteristics, especially the micropyle cap, using a microscope Moticam live module 20.

Although FAMACHA scoring system is only useful when dealing with the *Haemonchus contortus* parasite, in the present study it has been adapted to examine coocidiasis. FAMACHA score (FS) was used to determine the level of anaemia from eyelid colour scoring (Azlina, 2013). The level of anemia was determined by comparing the mucosa colour of the eye with the FAMACHA chart that ranks the scores from 1 to 5; score 1 = dark red eyelid membrane indicating no significant anemia, score 5 = white colour indicating severe anemia, scores 2 to 4 are light red, pink and pinkish white colours indicating increasing levels of anemia generally correspond to the parasite burden, and score 5 indicates severe anaemia.

Data on growth, $\log_{10}$ transformed mean OPG and haematological parameters were analysed using independent t-test to detect the differences between breed groups. All analyses were performed using IBM SPSS Statistic Version 22. Chicago, IL: IBM.

RESULTS AND DISCUSSION

The smallest *Eimeria* species identified was *E. parva* (13.6 x 12.1 µm) while the largest species was *E. ahsata* (28.6 × 18.8 µm) (Table 1).

Eimeria oocysts were positive in all purebreds and 90% of crossbred lamb samples (Table 2). Eight species of *Eimeria* were identified during coprological examination. For an overall prevalence in both breed groups, *E. parva* was the commonest of the identified species (31%), followed in order of frequency by *E. marsica, E. weybridgensis, E. pallida, E. bakuensis, E. faurei, E. ahsata* and *E. granulosa* with prevalence of 18, 17, 12, 11, 5, 4 and 2%, respectively. *E. marsica* (15%) and *E. parva* (23%) in purebred and crossbred Dorper lambs, respectively was the species present most. However, no highly pathogenic *Eimeria* species was observed in the study. Coccidiosis is present worldwide and thus it is difficult to link any particular geographical distribution with one or other species of coccidia. This is why not all *Eimeria* species infected sheep were found in the present study.

Table 1: Mean values of length and width of *Eimeria* species

Eimeria species	No. measured for length	Length (µm)	No. measured for width	Width (µm)
E. parva	31	13.6[a]	12	12.1[a]
E. pallida	12	14.3[a]	18	11.1[a]
E. marsica	18	15.9[ab]	31	11.2[a]
E. weybridgensis	17	20.2[bc]	17	13.3[a]
E. granulosa	2	20.7[bc]	2	14.3[a]
E. faurei	5	22.6[c]	11	16.9[ab]
E. bakuensis	11	23.4[c]	5	14.3[a]
E. ahsata	4	28.6[d]	4	18.8[b]

[a,b,c,d]Means within columns with different superscripts differ significantly at $P < 0.05$.

Table 2. Prevalence of Eimeria spp. In purebred and crossbred Dorper lambs

Eimeria species	Purebred	Crossbred	Overall
E. parva	8.0	23.0	31.0
E. pallida	5.0	7.0	12.0
E, faurei	5.0	0.0	5.0
E. weybridgensis	9.0	8.0	17.0
E. marsica	15.0	3.0	18.0
E. bakuensis	6.0	5.0	11.0
E. ahsata	2.0	2.0	4.0
E. granulosa	0.0	2.0	2.0

No clinical sign of coccidiosis wad seen in any of the lambs studied. The mean OPG in purebred Dorper lambs was 1,848 whereas in crossbreds it was 1,985 ($P>0.05$). Similarly with the growth parameters, there was no significant ($p>0.05$) difference between breed groups.

The results revealed that electrolyte loss in crossbred Dorper lambs was higher compared to purebreds. This may be due to presence of a high number of moderately pathogenic *E. parva* in crossbreds than in purebred Dorper lambs which had a high number of non-pathogenic species (*E. marsica*). Nevertheless, *E. parva* does not produce clinical signs of anaemia and diarrhoea in infected lambs.

In this study, only FAMACHA score 2 and score 3 were recored in the lambs. Both scores were compared with the mean values OPG and animals showing borderline anaemia score (score 3) had significantly ($p<0.05$) higher coccidia count that those with score 2. No significant ($p>0.05$) difference between purebred and crossbred Dorper lambs were observed for OPG and FAMACHA score.

CONCLUSION

Three Eimeria species, *E. bakuensis*, *E. ahsata* and *E. parva*, of moderate pathogenicity were observed in Dorper lambs. The study showed there was no significant difference in growth parameters, PCV and PP levels between purebred and crossbred Dorper lambs. However, coccidiosis appeared to affect body weight and electrolyte levels. Moreover, FAMACHA scores may assist in determining the severity of coccidiosis.

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DIVERSITY OF PARASITES IN BARN OWLS (*TYTO ABA*) IN BUKIT CHERAKA OIL PALM ESTATE, KUALA SELANGOR, MALAYSIA

Pakeeyaraj Nagalingam, [1,3]Shaik Mohamed Amin Babjee, [2,4]Jalila Abu, and [5]Puan Chong Leong

[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Clinical studies
[3]Wildlife Research Centre
[4]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
[5]Department of Forest Management
Faculty of Forestry
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Ten barn owls (*Tyto alba*) from Bukit Cheraka Oil Palm Estate belonging to Sime Darby were studied to determine the diversity of blood parasites and ectoparasites in this bird species. The birds were caught from their nest boxes using a net. Feather samples and damp cotton balls were taken from various parts of the birds followed by blood collection from the brachial vein. Bird nest materials were also taken from the nest boxes. Wet blood mount, buffy coat smear and thin blood smear of the blood samples were viewed under compound microscope for presence of blood parasites. Feather plucks, cotton ball swipes and nest materials were viewed under stereo microscope for presence of ectoparasites. The only blood parasite found was *Plasmodium* sp. (10%). The most common ectoparasite was the feather mites, *Glaucalges* sp. (100%), followed by lice, *Coplocephalum* sp. (22%), an unidentified mite species (11%) and the tropical blood sucking mite, *Ornithonyssus* sp. (9%).

Keywords: barn owl (*Tyto alba*), blood parasites, ectoparasites, *Plasmodium* sp., *Glaucalges* sp.

INTRODUCTION

The barn owls belong to the Tytonidae family alongside the Strigidae family that makes up the Order Strigiformes (nocturnal birds of prey) and simply known as the owls. Barn owls, one of our own raptors, were first introduced to serve as pest (rodents) control in oil palm plantations in Peninsular Malaysia. The role of barn owls in rat control is deemed important for the future of oil palm plantations as rats are expected to adapt and establish itself along with the rapid expansion of this

plantation crop in the country (Hoong, 2000) and cause damage to the plants and fruits.

Parasites are present in both free-living wild raptors and those in captivity. It was previous believed that parasites are harmless because of lack of information on the pathogenicity of parasites on their host. Parasitic diseases in the wild usually flare up when an animal is immunocompromised due to emaciation, infectious diseases, intoxications, young age and other causes (Cooper, 2008). Parasitism can affect the population size and cause losses in reproduction and nest defense of birds (Saggese *et al*., 2012). Heavy infestations may reduce hunting performance and survivability of the bird (Cooper, 2008).

The objective of this study was to identify common parasites of the barn owls in Selangor, Malaysia, to provide baseline information on the relationship with and effect of these parasites on host ecology, including habitat, diet and behaviour of these birds of prey. This information would be useful in the conservation of barn owls.

MATERIALS AND METHODS

Animal

The study was conducted at Sime Darby Oil Palm Estate, Bukit Cheraka, Kuala Selangor. The subjects, 10 barn owls were randomly selected. Sampling was done in the mornings. Capturing the owls involved the usage of a long net. Appropriate restraining method was used during blood collection and feather plucking. All laboratory screenings were done at the Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Blood collection and analysis

Approximately 1 mL of blood was aspirated from the brachial vessel of each bird using a 25G sterile needle and 1 mL sterile syringe into a vacutainer EDTA tube. A wet mount and two thin blood films were prepared. The wet mounts were directly viewed using a high power magnification on a compound microscope (100× and 400×) to observe for microfilaria. The thin blood films were air-dried and fixed in absolute methanol for about two minutes before flooding with 10% Giemsa solution for 30 minutes. The slides were dried before examination under a compound microscope (400× and 1000×) to screen for hematozoas. The remaining blood samples were also centrifuged at 200 × g for 5 minutes. The buffy layer was smeared onto a glass slide. This slide was viewed under high power magnification under compound microscopy to screen for trypanosomes.

Ectoparasite collection and analysis

Feathers were plucked from body, neck region and ventral parts of the barn owls. Cotton balls damped with 70% alcohol were used to swipe around the head and wing parts. Nest materials were also taken from the nest box. Ethanol (75%) was added to the containers with feather plucks and cotton balls for preservation. Each

feather and cotton ball sample was then examined under a stereomicroscope. Five grams of the nest material were taken and viewed under a stereomicroscope. Ectoparasites found were then mounted on clean glass slides using Hoyer's medium for further examination and identification.

RESULTS AND DISCUSSION

Blood Parasites

Only one of 10 Barn owls screened was infected with a *Plasmodium* sp. (Figure 1). This bird showed low parasitemia with not more than 8 parasites/10,000 erythrocytes in the thin blood smears. Previous studies showed that *Hemaproteus* especially in barn owls was the most commonly found hematozoa followed by leucucocytozoon. The zero prevalence of these blood parasites in this study can be explained by the lack of leucocytozoid vectors in the habitat of the owls. In a study by Young *et al*. (1993), Simulidae (black fly), the vector for *Leucocytozoon* sp. was found to be very low or absent in tropical areas. The sampling sites may lack Cullicoides and hippoboscid flies, the vectors for *Hemaproteus* sp. or the prevalence of the parasite among insects may be very low.

An important factor in the study that should considered is the sample size of these raptors, which is small. This may have contributed to the lack of significance of the blood parasites prevalence in the barn owls in this study. Furthermore, Krams *et al*. (2012) reported that microscopic examination is of low sensitivity in detection of blood parasites in case of very low parasitemia. Another possibility is the fact that the barn owls may have possibly developed resistance to the vectors.

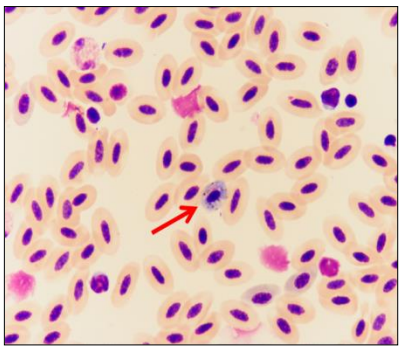

Figure 2: *Plasmodium* sp.

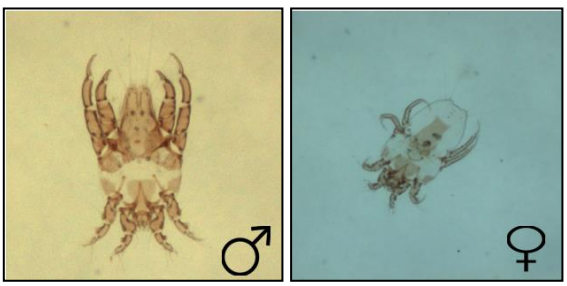

Figure 2: *Glaucalges* sp

Ectoparasites

Among the owls in this study, 55% were infested with one species of ectoparasite and 45% infested with a two ectoparasite species. The overall findings were among these owls there were presence of *Ornithonyssus* sp. (9%), unidentified mite (11%), *Colpocephalum* sp. (22%) and *Glauclages* sp. (100%). The feathers from all nine birds were found to harbour the feather mites, *Glaucalges* sp. (Figure 2). An

unidentified mite was found on feather of one female bird (Figure 3). From the cotton ball swipes, only *Glaucalges* sp. was seen in two of the birds. *Ornithonyssus* sp., a blood sucking mite, was found in one the nest materials of one of the birds (Figure 4). During handling of two birds, *Colpocephalum* sp. a chewing louse was also found (Figure 5).

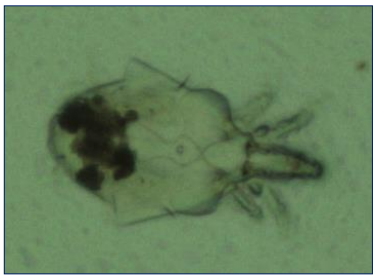

Figure 3: Unidentified mite

Figure 4: Ornithonyssus sp.

Figure 5: *Colpcephalum* sp.

The low prevalence of parasites in this study could be due to the site of the materials collected and the small amount samples obtained from the nest. Due to the structure of the nest box, the nest material with reach was mostly the material furthest from where the birds are nested. The *Colpocephalum* louse, a chewing louse lives in the body and wings of raptors were found during handling of birds. This showed that the methods used in the study were not suitable for collection of all ectoparasites in the barn owls. Ectoparasites such as the mosquitoes or flies are more mobile and usually fly off immediately during handling/feeding. Better techniques such as feather ruffling/dusting with insecticidal powder or chloroform treatment of body using anesthetic jars (Walther and Clayton, 1997) should have been employed to improve the sampling for parasites and provide greater diversity of ectoparasites obtained from the barn owls without need of sacrificing the bird.

The unidentified mite (Figure 3) in this study is closely related to the *Ereynetidae* family (Knee and Proctor, 2006). Based on the site where the mite was found, it can be assumed that it is a feather mite. The *Ornithonyssus* mite (Figure 4) found poses a threat to the barn owl population due to its blood sucking behaviour. Therefore it is recommended that the barn owls are treated for infestation of these mites. The best and most practical way is to treat the nest boxes with a safe insecticide. Cypermethrin, a pyrethrum, which is already being used in the plantation management is a good choice of drug. This insecticide can be used to fumigate/spray the nest boxes.

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COMPARATIVE ANTIBACTERIAL EFFICACY BETWEEN LEMON (*CITRUS LIMON*) AND POMEGRANATE (*PUNICA GRANATUM*) EXTRACT TOWARDS PATHOGENIC BACTERIA ISOLATES

Ravivarma Ramachandran, [1]Noordin Mohamed Mustapha & [1]Siti Khairani Bejo

[1]*Department of Veterinary Pathology and Microbiology*
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The aqueous extract of *Citrus limon* (lemon, CL) and *Punica granatum* (pomegranate, PG) are claimed to possess antibacterial, antifungal and antiviral properties. This study aims to ascertain the antibacterial properties of the whole fruit extracts with respect to the minimum bactericidal concentration (MBC) of CL and PG against selected bacteria. The well diffusion method was employed to determine the antimicrobial activity of CL and PG at different concentrations. The MBC was determined through 10 series of dilution using 96-well plate and cultured on the nutrient agar to examine for bacterial growth. The crude extract of CL showed antibacterial effect against all bacteria tested where the highest inhibiton occurred with *Streptococcus pyogenes*. Similarly, the crude extract of PG showed a bactericidal effect against all bacterial isolates except for *Escherichia coli* and *Klebsiella pneumonia.* The MBC of CL, at dilutions of 1:0 to 1:8 showed no significant ($p>0.05$) difference with the effect of Gentamicin (positive control), whereas for PG, at dilutions of 1:0 to 1:6, the effect was not significant ($p>0.05$) difference from that of Gentamicin. In conclusion, although both crude extracts of CL and PG had effective bactericidal activities, the effect of extract of CL may be stronger.

Keywords: *Citrus limon*, *Punica granatum*, antibacterial, minimum bactericidal concentration.

INTRODUCTION

Citrus limon, commonly known as lemon fruit, is a member of the family *Rutaceae* is known for its alkaloids from crude extracts of different parts of the fruit that have antibacterial and anticancer activities. Citrus fruit is also a good source of vitamins and a wide range of essential nutrients needed by the body. The fresh fruits and their juice contain high concentrations of flavanones and flavones (Al-Ani *et al*., 2009). *Punica granatum*, commonly known as Pomegranate, is plant which is a

member of the family *Punicaceae* and is grown in warm climate areas such as South-East Asia, the Mediterranean and the Americas. Local healers use pomegranate especially the fruit, peels and root as herbal remedies to cure various illnesses. Pomegranate juice was also shown to be effective antimicrobial and anti-inflammatory agent (Belal *et al*., 2009). Pomegranate is known for its tannins which have significant antimicrobial activity. Hydrolysable tannins found in the peels, membranes and piths of the fruit are the main polyphenols of pomegranate juice, account for 92% of its antioxidant contents (Dahham *et al*., 2010). The soluble polyphenolic content of pomegranate juice includes anthocyanins, catechins, tannins, gallic and ellagic acids (Fazeli *et al*., 2011).

The objective of the study was to determine the antibacterial effect of the whole extract of lemon and pomegranate against pathogenic bacteria isolates.

MATERIALS AND METHODS

Preparation of the fruit extracts

Fresh lemon (*Citrus limon*) and pomegranate (*Punica granatum)* were obtained from the wet market in Klang, Selangor, Malaysia. The fruits were washed with tap water, rinsed with sterile distilled water, dapped with 70% alcohol and allowed to dry at room temperature. The fruits were then placed in a sterile beaker, sealed with aluminium foil and stored under 4ºC for 24 hours. The fruits were cut into small pieces using a sterile knife and were grinded using an electrical blender without adding any water. The pure fruit extracts were obtained by centrifugation, separating the residues from the extract. The extract was stored at 4ºC.

Strain inocula

Escherichia coli, Enterococcus faecalis, Salmonella sp*, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumonia, and Pasturella multocida* cultures were obtained from the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The bacterial cultures were stored sealed with paper film at 4^{o}C.

Bacterial inoculation and incubation

The extract of the fruits was diluted into 4 concentrations, 1:0, 1:1, 1:3 and 1:6, using sterile distilled water. Bacterial cultures were suspended in 1 mL nutrient broth to obtain turbidity equivalent to the McFarland standard of 0.5. Within 20 minutes after the adjustment of bacterial inocula, a sterile swab was dipped inside the nutrient broth and pressed firmly against the test tube wall to remove excess fluid. The entire Mueller-Hinton (MH) agar surface was streaked with the swab three times, turning the plates 60^{o} between streaking, in order to obtain an even inoculation. Six wells were dug in the inoculated MH agar using a sterile pipette. Four of the wells were filled with 60 µL of extract of different concentrations of extract. Gentamicin disc was used as the positive control. The same procedure was repeated for all bacteria. The plates were incubated overnight at 37ºC. The next day,

the plates were examined for the zone of inhibition caused by the extracts and Gentamicin. The minimum bactericidal concentration (MBC) was evaluated using a 96-well plates using extract concentrations of 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, and 1:9. Gentamicin (0.3%) was used as positive control and distilled water as the negative control. The bacterial cultures were suspended into 1 mL nutrient broth to obtain turbidity equivalent to the McFarland standard 0.5. Within 20 minutes, 100 µL of bacterial suspensions were place in the respective wells, followed by 100 µL of the extract at their respective concentrations. The 96-well plates were shaken slowly and gently to obtain even mixtures of bacterial suspension and extracts and incubated overnight at 37ºC. The next day, one Mueller-Hinton agar plate was used for each bacteria and the plates were marked with 12 square box and labelled 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, Gentamicin and distilled water. The plates that were incubated were examined grossly for change in turbidity. A loopful of fluid from each well was taken and streaked onto the respective box on the respective agar plates. These plates were reincubated overnight at 37ºC. The next day, these plates were examined for bacterial growth and the MBC of the extract against each bacterium was determined.

RESULTS AND DISCUSSION

The statistical mean diameter of zone of inhibition shows that the lemon extract displayed antibacterial activity against all the bacteria tested, where the highest mean diameter of zone of inhibition was recorded against *Streptococcus pyogenes* and the lowest was against *Salmonella* sp. On the other hand, pomegranate extract exhibits antibacterial activity against all the bacteria except *Klebsiella pneumonia* and *E.coli*. The highest and lowest mean diameter of zone of inhibition by pomegranate extract was against *Pasturella multocida* and *Streptococcus pyogenes*, respectively. The diameter of inhibition for both extracts decreased with increasing dilutions.

The 1:0 dilution extract, that is 100% extract concentration, produced the highest inhibition among extracts of various dilutions. Overall pomegranate showed higher mean diameter of inhibition for all bacteria tested. Inhibition by pure lemon extract was greater than that produced by Gentamicin, pure pomegranate extract showed similar diameter of inhibition as Gentamicin.

The MBC showed that most bacteria tested were susceptible to lemon extracts of dilution 1:7 or less, while *Streptococcus aureus* and *Enterococcus faecalis* were susceptible lemon extract of 1:4 or less and 1:5 or less dilutions, respectively. There is no significant ($p>0.05$) difference between the antibacterial effects of lemon extracts at 1:8 and 1:0 dilutions and Gentamicin. The MBC of pomegranate extract showed that most of the bacteria tested were susceptible to 1:5 dilutions of pomegranate extract, whereas *Salmonella* sp and *Enterococcus faecalis* were susceptible to 1:2 or less dilutions only. There was no significant ($p<0.05$) difference between the effect of 1:6 and 1:0 dilutions of promengrate extract and Gentamicin.

Salmonella sp and *Klebsiella pneumonia* showed the lowest mean diameter of inhibition against lemon extract among bacterial species used in this study, which is similar to that the findings of Jayana *et al.* (2010), using lime juice. Lemon and lime belongs to the same family, *Rutacaea*, and the same genus, *Citrus.*

This antimicrobial effect of citrus fruit juices may be due to their citric acid, limonene, linalool, and turpinol contents. It has been proposed that antibacterial activity of the low pH organic acids is due to its undissociated structure, which is hydrophobic and has cytotoxicity effects (Ali, 2010). In pomegranate extract the phenols, tannins and flavonoids, which are their major active constituents are responsible for anti-bacterial and antifungal activities of the extracts (Dahham *et al.*, 2010). The tannin compounds can denature enzymes of bacterial (Furneri *et al.*, 2002) while phenols can be absorbed into the cell wall, displacing phospholipids and reorganise the membrane structure of the bacteria (Chen and Cooper, 2002), accounting for the antibacterial effects.

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EFFECT OF BLOWING ON QUALITY AND SPOILAGE OF ULTRA-HIGH-TEMPERATURE MILK

Siti Nadrah Kamaludin, [1]Latiffah Hassan & [2,3]Saleha Abdul Aziz
[1]Department of Veterinary Laboratory Diagnosis
[2]Department of Veterinary Pathology and Microbiology
[3]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Ultra-High-Temperature (UHT) processing of milk ensures that milk and milk-products are safe for consumption and extend its shelf-life. Recently, as part of guideline on how *Susu 1 Malaysia* should be consumed, the Ministry of Health had recommended that 'blowing' into milk should be avoided. In this study, nine Universiti Putra Malaysia students were selected to blow into three types of flavoured and non-flavoured of milk boxes. The UHT milk in 250 mL containers consisting of full-cream milk, chocolate and strawberry-flavoured milk were bought from a supermarket and sampling was performed consecutively for 5 hours. Total plate count (TPC) method was used to estimate the load of bacterial growth following treatment. The action of blowing into the milk resulted in higher number of bacteria colonies than milk that had not been blown into. Comparison using number of bacteria colonies between full-cream UHT milk and flavoured UHT milk showed higher bacteria colonies present in full-cream milk, even though the bacterial growth remained low throughout the 5-hour period. This study suggests that blowing will affect the microbiological quality of milk.

Keywords: UHT milk, TPC, spoilage

INTRODUCTION

Milk is produced by female mammals to nourish their young in the first days or weeks of life. It consists of a solution of dilute salts, a simple sugar, vitamins, fats in the form of globules and a complex system of proteins, mostly exists in the form of casein micelles. Milk products are available in a variety of processed forms in the market to reduce health-related risk when consuming dairy products. Such processing are pasteurisation and ultra-high-temperature (UHT) processing.

Ultra-high-temperature processing involves heating the milk at 135 and 145ºC for 1 to 10 seconds. The milk is then packaged in a pre-prepared aseptic packaging. The UHT milk is treated so that it will not spoil when stored at room temperature

and have an extended shelf-life. The spoilage rate of UHT packs vary from 1 to 4 per 10,000. A rate of 1 per 10 000 packs can be considered a reasonable commercial standard.

One Malaysia School Milk Program was established in September 2010 in an effort to improve the physical and mental well-being of the younger generation (Utusan Online, September 17, 2010). However, in 2012, there were a series of food poisoning incidents related to drinking milk in schoolchildren in Sabah, Sarawak and Peninsular Malaysia. The food poisoning was believed to originate from the supplied milk. In order to prevent more incidents, the Ministry of Health together with the Ministry of Education came out with a series of guidelines to milk handling and drinking. The aim was to educate children to exercise proper caution when drinking milk. One of the recommendations was to not to blow into the milk while drinking. However, how and where the guidelines were derived is unknown.

This project was conducted verify the recommendation of not blowing into milk and to determine if there is possibility of foodborne illness occurring as the result of this practice.

MATERIALS AND METHODS

Samples

Nine Universiti Putra Malaysia veterinary students were selected for the study. Three types of UHT milk of Brand A, in 250 mL containers, commonly consumed by children were selected, which included full-cream milk, chocolate-flavoured milk and strawberry-flavoured milk. Milk in containers were not refrigerated beforehand and left at room temperature.

The milk cartons were divided into two groups, treated and non-treated. Treatment referred to a process of blowing into the milk by subjects. The 9 students then insert the straw into the milk container and blew into it for 15 seconds. Control milk was not treated with blowing. Immediately, milk sample from each container were sampled for total plate count (TPC) and coliform plate count (CPC) using established methods (FDA, 1998). Series of sampling of the milk were done at 0 and 30 minutes, 1 and 3 hours for the 5 hour experimental period. The experiments were repeated for all milk types using the same set of students. Data were analysed using nonparametric Friedman, Wilcoxon and Mann-Whitney U tests (IBM SPSS Statistics 20) at significance level of 0.05.

RESULTS AND DISCUSSION

Growth was only seen on SPC agar and not on CPC agar. Milk that was blown into had higher cfu/mL compared to milk that was not blown. The mean difference was statistically significant ($p<0.05$) at the 5-hour duration, the types of treatment,

blowing versus not blowing, caused a statistically significant ($p<0.05$) changes in number of bacteria colonies.

Comparing between full-cream UHT milk with flavoured milk revealed that, full cream milk had higher number of bacteria colonies/mL (Figure 1). The association between types of milk and the number of bacteria colonies was also statistically significant ($p<0.05$), that is higher number of bacteria colonies from blown milk. Mann-Whitney U test revealed that full cream milk was significantly ($p<0.05$) different from flavored milk in terms of number of bacteria colonies present per mL. In both experiment, the number of bacteria colonies were still maintained low throughout the 5-hour study duration.

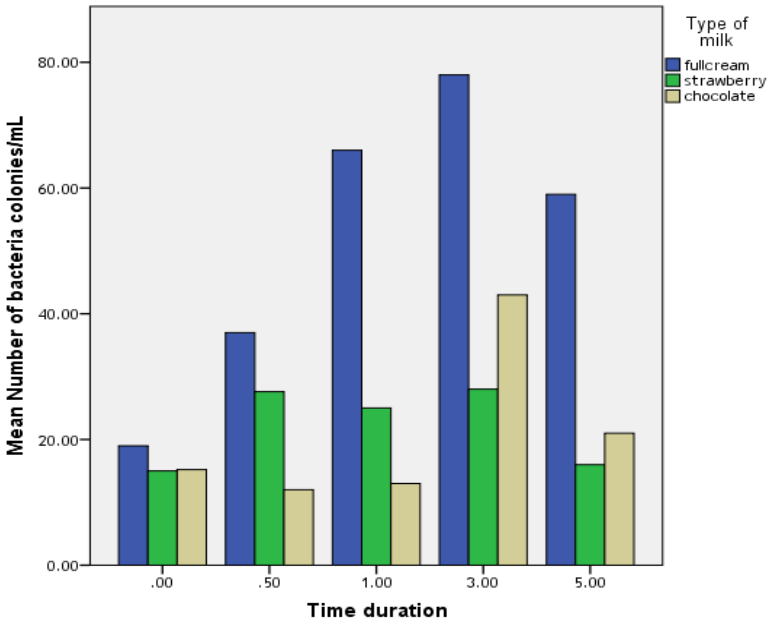

Figure 1: Comparison between milk type and the number of bacteria colonies

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INFLUENCE OF STREPTOCOCCOSIS IN HEAT STRESS ENVIRONMENT ON RED HYBRID TILAPIA (*OREOCHROMIS SP.*) OXIDATIVE STATUS

Wan Atikah Ab Karim Ghani & [1]Md Sabri Mohd Yusoff
[1]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Streptococcosis has become a global challenge to aquaculture industry. There are few references on the effect of heat stress on the oxidative status of red hybrid tilapia infected with *Streptococcus* spp. The effect of heat stress on commercial red tilapia infected with *Streptococcus agalactiae* was determined using plasma malondialdehyde (MDA) and erythrocyte superoxide dismutase (SOD) as stress-related biomarkers, histopathology, microbiology and polymerase chain reaction. Ninety healthy red hybrid tilapia, *Oreochromis* sp., obtained from Taman Pertanian Universiti, Universiti Putra Malaysia were used in the study. Ninety fish were randomly selected and weighed (±130 g), clinically examined and allowed to stabilise for 2 days before experimentation. The fish was divided into 3 groups of 30 fish each. Groups A and B was infected with 100 µL of 1×10^9 cfu/mL *S. agalactiae*, intraperitoneally (i.p). Group A also subjected to heat stress at 33°C. Group B was not stressed while Group C was not treated and served as control. Blood samples were collected at 6, 12, 24, 48, 72 and 96 hours post-challenge and subjected to MDA and SOD analyses. Tissue samples from the brain, eye and kidney were obtained for microbiology and histopathology examinations. Statistical analysis showed that the MDA and SOD were significantly ($P < 0.05$) difference between experimental groups with Group A rats generally showing the lowest MDA levels. Histopathology lesions were more severe in Group A than B. Based on these findings, heat treatment decrease the oxidative stress level and increase pathological changes of infected fish.

Keywords: *Streptococcus agalactiae*, Red hybrid tilapia, MDA, SOD

INTRODUCTION

Red hybrid tilapia (*Oreochromis* sp.) in Malaysia was first introduced in the mid 1980's. By the year 2000, red hybrid tilapia contributes 36% to total freshwater aquaculture production where 10% are from floating net cages. Although tilapia was first considered hardy and resistant to disease, mortality of tilapia of weights between 300 to 400 g was reported in Sungai Pahang (1997-1998). The same

problem was later observed in cages of Kenyir and Pergau Lake (Siti-Zahrah *et al*., 2004, 2008). Laboratory test revealed the Gram-positive *Streptococcus agalactiae* as the most common isolate from the infected fish.

MATERIALS AND METHODS

Fish

Ninety red hybrid tilapia (*Oreochromis* sp.) weighing 80 to 100 g were obtained from Taman Pertanian Universiti, Universiti Putra Malaysia were used in the study. The clinically healthy fish were allowed to stabilise for 2 days before dividing into 3 groups of 30 fish each. The tilapia, free from pathogens, was randomly assigned to five 200 L tanks for each experiment. Water temperature was set at 33°C with 12 hour light and 12 hour dark cycles, and aerated daily. The fish was fed *ad-libitum* with a commercial feed. Groups A and B was infected with 100 µL of 1×10^9 cfu/mL *S. agalactiae,* intraperitoneally (i.p). Group A also subjected to heat stress at 33°C. Group B was not stressed while Group C was not treated and served as control. Blood samples were collected at 6, 12, 24, 48, 72 and 96 hours post-challenge and subjected to MDA and SOD analyses. Tissue samples from the brain, eye and kidney were obtained for microbiology and histopathology examinations.

Bacteria

Streptococcus agalactiae used in this study was obtained from NaFisH, Penang, Malaysia. Bacteria were cultured on blood agar and further subcultured into the brain heart infusion broth (BHIB) in a 30°C shaking incubator for 18 hours. Following incubation, the bacteria concentrations were determined using the standard plate count technique (Alcamo, 1997). Approximately 1 mL of the broth was serially diluted 10-fold before 0.1 mL of each dilution was poured and spread onto blood agar and incubated for 24 hours at 30°C. Following incubation, the number of colonies, particularly from plates containing between 25 to 250 colonies, was counted before the concentration was expressed as colony froming unit. The stock solution was then diluted 10-fold using phosphate-buffered saline (PBS) and the inoculums taken immediately.

Malondialdehyde

The plasma malondialdehyde (MDA) concentration was assayed according to the method described by Ohkawa *et al*. (1979), with modifications.

Superoxide dismutase

The erythrocyte superoxide dismutase (SOD) was measured according to Marklund and Marklund (1974).

Histopathology

Specimen were sacrificed by pitting technique and the eyes, brain, kidneys, spleen, liver and gills tissue samples obtained, fixed in 10% formaldehyde, and processed using a standard histological technique. Sections were stained with haematoxylin and eosin (H&E).

Polymerase chain reaction

Samples were taken from cultured colonies positive for the bacteria. For identification of *S. agalactiae*, total cellular DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) according to manufacturer's protocol. The extracted DNA was then further evaluated by PCR for *S. agalactiae*-specific section (Firdaus-Nawi *et al*., 2013).

RESULTS AND DISCUSSION

Based on the results, the MDA concentration in Group B is significantly ($p < 0.05$) higher than in Group A until 48 hour post-challenge (pc) (Figure 1). However, this result was not as what as expected since Group A was predicted to have higher MDA concentrations than Group B fish because of heat exposure. Heat stress should cause oxidative damage markers, such as MDA, to increase. Nevertheless, fish may tend to adapt to oxidative conditions to which they are exposed. However, the MDA concentration of Group A at 72 hours pc was slightly higher and increased ($p < 0.05$) significantly exceeding that of Group B. The finding correlated with the 6.5% mortality observed in Group A fish at 72 h pc. There was no mortality either Group B or C.

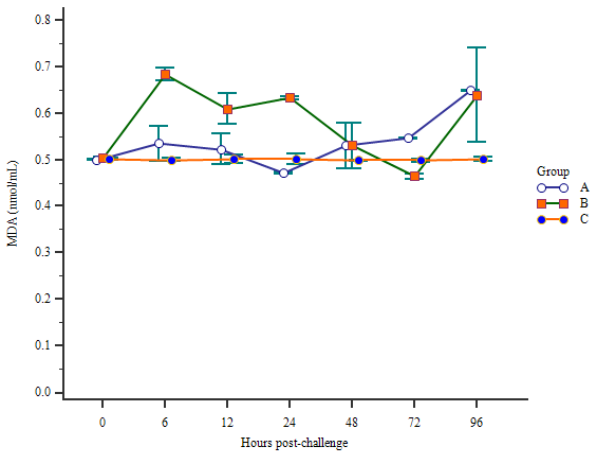

Figure 1: Plasma malondialdehyde activities for groups A, B, C and D following challenge and heat stress. Values are presented as mean±SEM.

The SOD concentration in Group B fish was significantly ($p < 0.05$) lower than in Group A fish until 48 hour pc (Figure 2). Similarly, this finding is unexpected since it was predicted that Group A, which was heat-stressed, would have lower SOD concentrations than group B fish. Induction of antioxidant enzymes is an important defense against oxidative stress in biological systems (Parihar *et al*., 1997). However, the SOD concentrations in Group B fish at 48 hours pc was lower than in Group A. The SOD concentration in Group A decreased significantly ($p < 0.05$) at 72 hours pc, correlating the mortality of fish in this this group.

The reason behind the changes in MDA and SOD concentrations may be the gradual water temperature elevation that had caused faster and higher amount of anti-oxidant production that broke down the MDA.

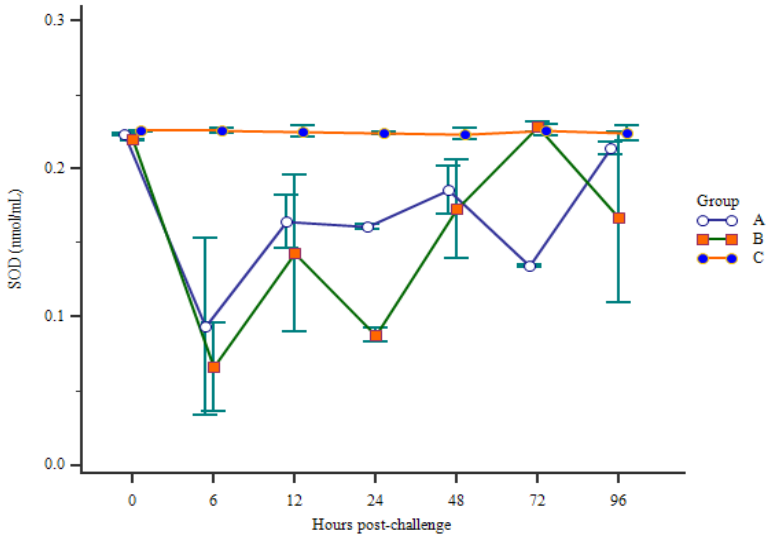

Figure 2: Erythrocyte superoxide dismutase activities for groups A, B, C and D following challenge and heat stress. Data are presented as Mean±SEM.

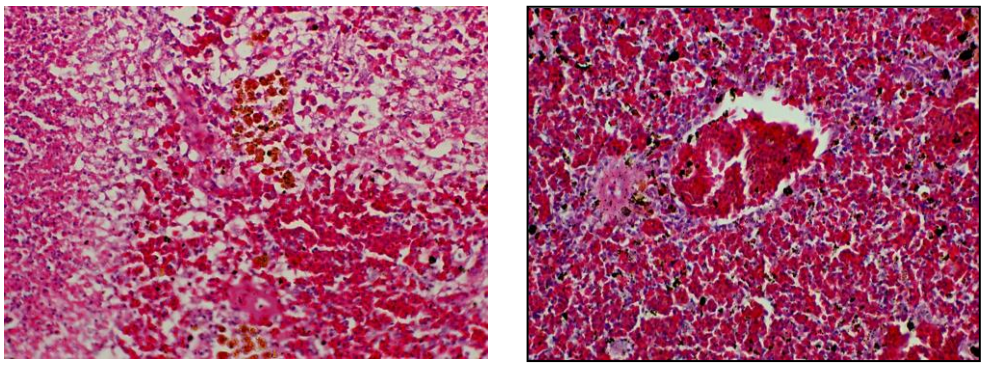

Figure 3: Severe congestion and necrosis of the spleen. H&E (100 × magnification).

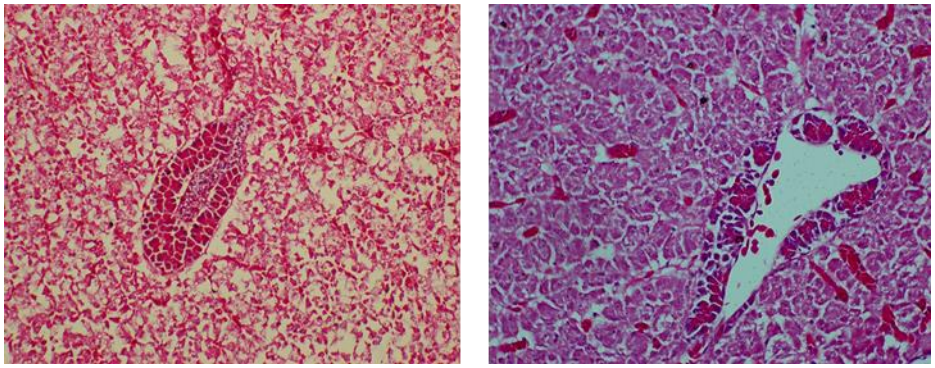

Figure 4: Hepatic congestion, severe vacuolation, and mild vasculitis at the central veins. H&E (100× magnification).

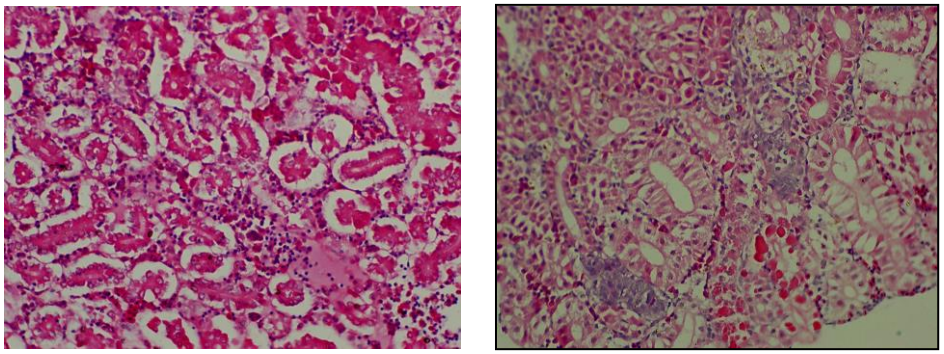

Figure. 5: Tubular degeneration and necrosis, interstitial haemorrhages, influx of inflammatory cells and bacterial colonies. H&E (100× magnification).

Bacteriology examination of diseased fish revealed the presence of Gram-positive cocci in pairs and/or long chains, suggestive of *Streptococcus* spp. To confirm the identification API rapid ID 32 Strep (BioMerieux, France) and PCR analyses were done. Histopathologically there were haemorrhages and congestion of spleen (Figure 3), severe vacuolation, congestion and mild vasculitis with necrosis of the liver (Figure 4) and tubular degeneration with haemorrhage of the kidneys (Figure 5). It was concluded that streptococcosis caused the lesions in various organs through septicaemia. The findings are similar to those reported elsewhere (Suanyuk *et al*., 2008; Filho *et al*., 2009; Zamri-Saad *et al*., 2010). Group A in this study showed more severe lesions than Group B fish and the severity increased with time.

In conclusion, the oxidative stress level of heat-stressed infected fish was lower non-heat-stressed infected fish at 48 h pc. Heat stress also produced more severe histopathological lesions in the organs that increased with time and caused mortality

of infected fish under heat stress. Thus, biomarkers of oxidative stress are useful diagnostic tools in the evaluation of the welfare and health status of fish.

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SLEEPING PATTERN OF CAPTIVE MALAYAN TAPIR (*TAPIRUS INDICUS*)

Wazirotul Adilah Darwis Harahap, [1,3]Tengku Rinalfi Putra Tengku Azizan & [2]Nurul Hayah Khairuddin

[1]Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Clinical Studies
[3]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Malayan tapir (*Tapirus indicus*) is a large monogastric animals found in Southern Thailand and Myanmar through to Malaya Peninsula and the Indonesian island of Sumatra. Tapirs are primarily nocturnal. Their activity cycle include resting or hiding during the day in the wild, and sleep between midnight and dawn. Individuals maintain regular pattern of sleep during the day and are active during the evening or night. This study was conducted in PERHILITAN Wildlife Conservation Centre in Sungai Dusun, Selangor, Malaysia. Two healthy adult tapir, a male and a female were selected for this study. The tapirs are kept in an approximately 0.15 acre of green area equipped with a night stall. Focal observation method by video camera was used in this study. Five camera traps were placed in each of the tapir's enclosure. The cameras were set for 7 days for each tapir to capture tapir movements within the area. The result showed the male tapir spent up to 6 hours of sleep during early morning, afternoon, evening and at night while the female tapir sleep up to 10 hours over 24 hours in the evening until midnight. From the observation the tapir experienced rapid eye movement and non-rapid eye movement sleep. In conclusion, the captive Malayan tapirs are more tolerant to conspecifics and demonstrate crepuscular rather than completely nocturnal behaviour.

Keywords: *Tapirus indicus*, sleep pattern, sleep duration, camera trapping

INTRODUCTION

The Malayan tapir (*Tapirus indicus*) is the largest of the five species of tapir and the only one native to Asia. This species can be found in the Malay peninsular, and Sumatra Island. These large mammals with large, stocky bodies with a prominent, prehensile proboscis can be 1.8 to 2.5 m in length, 0.9 to 1.1 m in height and 250 to 540 kg in weight. Although generally considered solitary nocturnal animals,

Malayan tapirs are more tolerant of conspecifics and demonstrate crepuscular rather than completely nocturnal behavior (Huffman, 2004). Individuals maintain regular pattern of sleep during the day and activity during the evening/night (Barongi, 1993).

Every animal demonstrate sleep which is a universal behavior. Sleep is described as a rapidly reversible state of immobility and greatly reduced sensory responsiveness (Siegel, 2008). Sleep is relevant as a diagnostic parameter because sleep disturbance occur in psychiatric illness and also stereotypic behavior.

This preliminary study was conducted to establish baseline data of sleeping pattern of healthy captive Malayan Tapirs in Malaysia.

MATERIALS AND METHODS

Animals

The study was carried out in Sungai Dusun Wildlife Conservation Centre, Kuala Kubu, Selangor, Malaysia, a facility operated by the Department of Wildlife and National Parks, Malaysia (PERHILITAN). There were 7 tapirs at the centre at the time of the study. The study was carried out for two weeks. In this study, 2 captive adult tapirs, a male (Boy) and a female (Mala), were chosen as subjects. The age, body condition and health status was similar for both animals. The tapirs performed similar daily activities.

Sleeping Pattern

The variable that used in this study was body position as an indicator of sleep state. In this study, non-rapid eye movement (NREM) sleep was characterised by the sternal recumbency, lowered head and neck posture and touching the ground with semi- or fully-closed eyes and occasional snout and ear movement. During rapid eye movement (REM) sleep, the tapir should be in lateral recumbency, the muscle completely relaxed and twitching of ears and extremities may be observed. Unintentional sounds such as snoring or whistling may be heard. Eyes are kept shut and sometimes the mouth will open slightly.

Experimental Design

To eliminate observer factor in this study, 5 digital infra-red camera traps were placed in each of the tapir's enclosure at various places and one placed at the sleeping site for 7 days for each enclosure 24 hours/day. The cameras were set at high sensitivity to capture 30 second video whenever movement is detected. The type of sleep was identified, and characteristics of each type of sleep were recorded through focal observation. To analyze the data, videos that include sleeping characteristics were taken into account. Sleeping bout is defined as the time on the video when the tapir first lay down until the time it stood up from its bedding site. From the data duration, frequency of sleep was measured.

RESULT AND DISCUSSION

Sleeping frequency

The tapirs did not sleep in long stretches. Figure 1 shows the frequency of sleep over 24 hours. Male tapir usually sleep 4 to 5 times a day while female tapir sleep about 18 to19 times a day.

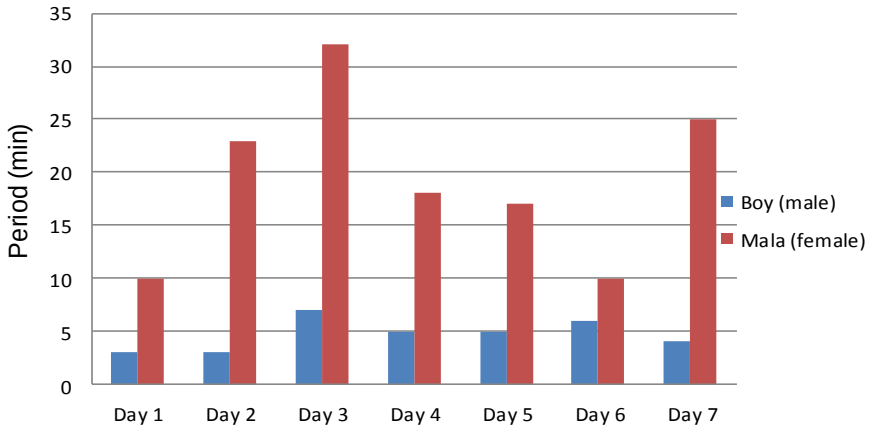

Figure 1: Sleeping frequency of tapir

Total sleep time

Figure 2 shows that the female tapir slept up to 10 hours while the male almost 6 hours a day.

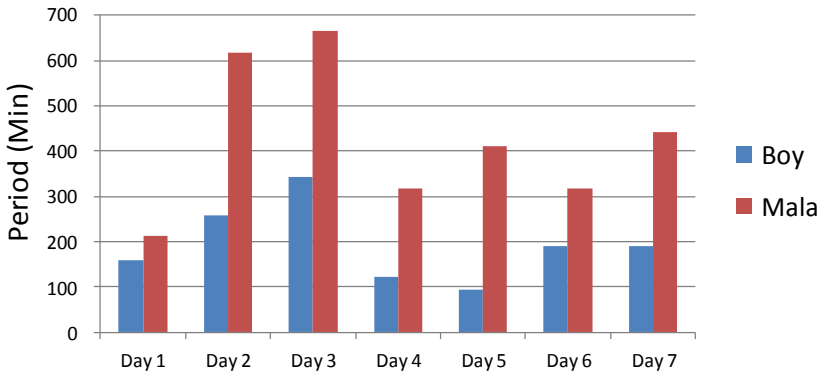

Figure 2: Tapir total sleep time

Rapid eye movement and non-rapid eye movement

The female tapir usually have higher REM sleep than NREM sleep (Figure 3). On average, the male tapir displayed REM sleep for 1.7 hours and NREM sleep for 1.3 hours per day. The female tapir displayed REM sleep for 4.3 hours and NREM sleep for 3.4 hours per day.

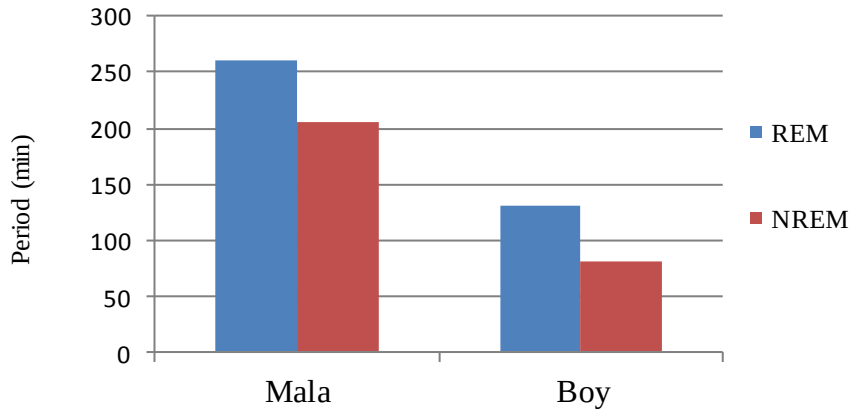

Figure 3: Rapid eye movement and non-rapid eye movement in female and male tapir

In this study there are a number of factors affecting sleep of the tapir such as the location of the enclosure, lighting effect, human activity within the surroundings and fight and flight reflex. The nature of the enclosure is that female section situated at the back is much more serene than the male's section closer to the main walkway.

Gender of the animal is said to be a factor that can affect the sleep of the animal. It is known that sex do influence the length of sleep bouts, but not sleep pattern (Santymire *et al*., 2012). The central nervous system was shown to contain gonadal hormone receptors and gonodal hormones affect sleep (Manber and Armitage, 1999).

The Tapir is a herbivore and the prey and predator relationships in the wild, it is expected that REM sleep is less than NREM sleep. However, in this study there is no predator threat to the captive tapirs, thus the sleep pattern of the tapir should not be significantly affected. There is also possible relationship between REM and NREM sleep in tapir with food availability. In the wild, food availability is not constant therefore, tapir would have to spend more time walking and searching for food rather than sleep. In captivity, the supply of food is constant and given at fixed time of the day, thus the tapir have greater time for go into REM sleep.

The findings of this study may provide baseline data that would help future researchers to study tapir sleep in captivity in a more comprehensive manner.

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REPRODUCTIVE TRACT TUMOURS IN PET DOGS AND CATS REFERRED TO THE VETERINARY LABORATORY SERVICES UNIT, FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA: A RETROSPECTIVE STUDY

Wendy Liew Wei-Qin, [1]Rasedee Abdullah, [2]Gayathri Thevi Selvarajah & [3]Mohamed Ariff Omar

[1]Department of Veterinary Laboratory Diagnosis
[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

Documentation on the occurrence of reproductive tract tumours of dogs and cats in Malaysia is poor. This study was conducted to determine the frequency and type of reproductive tract tumours in dogs and cats referred to the Histopathology Laboratory, Veterinary Laboratory Services Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia from 2008 to 2013. The objectives of this study were to determine the association of age, neuter status and haematological parameters with occurrence of reproductive tract tumours in cats and dogs. The findings from this study showed that reproductive tumours were more frequent in dogs (89%) than cats (11%). In dogs, the most common tumour types were testicular tumours (46.1%) with Sertoli cell tumour and seminoma occurring at equal frequencies of 44.4%. Cryptorchidism was more frequent in Sertoli cell tumour cases (75%). Both Sertoli cell tumours (75%) and seminomas (62.5%) occurred more frequently in middle-aged dogs (6-10 years old). There was a significant ($p<0.05$) positive association between cryptorchidism and type of testicular tumours.

Keywords: reproductive tract tumours, cats, dogs, cryptorchidism

INTRODUCTION

Ovarian tumours are now uncommon in dogs and cats due to the practice of routine ovariohysterectomy (OHE) (Saba and Lawrence, 2013). Ovarian tumours are rare in cats while uterine tumours are rare in dogs (Brodey, 1970; Miller *et al.* 2003). Testicular tumours are the most common tumours of the canine male genitalia comprising 90% of all cancers in the male reproductive tract with an overall prevalence of 6 to 27% (Cotchin, 1964). Prostatic tumours are relatively uncommon

in dogs with a low prevalence of less than 1% (Weaver, 1981). Although it is known that various tumour types can affect the canine penis, prepuce and scrotum, there is a lack of information on the incidence of these tumours in the cat (Saba and Lawrence, 2013).

Currently there was no information on prevalence of reproductive tumours in dogs and cats in Malaysia. Thus, this retrospective study is a first report providing preliminary information on occurrence of reproductive tumours in dogs and cats presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM).

MATERIALS AND METHODS

Data retrieval

The identification, age, sex, neuter status and breed of the dogs and cats diagnosed with reproductive tract tumours were obtained from the records kept at the Veterinary Laboratory Services Unit and UVH, UPM. From the same records, information on the location of the tumours was extracted. The biopsy reports were scrutinised and the final diagnosis for the respective tumour cases obtained.

Statistical analysis

The frequency and percentage of reproductive tumours of dogs and cats are obtained the association between haematological parameters and reproductive tumours determined using the Chi Square test. Significance values were determined at 95% confidence and p values <0.05 was considered significant. All the statistical analyses were done using SPSS software version 19.0 (SPSS, IBM Inc, USA).

RESULTS

For the period of study (2008-2013) there were 44 reproductive tumours for dogs and cats (Table 1). The majority of the testicular tumours involved the penis, prepuce and scrotum. There were equal proportions of Sertoli cell tumour and seminoma cases (n=8). In sertoli cell tumour cases, 75% (6/8) of the dogs were cryptorchids while 25% (2/8) had scrotal testes. With seminoma 87.5% (7/8) of the dogs had scrotal testes while 12.5% (1/8) had cryptorchid testes. There was a significant ($p<0.05$) association between cryptorchidism and type of testicular tumours.

Dogs with Sertoli cell tumours 25% (2/8) were in the young age category (<6 years) and 75% (6/8) middle aged (6-10 years). Among dogs with seminoma, 12.5% (1/8) were of young aged (< 6 years), 62.5% (5/8) were middle aged (6-10 years) and 25% (2/8) old aged (> 10 years). There was no significant association ($p>0.05$) between the type of testicular tumours and the age of affected dogs.

In dogs with testicular tumours < 10 years, cryptorchidism 64% (9/14) was more frequent than normal testes (36%; 5/14). In dogs >10 years old, cryptorchidism

(50%; 1/2) and normal testes (50%; 1/2) were of equal frequencies. There was no significant ($p>0.05$) association between cryptorchidism and age of dogs with testicular tumours.

Table 1: Reproductive tumours in dogs and cats

Anatomical site	Number of cases	
	Dog	Cat
Testis	18	3
Prostate gland	2	-
Penis, prepuce, scrotum	10	-
Ovaries	1	-
Uterus	1	1
Vagina + vulva	7	1
Total	39	5

DISCUSSION

In male dogs, testicular tumours at 5.5% were of the highest frequency among all dog tumours, followed by tumours of the penis, prepuce and scrotum (3.1%) and prostate (0.6%). Testicular tumours in male dogs were most common among all dog reproductive tumours (Saba and Lawrence, 2013). In female dogs, vagina/vulva tumours showed the highest frequency at 2.1%, followed by ovarian and uterine tumours, each at 0.3%. This is consistent with the findings of Brodey (1967), which showed that vagina and vulva tumours accounted for the highest proportion among canine female reproductive tumours.

Reproductive tumours in the cat were generally rare (Saba and Lawrence, 2013). In this study, testicular tumours was of the highest frequency (3.3%), followed by uterine and vagina tumours, each at 1.1%.

In dogs, there were equal frequencies of sertoli cell tumour and seminoma. Both types of tumours were more frequent in dogs with cryptorchid testes (Liao *et al*. 2009). In that study, it was shown that there was significant association between cryptorchidism and the type of testicular tumours. We could not make the same conclusion in our study because of the small sample size. There was also no significant association between age of dogs and cryptorchidism, although most dogs with cryptorchid testicular tumours were <10 years old. What the finding suggests is that cryptorchidism may contribute to and/or speed-up tumourigenesis of testicular tumours.

CONCLUSION

Reproductive tumours are generally uncommon in both dogs and cats. In dogs, the most common reproductive tumours were testicular tumours. Cryptorchidism was more frequent in Sertoli cell tumour than seminoma cases and most frequently seen in middle-aged dogs. In cats, all reproductive tumours were uncommon.

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SEED DISPERSAL SIMULATION IN CAPTIVE MALAYAN TAPIR (*TAPIRUS INDICUS*)

Zu Azuwana Musa, [1,2]Tengku Rinalfi Putra Tengku Azizan & [1]Hasliza Abu Hassim

[1]Department of Veterinary Preclinical Sciences
[2]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Tapir plays an important role in facilitating recruitment of seeds over long distances therefore influencing the diversity of plant species in the ecosystem. The capacity of Malayan tapirs *Tapirus Indicus* to disperse various types of small to large seeds was assessed. The ability of tapir to be a potential seed disperser was tested by studying their ability to disperse the seeds of four fruited plants with seeds of 0.2 to 13.5 cm in lengths. Data from feeding trials and camera trapping was combined to assess the effect of tapir consumption on seed survival and to estimate the gut retention time. Two tapirs were selected for this study. Twenty-seven tapir faecal dumps were collected and filtered during a 7-day period. Seed survival rate through gut passage was high for small-seeded plants (80% for *Psidium guajava*). However seeds of cempedak (*Artocarpus integer*) and longan (*Dimocarpus longan*) were not found in the faeces. The seed of Mango (*Mangifera indica*) was found partially digested in the faeces. Seed gut passage time was between 23.0 -125.5 hours for guava. The distance from ingestion of seed to the defecation site was approximately 35 meters in the captive environment. In conclusion, from this simulation study, the Malayan tapir is a potential long distance disperser of seeds due to its long gut retention period. Malayan tapir is more effective in dispersing small seeded plants but they can also potentially be seed predators for large seeded plants.

Keywords: *Tapirus indicus*; seed dispersal; gut retention times; feeding trials; camera trapping.

INTRODUCTION

Seed dispersal is a key stage in the life cycles of plants with profound implication for the succession, regeneration and conservation of ecosystems (Wang and Smith, 2002). The Malayan tapir *(Tapirus indicus)* is the largest species of tapir weighing about 350 kg. It is the only representive of the family Tapiridae in the Old World. Tapirs are among the most primitive extant large mammals (Feldhamer *et al.*,

2007). Today, there are only 4 extant tapir species, all of the genus *Tapirus*. Large bodied frugivores play an important role in shaping the structure of plant communities at different scales and maintaining plant diversity because they are able to ingest more fruits, eat larger seeds and disperse them to larger distances then small bodied frugivores (Jordano, 2007).

MATERIALS AND METHODS

Two tapirs were selected for the studies, which were Boy (male) and Mala (female). Four plant species representing broad range of fruits and sizes were used in this study. The fruit were guava (*Psidium guajava*) representing small seeds, longan (*Dimocarpus longan)* the medium sized seed while cempedak (*Artocarpus integer)* and mango (*Mangifera indica)* representing large sized seeds. During each feeding trial, the tapir were fed fruits with known numbers of seeds and subsequently all faeces were collected to retrieved defecated seeds and estimate seed survival rates and gut passage times. The numbers of seeds in the fruits fed to the tapir were estimated from direct count of one-seeded fruits such as longan (*Dimocarpus longan)*, cempedak (*Artocarpus integer)* and mango (*Mangifera indica)*. While for guava (*Psidium guajava*) the control fruit was used to estimate the mean number of seeds per fruit.

Five camera traps were placed for two weeks around the enclosure of the tapir. The camera was set up for 30 seconds of video recording per track. This was used to monitor the movement and behaviour of the animal, specifically to record defecation behaviour. Real-time focal observation was also carried out for 4 days.

Twenty-seven tapir faecal dumps were collected and filtered during the period of seven days. Entire faeces were collected in one per plastic bag. All boluses were broken open by hand and the seeds separated from the faecal material. Then the faecal material were sieved using two different mesh sizes with the aid of high pressure water to retrieve all defecated seeds. The seeds were then dried with tissues paper and counted. The movement pattern of the tapirs was observed and the location from eating site until defecating site were plotted sequentially to obtain the movement pattern. The distances in the movement pattern were measured.

RESULTS AND DISCUSSION

The captive tapir ingested a total of 1624 seeds and defecated 800 seeds which were only guava seeds. During the study, tapir did not spit the mango, longan or cempedak seeds, but 0.5% of guava seeds were left for both tapirs. The result showed that Malayan tapir had capability to disperse the small seeds, such as guava seeds and the survival rate of the seeds in the gut passage was high at approximately 80%. This high survival rate may be due to the small size of the guava seeds and the daily diet that was high in fibre, such as leaves and stem. The hardest foods to be

digested in the stomach are large food particles and fibres. With the presence of the large food particles and fibres in the stomach, the guava seeds escape the digestive enzyme and exit the stomach relatively intact. The survival of guava seeds is also influenced by the ability of the seeds to escape the crushing action of the teeth because of their seed-coat hardness. Gut passage times calculated from the time fruit was given until the seeds found in the faeces were between 23 to 125.5 hours. Gut retention time for guava seed are relatively longer at approximately 5 days, which is similar to a previous study that showed that mangosteen and papaya seeds have shorter retention times than guava seed at 4 and 3 days, respectively (Campos–Arceiz *et al.,* 2012). Therefore, with longer gut passage times, the tapir is more capable of digesting seeds hence reducing dispersion of viable seeds.

The longan and cempedak seeds were not found in the faeces of the tapir, although the skin of these fruits was seen. Since the duration of the study was short, time may be the limiting factor to the discovery of these seeds in the faeces. According to Campos-Arceiz *et al.,* 2012, longan takes about 5 days while cempedak takes about 7 days to be found in the faeces. The seed survival is influenced by the gut passage times. It takes the cempedak seeds 235.5 hours to travel through the gut passage. Because of the longer gut retention time, bigger seeds do not survive the action of the digestive enzyme. This accounts for lack of mango seeds in the faecal samples of the tapir.

The distance of tapir moved from time of seed ingestion until defecation was approximately 35 meters. This distance was relatively short for the tapirs; however the result from this study is supported by Williams and Petrides (1980), who showed that the Malayan tapir often have overlapping home ranges and move relatively short distances. In the present study the short distances travelled by the tapir was influenced by captive environment and behaviour of these animals. The tapirs mostly defecated at the same site every day, which can affect the seed dispersal distance by this animal.

In conclusion, the Malayan tapir is a potential long distance disperser of seeds because it has a long gut retention period for seeds. Although the Malayan tapir is also more effective in dispersing small plant seeds, it has potential as seed predators for large seeded plants.

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ASSESSMENT OF *BIOJADI*® PROBIOTIC IN AFRICA CATFISH (*CLARIAS GARIEPINUS*, Burchell, 1822) AS IMMUNOMODULATOR AND GROWTH PROMOTER

Zuraini Abdul Sukor, [1,2]Hassan Hj. Mohd Daud & [2]Mohd Fuad Matori
[1]Department of Veterinary Clinical studies
[2]Aquatic Animal Health Unit
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Probiotic is the beneficial microorganisms that used in aquaculture as growth improver and promote disease resistance. The aim of this study is to test the potential benefits of a probiotic preparation as a diet supplement in African catfish (*Clarias gariepinus*) fingerlings. In the study, three groups consisting of 20 fingerlings each, has an average weight of 10-20 gm. Fingerlings in control group fed with crushed pellet without probiotic, while treatment 1(T1) and treatment 2 (T2) groups fed with (i) probiotic at recommended dose (10 gm of feed mixed with 0.3 ml probiotic) and (ii) 2x of recommended dose (10 gm of feed mixed with 0.6 mL of probiotic). Results showed that at α=0.01, there were no significant difference between the groups in survival rate and growth performance. Culturing *BioJadi*® probiotic on TSA, at 30^{o}C aerobically, showed no growth. pH determination of probiotic revealed acidic pH i.e. 4.2. Disc diffusion test with eight different concentrations tested probiotic's ability to inhibit the growth of *Aeromonas hydrophila*. Results revealed that the antimicrobial activity with widest zone of inhibition (ZI) was at 7 times the strength of recommended concentration and the ZI was directly proportional to concentration of the product. However, in this study concentrations used for T1 and T2 groups were lower than the ideal dose. Therefore, BioJadi® probiotic can benefit as immunomodulator and growth improver.

Keywords: probiotic, *Aeromonas hydrophila*, antimicrobial effect, survival rate, growth performance, African catfish fingerlings.

INTRODUCTION

A number of wild fish species become more attractive as potential aquaculture species such as tilapia (*Oreochromis* sp.) and African catfish (*Clarias gariepinus*) (Taoka *et al*., 2006). In Malaysia, African catfish has become very popular among farmers due to their high growth rates, hardiness, and ease of culture as well as

availability of its fry (Kechik, 1995). Thus, African catfish has been one of the important cultured fish species. Due to increasing consumer demands can no longer be met by wild capture fisheries alone and the needs has to comply with high production aquaculture fisheries. However, these practices induced stress thus causing the fish to become more susceptible to diseases. *Aeromonas hydrophila* is one of the common pathogenic bacteria that cause high fatality to different life stages of fresh water fish (Rengpipat *et al.,* 2008). *Aeromonas hydrophila* are the toxin-producing, motile, gram negative rod- shaped bacteria which is ubiquitous in open water and can cause Motile Aeromonas Septicaemia (MAS) which is a serious disease in fresh water fish with manifestation of skin ulceration and mortality. Therefore, several alternatives suggested including the use probiotics. Probiotics are live microbial feed supplement that are beneficial to the host by providing inhibitory compounds; compete for chemicals and adhesion sites with pathogenic bacteria and help to balance the microbial community in the fish (Reneshwary *et al*. 2011). Therefore, this study aim to test the protective level of BioJadi® probiotic through presence of antimicrobial activity against *Aeromonas hydrophila* and rate of survivability as well as a growth promoter.

MATERIALS AND METHOD

Animals and feeding trial

This study conducted at the Aquatic Animal Health Unit (AAHU), Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Sixty African catfish fingerlings with mean first weight ranging from 10-20g each of 10-14 cm in total body length purchased from a local fish farm in Balakong, Selangor, Malaysia. Fingerlings acclimatised to laboratory conditions for one day before the experiment. Twenty fingerlings were randomly placed in each of 50 L glass aquarium. The three aquarium with experimental conditions include; (i) control A :fingerling fed with commercial diet in crushed pellet form, (ii) treatment level 1: fingerlings fed with Biojadi® probiotic in recommended dose together with commercial diet to proportion of 10 g of crushed pellet mixed with 0.3 mL *BioJadi*® probiotic in 2 mL of distilled water; and (iii) treatment level 2: fingerlings fed with double the recommended dose of 10 g of crushed pellet mixed with 0.6 mL Biojadi® probiotic in 2 mL of distilled water. These fingerlings fed twice daily with commercial diet contained 32% of crude protein and 6% of crude fiber starting from the acclimatization period until the last day of experiment.

BioJadi® probiotic

A loopful of BioJadi® probiotic streaked on TSA and incubated at 30^0C for 24 hours aerobically. Each strength of BioJadi® probiotic concentration prepared by mixing the probiotic solution (0.3, 0.6, 1.2, 2.4, 4.8, 9.6, 19.2 and 38.4 mL) with 2 mL of sterile sodium chloride ($NaCl_2$) solution for each strength. The pH determined using

Orion® 2-Star Benchtop pH meter that calliberated using one pint of pH 4.01, 7.00 and 10.01 buffers provided by the manufacturer.

In vitro challenge
This in vitro challenge test was done using disc diffusion test method (Amin et al., 2013). A sterile blank disc immersed in each strength of BioJadi® probiotic for 30 seconds to allow complete absorption of probiotics into the disc. The immersed disc placed on newly spread *A.hydrophila* on TSA and incubated at 30^{o}C for 24 hours aerobically to determine any presence of zone of inhibition (ZI) around the disc.

RESULTS AND DISCUSSION

No growth observed after innoculation with BioJadi ® probiotic on TSA and this can be explained due to TSA itself which was a basic nutrient agar that might not able to promote the growth of the bacteria. Each BioJadi® probiotic concentrations were acidic with mean pH of 4.01. Low pH associated with production of organic acids by the probiotics itself as reported by Sugita *et al.* (1997). Zone of inhibition (ZI) of probiotic against *A. hydrophila* does not necessarily elicit the same result as the fish challenged with LD_{50} of *A. hydrophila*. In this study, BioJadi ® probiotic did show antimicrobial activity with the widest ZI shown at 7 times the strength of recommended probiotic concentration. Some bacteria exhibit antimicrobial activity by secreting non-specific antimicrobial substances such as short-chain fatty acids, hydrogen peroxide, bacteriolytic enzymes and toxins, i.e: bacteriocins and bacteriocin-like inhibitory substances (BLIS). Grossly, there was an increased in survival rate of the African catfish fingerlings when the strength of BioJadi® probiotic concentration increased. However, there was no statistically significant difference between the treatment groups as determined by Kruskal-Wallis, as $p = 0.05$, $\alpha > 0.01$. Generally, the fingerlings showed increased in growth performance with regards to body weight, however, there was no statistically significant difference between different strength of concentrations of BioJadi® probiotic used as determined by one-way ANOVA, $p = 0.065$, ($\alpha > 0.01$). Visually, there was an increased in survival rate and growth performance of the fingerlings. However, the statistical analyses showed that increase in the strength of BioJadi ® probiotic concentration did not give any significant difference to both parameters. These could be due to several factors such as variation in fish species, route of administration, and dosage of probiotic administered to the fingerlings as well as presence of co-infection which was *Citrobacter freundii* infection in the fingerlings used in this study.

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FATTY ACID COMPOSITION OF SELECTED FRESHWATER FISH IN MALAYSIA

Ahmad Afifi Abdul Ghani, [1]Hasliza Abu Hassim & [1]Mohamed Ali Rajion
[1]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

A comparative study on the fatty acid contents particularly of essential fatty acids (omega-3; omega-6) between two species of selected freshwater and marine fish under different conditions (raw and boiled) was carried out. Two species of freshwater fish, the walking catfish (*Clarias batrachus*, "*keli*") and red tilapia (*Oreochromis* sp., "*tilapia merah*") and two species of marine fish, the mackerel (*Rastrelliger kanagurta*, "*kembung*") and Indian scad (*Decapterus russelli*, "*selayang*") were used in this study. In raw and boiled fish samples, the content of omega-3 (alpha-linolenic acid; eicosapentaenoic acid; docosahexaenoic acid) was highest in mackerel followed by Indian scad, red tilapia and walking catfish. The content of omega-6 (linoleic acid; arachidonic acid) was highest in walking catfish followed by red tilapia, mackerel and Indian scad. There was a significant ($p<0.05$) difference in fatty acid contents between freshwater and marine fishes and between fish species. However, there was no significant difference in fatty acid content between raw and boiled fish. In conclusion, the fatty acid content was nat influenced by preparation method but by the fish species.

Keywords: essential fatty acid, freshwater fish, marine fish, raw fish, boiled fish

ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY OF BACTERIA FROM PRE- AND POST- SURGICAL SKIN PREPARATION OF CATS

Ahmad Shafiq Saadan, [1]Wan Mastura Shaik Mossadeq, [2]Siti Khairani Bejo & [3]Loqman Mohamad Yusof

[1] Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Pathology and Microbiology
[3]Department of Companion Animal Medicine and Surgery
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Surgical site infections (SSIs) are risks in any surgical procedure in veterinary medicine whereby bacterial contaminations are the precursors of SSIs. Therefore, identification of bacteria at pre- and post-surgical skin preparations and determination of their sensitivity toward antibiotics are important in the prevention of SSIs. The objectives of this study are to isolate and identify common bacteria from pre- and post- surgical skin preparations of female cats presented for ovariohysterectomy and to determine the sensitivity of common bacteria isolated from pre- and post- surgical skin preparations, against the common antibiotics used in University Veterinary Hospital (UVH), Universiti Putra Malaysia.Six healthy female cats aged 6 month to 3 years old presented for ovariohysterectomy at UVH, were selected. Swab samples were taken from the proposed incision site (midline laparotomy between 3rd and 4th teats) during pre- and post-surgical skin preparations. Bacteria isolation and identification was performed in accordance to Kirby-Bauer antibiotic sensitivity test. Eighteen isolates were identified from pre-surgical skin preparation samples while there was no bacteria growth in post-surgical skin preparation samples. There was a significant ($p<0.05$) difference in number of bacteria isolated between pre- to post-surgical skin sample. The two most common bacteria isolated from pre-surgical prepared skin were *Staphylococcus pseudintermedius* (29%) and *Staphylococcus hyicus* (29%). Most bacteria isolated from pre-surgical skin preparation samples were sensitive to amoxicillin-clauvanic acid (78.57%), gentamicin (75%), and amoxicillin (71.43%), and showed low sensitivity to clindamycin (64.29%), enrofloxacin (58.33%) and marbofloxacin (58.33%). Three multi-drug resistant *Staphylococci* were isolated from pre-surgical skin preparation samples. Based on the results, the recommended prophylatic antibiotics for prevention of SSIs for surgical procedures of midline laparotomy in cats are gentamicin, amoxicillin, and amoxicillin-clauvanic acid.

Keywords: bacteria, antibiotic sensitivity, surgical skin preparation, cats, ovariohysterectomy, University Veterinary Hospital

AMINO ACID COMPOSITION OF SELECTED FRESHWATER FISH IN MALAYSIA

Amir Ahmad, [1]Hasliza Abu Hassim & [1]Anjas Asmara Samsudin
[1]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The objective of this study was to analyse and compare the amino acids contents in raw and boiled fish of two Malaysian marine fishes; the Indian mackerel "kembong" (*Rastrelliger kanagurta*) and sardine "selayang" (*Sardina pilchardus*) as well as two Malaysian freshwater fish; the walking catfish "keli" (*Clarius batrachus*) and red tilapia "tilapia merah" (*Oreochromis* sp.). Nine ssential amino acids, namely histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and arginine were determined in all fishes. The results showed that there were a significant ($p<0.05$) difference in amino acid contents between Malaysian marine and freshwater fishes. The amino acids content, except lysine, was higher in Marine fish than freshwater sample. There was also a significant ($p<0.05$) difference in amino acids content between raw and boiled fish. The amino acid content in boiled fish was lower than raw fish sample. In conclusion, the amino acids content in Malaysian marine species differ from that of freshwater fishes. The boiling method had altered the quantity of amino acids in the fishes.

Keywords: amino acid, fish, ovariohysterectomy

DETECTION OF FELINE HERPESVIRUS AND FELINE CALICIVIRUS IN CATS WITH FELINE UPPER RESPIRATORY TRACT DISEASE PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

Chai Hui Ling, [1]Malaika Watanabe, [2]Zeenathul NazariahAllaudin & [3]Puteri Azaziah Megat Abdul Rani

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
[3]Department of Companion Animal Medicine and Surgery
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Feline herpesvirus (FHV) and feline calicivirus (FCV) are the two most important aetiological agents of feline upper respiratory tract disease and are known to affect cats worldwide. Twenty-four blood and oropharyngeal samples were obtained from cats exhibiting clinical signs of feline upper respiratory tract disease (FURD) presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia between January and February 2014. Reverse transcription polymerase chain reaction (RT-PCR) and polymerase chain reaction (PCR) were performed to detect FHV and FCV from the oropharyngeal swabs using NorgenBiotek feline calicivirus RT-PCR and feline herpesvirus PCR detection kits, respectively. Virus isolation was carried out in African Green Monkey Kidney (VERO) and Crandell Rees feline kidney (CRFK) cell lines and observation of the cytopathic effect (CPE) confirmed virus isolation. None of the cats were RT-PCR positive for calicivirus while 45.8% (11/24) were positive for herpesvirus. As for virus isolation in cell cultures, 37.5% (9/24) showed CPE in VERO cells while 16.7% (4/24) showed CPE in CRFK cells. The majority of the cats sampled were unvaccinated (23/24), further highlighting the importance of vaccination against these viruses. This is the first time feline herpesvirus and feline calicivirus was detected in Malaysia using molecular techniques.

Keywords: feline herpesvirus, feline calicivirus, PCR/RT-PCR, detection, cell culture

PREVALENCE OF BLOOD PARASITE AMONG DAIRY CATTLE IN AND AROUND KENINGAU AREA, SABAH, MALAYSIA

Freska Sambrani, [1]Murugaiyah M. & [2]Yeo Boon Kiat
[1]Department of Veterinary Clinical Studies,
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Jabatan Penternakan Haiwan dan Pertanian Ternakan
88999 Kota Kinabalu, Sabah, Malaysia

ABSTRACT

A study to determine the incidence of blood parasites in 327 dairy cattle was conducted in and around the Keningau area, Sabah, Malaysia. The sampling technique used was the two stage cluster sampling method. Parasitological examinations included wet mount, Giemsa thin blood film smear and buffy coat methods. Hematocrit centrifuge technique was used to determine the packed cell volume and presence of motile parasites. The overall prevalence of blood parasites in the dairy cattle was 14.98% (49/327) with absence of the *Trypanosome* spp. *Babesia* spp. infestation was 0.31% (1/327), *Anaplasma* spp. infestation was 2.75% (9/327), and microfilaria infestation was 4.89% (16/327). The most prevalent blood parasite was *Theileria* spp. at 7.03% (23/327). There was a significant ($p<0.05$) difference in packed cell volume of animals with blood parasites. This study revealed that the prevalence of blood parasites among the dairy cattle population in and around Keningau area was low.

Keywords: dairy cattle, blood parasites, occurrence, surveillance protocol

CYTOLOGICAL STUDY OF *SIMONSIELLA* SP. IN ORAL CAVITIES OF ANIMALS

Lee Yee Cheng, [1]Gayathri Thevi Selvarajah, [2]Rasedee Abdullah & [3]Mohamed Ariff Omar

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Laboratory Diagnosis
3Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The proteobacteria of genus *Simonsiella* is a host-specific member of the *Neisseriaceae* family and is a morphologically unique prokaryote found in the oral cavities of warm-blooded vertebrates. Although commonly associated with epithelial cells, the role of these organisms in their specific hosts has not been studied and prevalence in the oral cavities of animals with oral pathologies is not known. Thus, the aim of this study is to firstly describe the prevalence and morphological characteristics of *Simonsiella* from the oral cytology of various species of animals. Next, the occurrence of *Simonsiella* in associations between the age, sex and oral diseases of cats and dogs were determined. Oral swabs were collected from 178 animals, smeared onto clean glass slides, stained and examined using light microscopy. Prevalence rate of *Simonsiell*a for dogs was 90.7%, cats 59.7%, horses 40% and cattle 60%. One sample from a Siberian Tiger was positive whereas all reptile samples were negative for *Simonsiella.* ANOVA analysis of the bacterial filament widths revealed significant ($p<0.05$) differences between the bovine samples and canine, feline and equine samples. Detection of *Simonsiella* was also associated with cats of less than 2 months of age ($p<0.05$) but neither associated with sex nor oral disease. The possible explanation to this finding is lack of transfer of the organism from the environment to the neonate oral cavity. The study also documented the first observation of *Simonsiella* in a tiger. In conclusion, *Simonsiella sp*. is non-pathogenic and cannot be morphologically characterised according to host species.

Keywords: *Simonsiella*, oral, prevalence, morphology, bacteria

NOCICEPTION AND ENCEPHALOGRAM CHANGES IN MINIMALLY ANAESTHETISED DOGS

Leow Wei Chean, [1]Goh Yong Meng & [2]Chen Hui Cheng
[1]Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This study was designed to determine the threshold for electroencephalographic evidence of nociception in minimally anaesthetised dogs following noninvasive physical manipulation. Six dogss were included in this study, and noxious stimulation will be given before and after administration of tramadol, at 4 mg/kg body weight and 20, 40, 60 and 80 miliamperes (mA). Baseline electroencephalogram (EEG) recording was done 10 minutes before stimulation. This is then followed by EEG recording 10 minutes for each stimulation level before and after treatment. It was found that both total power and median frequencies of the EEG waves showed changes before and after tramadol was given. It was also shown that tramadol exhibited evident analgesic activities when the stimulus signals were less than 60 mA. Beyond the 60 mA stimulation threshold, tramadol showed diminishing analgesic effects. In conclusion, dosage and type of analgesics, as well as the intensity of nociceptive signals are major determinant factors in the success of an analgesia regime.

Keywords: nociception, electroenchephalogram, median frequency, total power, tramadol

COMPARISON OF SEMEN QUALITY AND CHROMOSOMAL KARYOTYPE OF GAUR × BRAFORD AND BRAFORD BULLS

Liwina Thamby Pillai, [1]NurHusien Yimer Degu, [1,3]Abd Wahid Haron, [1]Rosnina Yusoff, [2]Mohamed Ariff Omar, [1]Yap Keng Chee, [1]P. Ganesamurthi & Muhammad Naim Kassim

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Preclinical Studies
[3]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

ABSTRACT

The *Selembu* is a cross between Malayan Gaur or 'Seladang' (*Bos gaurus hubbacki)* and domestic cow (*Bos indicus/taurus*). Despite the impressive growth performance of the *Selembu*, its fertility is questionable. The objective of this study was to compare the semen quality and chromosomal karyotype of *Selembu* and Braford bulls. The bulls used in this study were of Gaur × Braford and Braford breeds. Semen samples were collected using electro-ejaculation technique at 2-day intervals and evaluated for quality using Computer-Assisted Sperm Analyzer and standard Eosin - Nigrosin staining technique. Short-term lymphocyte culture was performed to obtain metaphase chromosomes. Thirty good metaphase spreads were selected and analysed for morphology, number and karyotyping using Giemsa stain. Video Test Kayo 2.1 and 3.1 softwares were used to create a karyogram to further chromosomal differentiation between the two breeds. There were significant differences between the two breeds with the Braford showing better semen colour, consistency, concentration, percentage of progressive spermatozoa, general motility and live spermatozoa. The Braford and *Selembu* bulls had a diploid 2N chromosome number of 60 and 58, respectively. The Selembu had one submetacentric autosome unlike the Braford bull, which did not have any submetacentric autosomes. In conclusion, *Selembu* bull demonstrated significantly lower quality semen compared to Braford bulls, with a remarkable difference in chromosome number and morphology.

Keywords: *Selembu,* Gaur, Braford, semen quality, karyotype

PREVALENCE OF CANINE HEART DISEASE AND OWNER COMPLIANCE WITH LONG-TERM TREATMENT OF CHRONIC HEART DISEASE IN DOGS: A RETROSPECTIVE STUDY

Low Jo Ann, [1]Khor Kuan Hua & [1]Malaika Watanabe
[1]Department of Veterinary Clinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This retrospective study revealed 35 (90/2952) of the dogs presented to the University Veterinary Hospital (UVH), Universiti Putra Malaysia from 2011 to 2013 was diagnosed with canine heart diseases. Chronic degenerative vulvular disease (n=65) was most frequently diagnosed, followed by cardiomyopathy (n=9), congenital heart disease (n=1) and pericardial effusion (n=1). The progression of the heart diseases was further subdivided into 4 stages based on clinical signs, heart auscultation, radiographic and echocardiographic findings. The majority of the canine heart patients presented were in stages 3 (36.8%) and 4 (42.1%), followed by stage 1 (13.2%) and 2 (7.9%). Owner and dog compliance with the long-term treatment of canine heart diseases were evaluated using a questionnaire-based telephone survey. Only 65 out of 76 owners with dogs diagnosed with heart diseases participated. Owner compliance with long-term treatment and daily medication for their dogs with heart diseases were 86% (43/50) and 67% (29/53), respectively, with increasing trend from stages 1 to 4. Dog compliance with the daily medication prescribed was 44% (22/50), while 3 dogs were reported to develop behaviour changes as a result of long-term daily medication.

Keywords: canine heart disease, prevalence, owner compliance

AEROBIC NORMAL INTESTINAL BACTERIAL FLORA OF COMMON MARMOSETS (*CALLITHRIX JACCHUS*)

Lya Hoklai Hat, [1]Zunita Zakaria & [2]Siti Khairani Bejo
[1, 2]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

ABSTRACT

The common Marmosets (*Callithrix jacchus*) are a growing population of captive wildlife in Malaysia. This study aimed to survey the normal intestinal aerobic bacterial flora of the common marmoset. Fresh fecal samples were collected from 10 captive marmosets at the Underwater World Langkawi, Kedah and subjected for aerobic bacterial isolation and identification. Twelve species of bacteria consisting of, *Staphylococcus* sp. (70%), *Escherichia coli* (70%), *Achromobacter* sp. (70%), *Pantoea agglomerans* (40%), *Enterococcus faecalis* (40%), *Streptococcus bovis-streptococcus equinus* complex (40%), *Enterobacter gergoviae* (30%), *Proteus mirabilis* (10%), *Proteus pennerii* (10%), *Klebsiella pneumoniae* (10%), *Staphylococcus saprophyticus* (10%) and *Enterococcus durans* (10%) were isolated. Most of these isolates are commonly found as part of the normal intestinal flora in primates. The isolates were then subjected to antibiotic susceptibility tests against enrofloxacin, oxytetracycline, streptomycin, clindamycin, amoxicillin and trimethoprim-sulfamethoxazole. Out of the 12 bacterial isolates, 4 were multi-drug resistant, which are *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Achromobacter* sp.. This study provided us with information on the types of normal bacteria present within the gastrointestinal tract of common Marmoset and these information can be useful for future reference.

Keywords: intestinal bacterial flora, isolation and identification, common Marmoset (*Callithrix jacchus*), multi-drug resistant

ANTIMICROBIAL RESIDUES IN KIDNEYS OF ANIMALS SLAUGHTERED IN ABATTOIR AND NON-ABATTOIR SLAUGHTER HOUSES IN NEGERI SEMBILAN, MALAYSIA

Madihah Mohd Zin, [1]M. Murugaiyah, [2]Ahmad Salleh, [2]Norfaizah Abdul Hamid & [2]Mazura Mohd Rodzi

[1] Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Veterinary Services
Ministry of Agriculture and Agro-based Industry, 62624 Putrajaya, Malaysia

ABSTRACT

There is an association between antibiotic administration in farm animals and increase of bacterial resistance in humans. In this study, the screening of antibiotic residues was done on the kidneys of animals slaughtered in Negeri Sembilan, Malaysia. This study was conducted using the microbial inhibition six-plate method to obtain baseline data on the antibiotic residues in the slaughtered animals. In the agar diffusion method 6 six media were used to culture the bacteria that were relatively susceptible or resistant towards penicillin, sulphadimidine, streptomycin, erythromycin, oxytetracycline and cyclofloxacin at specific pHs. Forty and 15 kidney samples were obtained from the abattoir (Senawang, Negeri Sembilan Abattoir) and non-abattoir slaughter houses, respectively. The results of the study revealed a significant ($p<0.05$) association between types of slaughter house and antibiotic residue, where samples from non-abattoir slaughter houses were tested 7 times more positive for the residue. There was no significant ($p>0.05$) difference in the type of residue among samples tested positive for the antibiotics. From this limited study, it can be concluded, based on antibiotic residue, that the safety for human consumption of meat from the Negeri Sembilan abattoir and non-abattoir slaughter houses is questionable.

Keywords: antibiotic residue, kidneys, abattoir, non-abattoir slaughter houses

PRELIMINARY OBSERVATION ON STRESS LEVEL AND PATTERN IN BUFFALO CALVES FOLLOWING WEANING

Mazrul Fahmi Mahadzir, [1,3]Mohd Zamri Saad & [2]Hasliza Abu Hassim
[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Preclinical Sciences
[3]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This study was carried out at the Buffalo Research and Breeding Farm, Telupid, Sabah. Eighteen Murrah crossbred buffalo calves were selected to assess the stress level following weaning. The calves were divided into two groups: 1) early weaning (EW; n = 9); and 2) late weaning (LW; n = 9). The early weaning calves were 3-months old while the late weaners were 6-months old. All animals were kept in individual pen under feedlot system throughout the 5-week study period. Blood samples were collected from venipuncture of the jugular vein prior to weaning and on days 7, 14, 21 and 28 post-weaning. Blood were collected into EDTA tube for blood parameters measurement and white blood cell (WBC) differential count, particularly the neutrophils and lymphocytes for stress leukogram determination. Statistical analysis revealed no significant ($p>0.05$) differences in the total counts of neutrophil and lymphocyte of both groups compared to pre-weaning stage. In fact, all the blood parameters were within normal range.

Keywords: buffalo calves, weaning, stress, neutrophil, lymphocyte

COMPARISON OF MUSCLE FIBRE CHARACTERISTIC AND MEAT NUTRITIONAL QUALITY OF PEKIN DUCK IN OPEN-HOUSE FREE-RANGE AND CLOSED-HOUSE SYSTEM

Mohamad Azrin Yahaya & [1] Lokman Hakim Idris
[1] Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Meat fibre characteristic and nutritional analysis of commercial pekin duck of Cherry Valley strain and reared at the Perak Duck Food Companies Malaysia under open-house free-range and close-house systems were investigated. Ten matured 54-day-old ducks given the same commercial diet from each different rearing system was selected, slaughtered, defeathered, dressed and finally blast-frozen. Breast muscle (Pectoralis major) was dissected and sampled for proximate analysis of crude protein using Kjedahl method and moisture, fry matter and ash using the AOAC official method of analysis. The microstructure of the muscle fibre was determined by dissection of the breast muscle, fixing the samples in 10% formalin embedded, trimmed, sectioned, stained with hematoxylin and eosin stain and analysed using Image Analyzer to determine muscle bundle area, number of muscle fibre and diameter of muscle fibre. The result from the proximate analysis revealed no significant ($p>0.05$) difference between the rearing systems. The crude protein was 20.75±2.40 and 20.94±2.09%, moisture was 77.45±1.14 and 77.07±0.67%, ash was 0.99±0.19 and 1.06±0.32% and dry matter was 22.55±1.14 and 22.73±0.20% for meat from open-house free-range and close-house systems, respectively. The microstructure of the muscle for the muscle bundle area and number of muscle fibre was not significantly ($p>0.05$) different between rearing systems. However the muscle fibre diameter of meat from the open-house were significantly ($p<0.05$) greater at 19.50±2.80 µm than that from close-house system at 16.33±0.32 µm. Based on the result, the open-house free-ranged system produced duck meat of much larger muscle fibre than those from close-housed system. Other parameters such as nutritional analysis and muscle fibre characteristic in term of muscle bundle area and muscle fibre number were similar for ducks from the two systems of rearing.

Keywords: Pekin duck, open-house-free-range, closed house system, proximate analysis, muscle microstructure.

EFFECT OF DIETARY PROTEIN SOURCES ON GROWTH PERFORMANCE AND INTESTINAL MICROFLORA OF BROILER CHICKEN

Mohd Syakir Abdul Hamid, [1]Yusof Hamali Ahmad & [2]Siti Khairani Bejo
[1] Department of Veterinary Preclinical Sciences
[2]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The study was designed to examine the effect of feeding diets containing different protein source on the performance and intestinal microflora in broiler chickens. The three sources of protein in the treatment diets were soybean meal (SBM), fish meal (FM) and cricket meal (CM). One hundred and eighty, unsexed 21 day old Cobb crossbred chicks were randomly allocated to three treatment diets in a completely randomised design with six replicates per treatment. Each replicate contained ten chicks. Results of the study showed that broilers fed FM diet had significantly higher ($p<0.05$) mean daily weight gains (DWG) and lowest feed conversion ratios (FCR). The mean daily weight gain of broilers fed SBM, FM and CM were 37.03, 37.72 and 36.15 g, respectively. The coliform population count in the ileum was found to be highest for the SBM diet ($p<0.05$). The serum cholesterol, total protein, high density lipoprotein (HDL) and low density lipoprotein concentrations in the broiler chicken were not influenced by any treatment diets. The serum albumin concentration was highest in the broiler fed with CM diet ($p<0.05$) compared with SBM and FM diets. Results from this study showed that broilers fed FM recorded the highest DWG and lowest FCR value suggesting that protein sources influenced performance and coliform population of broilers.

Keywords: broiler, performance, microflora, blood characteristics

EFFECT OF USING HONEY INCORPORATED INTO TRIS EXTENDER ON CRYOPRESERVATION OF BULL SPERMATOZOA

Muhammad Naim Md Kassim, [1]Nurhusien Yimer Degu, [1]Rosnina Yusoff & [1,2]Abdul Wahid Haron

[1]Department of Veterinary Clinical studies
[2]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Honey contains several simple sugars and other trace elements that are beneficial to bull spermatozoa during cryopreservation. The aim of this study was to evaluate the effect of different concentrations of honey incorporated into Tris extender for bull semen cryopreservation. Semen from 4 bulls was collected via electroejaculation. The semen was physically and microscopically evaluated before diluting with 4 solutions of Tris-based extender containing 0 (control), 2.5, 5 and 10% honey. The processed semen was chilled at 5°C for 3 hours to equilibrate, packed into 0.25 mL straws, frozen and stored for 24 hours in a cryogenic tank containing liquid nitrogen. The straws were thawed at 37°C for a minimum of 45 seconds before evaluation. Semen quality parameters used included general and progressive motility, which was determined by a computer-assisted semen analyser, livability and sperm morphology by the eosin-negrosin staining technique. The results revealed that significantly ($p<0.05$) better cryopreservation can be achieved with Tris extender containing 2.5% honey than with conventional Tris or other Tris-based extenders. The advantage of incorporating honey into Tris extender is that it provides additional source of energy from the simple sugar content, which is believed to support the metabolic demand of the spermatozoa. Honey at 2.5% concentration is optimum for spermatozoa crypreservation because at this concentration the intracellular fluid is withdrawn adequately to prevent crystal ice formation during the procedure. However, addition of honey of concentrations higher than 2.5% appeared to be detrimental to the spermatozoa, probably due to the removal of large amount of intracellular fluids that can cause excessive dehydration to spermatozoa.

Keywords: bulls, semen cryopreservation, honey, Tris extender

EFFECT OF EARLY AND LATE WEANING ON PERFORMANCE OF FEEDLOT BUFFALOES

Muhammad Syafiq Shahudin, [1]Mohd Zamri Saad & [2]Hasliza Abu Hassim
[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This preliminary study was done to determine the effect of post-early and late weaning on performance of feedlot buffaloes with respect to the body weight gain and feed intake pattern. This study was conducted in Telupid Buffalo Farm, Sabah, Malaysia. Eighteen buffalo calves used in this study were allocated into 2 groups according to their weaning age; one group were weaned at age of 3 months (early weaning) calves of the second group were weaned at age of 6 months (late weaning). Following weaning, the calves were then kept under a feedlot system and fed 2 kg of cut grass/animal/day and supplemented feed at 1.5 kg/animal/day throughout the study period. Feed intake was recorded daily while the body weight was measured every two weeks for 4 weeks. In this study, the results showed that there was a significant ($p<0.05$) difference in the initial body weight between early and late weaning calves, whereas there was no significant ($p>0.05$) difference in final body weight, feed intake, average daily gain and feed efficiency between groups.

Keywords: buffalo calves, early weaning, late weaning, feedlot

CHANGES IN REPRODUCTIVE HORMONES AND ORGANS OF FEMALE MICE EXPERIMENTALLY INFECTED WITH *BRUCELLA MELITENSIS* AND ITS LIPOPOLYSACCHARIDE

Nafisah Mydin, [1,3]Faez Firdaus Jesse Abdullah & [2,3]Zamri Saad
[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Laboratory Diagnosis
[2]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Brucella melitensis is a small gram-negative bacterium of the genus *Brucella* which is an aerobic facultative intracellular coccobacilli that causes Brucellosis in small ruminants and human. Its lipopolysaccharide (LPS) is the major virulent determinant of the Brucella species. Little is known about the effects of *B. melitensis* on the reproductive hormones of animals. Thus, this study is designed to observe the histopathological changes in the reproductive hormone-producing organs (pituitary gland and ovary) and to determine the progesterone and estrogen concentrations in female mice orally infected with *B. melitensis* and its LPS. Twenty four female mice of same age were used as animal models in this study. These mice were divided into 3 groups of 8 mice each, treated with either PBS, Brucella and LPS group orally inoculated with 0.4 mL of phosphate-buffered saline (PBS) of pH7.0; 0.4 mL of 1×10^9 cfu *B. melitensis* or 0.4 mL LPS from 1×10^9 cfu of *B. melitensis*. All groups were observed for 10 days. Brucella group showed normal to mild cellular changes in the pituitary gland and ovary while LPS group showed absent to mild cellular changes. By comparing hormone radioimmunoassay with the PBS (control) group, the progesterone level decrease by 13% in Brucella group and 44% in LPS group. Estrogen level decreased by 33% in Brucella group and increased by 4% in LPS group. Therefore, oral inoculation of *B. melitensis* and its endotoxin produced some changes in female reproductive organs and reproductive hormones.

Keywords: reproductive organ, reproductive hormones, *B. melitensis*, lipopolysaccharide, oral inoculation, cellular changes

SEROLOGICAL PREVALENCE AND HAEMATOLOGICAL PROFILE OF FELINE IMMUNODEFICIENCY VIRUS-INFECTED CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

Nur Afiqah Mohamaddiah, [1]Khor Kuan Hua, [2]Siti Suri Arshad & [2]Farina Mustaffa Kamal

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Feline immunodeficiency virus (FIV) is a retrovirus of the genus Lentivirus, and is among the most common infectious diseases of cats. In this study, 55 client-owned cats presented to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM), were sampled during a 3-week period. The inclusion criteria were semi-roamer and outdoor cats, and more than 6 months old. Blood samples were collected for serological analysus using the SNAP Combo FeLV antigen/ FIV antibody test kit (IDEXX, USA). Complete blood counts were performed at the Haematology and Clinical Biochemistry laboratory, Veterinary Laboratory Services Unit, Faculty of Veterinary Medicine, UPM. Of the 55 cats tested, 13 (23.6%) were tested positive for FIV antibodies. Chi-square analysis revealed significant ($P<0.05$) association between neuter and health status of the cats with FIV seropositivity. Mann-Whitney analysis revealed that the erythrocyte count, hemoglobin concentration and packed cell volume (PCV) were significant lower in FIV-infected cats than those that were seronegative. In conclusion, feline immunodeficiency virus infections were more likely to occur in intact than neutered cats, and in sick than healthy cats. Erythrocyte count, hemoglobin concentration and PCV were lower in FIV-infected than seronegative cats, although still within the normal range.

Keywords: cats, FIV, UVH, UPM, prevalence, risk factors, haematological profile, SNAP Combo test kit, IDEXX.

THERAPEUTIC EFFECT OF *PYHLLANTHUS NIRURI* LEAF EXTRACT ON GENTAMICIN-INDUCED RENAL INJURY IN MICE

Nur Syamila Zabri, [1]Hazilawati Hamzah, [1]Noordin Mohamed Mustapha & [2]Mohd Rosly Shaari

[1]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Strategic Livestock Research Centre
Malaysian Agricultural Reasearch and Development Institute
43400 Serdang, Selangor, Malaysia

ABSTRACT

Phyllanthus niruri or in Malay known as *Dukong Anak* is a common traditional herb used to treat hepatic and renal diseases. This study investigated the therapeutic effects of ethanolic extract of *P. niruri* leaves on gentamicin-induced renal injury in mice. Ninety-two mice were equally divided into five groups named control, gentamicin, and gentamicin and treated with three levels of *P. niruri* extract at doses of 75, 150 and 250 mg/kg body weight. Control and gentamicin groups were treated intraperitoneally with normal saline and gentamicin (100 mg/kg body weight) once daily for six day. The *P. niruri* leaf extract was given orally via oral lavage, daily, until the mice were sacrificed at days 7, 10, 13 and 16 of the experimental period. Renal biochemistry parameters and histological lesions of the kidneys were evaluated. The results showed that at day 16 the BUN concentrations of mice treated with the three different doses of *P. niruri* leaf extract were significantly ($p<0.05$) lower than that of the gentamicin group. There was no significant ($p>0.05$) difference in serum creatinine concentration and renal histology among groups. The study suggests that *P. niruri* leaf extract has therapeutic potential for the treatment of gentamicin-induced renal injury in mice.

Keywords: *Phyllanthus niruri*, gentamicin, blood urea nitrogen, creatinine, renal injury.

PREDILECTION SITE OF *ARCANOBACTERIUM HAEMOLYTICUM* ADMINISTERED BY DIFFERENT ROUTES IN MICE

Nur Syariha Abdul Halim, [1]Md Sabri Mohd Yusoff, [1]Tanko Polycarp Nwunuji & [1]Opeyemi Onilude Mayowa

[1]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The present study was conducted to determine the predilection site of *Arcanobacterium haemolyticum* in mice infected using different routes of infection. Thirty 2-month-old female mice used in this study were supplied by the Animal Resources Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The mice were divided into 3 groups. Group A (13 mice) was challenged with 0.3 mL of 1×10^9 cfu *A. haemolyticum* through the intranasal route. Group B (13 mice) were injected with 0.3 mL 1×10^9 *A. haemolyticum* subcutaneously. The remaining 4 mice (Group C), which formed the control group was not challenged. The mice were humanely euthanised day 3, 7 and 10 post-challenged. Four mice each were selected from Groups A and B to be euthanised. The bacterial culture and identification were carried out using standard biochemical tests. Various organs were collected for histological evaluation. The results showed that the intranasal administration of *A. haemolyticum* had more bacterial cultures on day 7. However, the parameters observed for the different routes were not significantly different. Histology evaluation showed that Group A had histological changes in the lung, but Group B showed more changes in the liver.

Keyword: *A. haemolyticum*, intranasal, subcutaneous, histopathology

ANTIBACTERIAL EVALUATION OF ETHANOLIC EXTRACT OF *MELASTOMA ALABATHRICUM* LEAVES AND ITS EFFECT ON HAEMATOLOGICAL PROFILE

Ong Chia Lin, [1]Hazilawati Hamzah, [1]Siti Khairani Bejo & [2]Mohd Rosly Shaari

[1]Department of Veterinary Pathology and Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Strategic Livestock Research Centre
Malaysian Agricultural Reasearch and Development Institute
43400 Serdang, Selangor, Malaysia

ABSTRACT

Melastoma malabathricum, under Melastomaceae family, locally known as *Sendudu* or *Kenduduk* has been used in Malay traditional medicine with impressive range of medicinal values. The ethanol extract from the leaves of *M. malabathricum* was evaluated for its antibacterial activity and its effect on haematological parameters. Antibacterial evaluation consisted of *in vitro* disc diffusion and broth microdilution methods and *in vivo* topical dermal application of cream formulation (4, 2, 1% w/v) on *Staphylococcus aureus*-induced dermatitis in murine model. Disc diffusion method showed optimum inhibition zone of 13.85±0.25 mm in diameter against *S. aureus* at the concentration of 4% *M. malabathricum* extract, 10.53±0.14 and 9.23±0.37 mm at the concentration of 2 and 1% *M. malabathricum* extract, respectively. The minimum inhibition concentration and minimum bactericidal concentration of *M. malabathricum* extract against *S. aureus* were 0.390 and 0.780 mg/mL, respectively. Five days of topical dermal application with 1% *M. malabathricum* extract significantly ($p<0.05$) reduced bacteria concentration at infection sites. There were no significant ($p>0.05$) changes in the haematology particularly the leucocyte parameters among groups.

Keywords: *Melastoma malabathricum*, antibacterial activity, haematological parameters

CHANGES IN FEMALE REPRODUCTIVE HORMONES AND ORGANS OF FEMALE MICE AFTER ORAL INOCULATION WITH *PASTEURELLA MULTOCIDA* TYPE B AND ITS LIPOPOLYSACCHARIDE

Rosmawati Zainal & [1,2]Faez Firdaus Jesse Abdullah
[1]Department of Veterinary Clinical Studies
[2]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Haemorrhagic septicaemia (HS) is an important fatal disease involving cattle and buffalo caused by *Pasteurella multocida* type B in Asia. There is lack of knowledge about cellular changes of female reproductive organs, pituitary gland and hormones in animals infected with *P. multocida* and its endotoxin. In this study, 24 healthy mice were divided into 3 equal groups of 8 mice each. Each of the group was inoculated orally with 0.4 mL of phosphate-buffered saline (PBS), 0.4 mL of 1×10^9 colony forming unit (cfu) of *P. multocida* type B (HS) and 0.4 mL of lipopolysaccharides from 1×10^9 *P. multocida* lipopolysaccharides (LPS). The mice were then observed for clinical signs during the 10 day-period post-inoculation. Blood was collected via cardiac venipuncture from the mice showing severe clinical signs and survived after 10 days post-inoculation, for hormone analysis. The pituitary gland and reproductive tract were collected from euthanised mice for microscopic examination. The lesions observed include degeneration and necrosis, congestion or haemorrhages, presence of inflammatory cells and oedema. The mice in the treatment group showed significantly ($p<0.05$) greater cellular changes in most lesions of the reproductive organs than the control. On the other hand, mice of the treatment group showed significant ($p<0.05$) degeneration and necrosis in pituitary gland compared to controls. The progesterone concentration increased by 107% in the HS and 217% in the LPS group mice. The estrogen concentrations decreased in the HS and LPS groups by 16 and 8% respectively. Therefore, oral *P. multocida* type B and its lipopolysaccharides inoculations caused cellular changes in the female reproductive organs and some changes in the reproductive hormone profile.

Keywords: mice, *Pasteurella multocida* type B, lipopolysaccharides, progesterone, estrogen, female reproductive organs, pituitary gland.

COLOUR, SHEAR FORCE, DRIP AND COOKING LOSS OF PORK

Rufina Lee Ling Jie & [1]Peck Toung Ooi
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

For years, assessment of pork quality in Malaysia was mostly done subjectively and no proper objective measurements have been carried out. This study evaluates a few traits of pork quality, including pH measurement, drip and cooking loss, colour determination and shear force. The results showed that the average sample pH was 5.88, which falls within ideal range of 5.6 to 5.9 stipulated by the National Pork Board /American Meat Science Association, 1998. Average drip and cooking loss of samples were 9.98 and 27.13% respectively, which fell within acceptable and good range according to international standards. Warner-Braztler reading for pork sample firmness was 7.12kg, which was higher than the international target of 3.2 kg. In the colour determination, the average lightness, redness, yellowness and hue angle values of the samples are 45.135, 6.771, 15.274 and 48.172 respectively. These indicate that the pork samples used in this project were darker, more reddish and yellowish compared to international standards. Overall, Malaysian pork samples are within international standards. However, pork samples used in this project were limited to a top-tier farm, and overall standard of pork quality from other farms in Malaysia is still unknown. Efforts should be taken to investigate the pork quality in other farms for the information to pork producers in Malaysia on the quality of their end-product and to improve where necessary.

Keywords: pork quality, pH, drip and cooking loss, colour, shear force

DIURNAL ACTIVITY BUDGET OF CAPTIVE DHOLES (*CUON ALPINUS*) AT A LOCAL ZOOLOGICAL PARK IN PENINSULAR MALAYSIA

Sandy Ling Choo, [1]Abdul Rahim Mutalib & [2,3]Sumita Sugnaseelan
[1]Department of Veterinary Laboratory Diagnosis
[2]Wildlife Research Centre
Faculty of Veterinary Medicine
[3]Department of Animal Science
Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

This study was undertaken to document the diurnal activity budget of captive dholes (*Cuon alpinus)* (n = 8) at a zoological park in Peninsular Malaysia, with the aim of contributing to the better understanding of factors affecting the welfare and biology of these animals in captivity. An ethogram was established and data were collected *via* direct visual observation of the subjects in an open, naturalistic exhibit. Instantaneous scan sampling was performed at 10 minute interval, between 0900 and 1800 hours over a period of 31 days; the first 3 days for ethogram validation, the next 7 days as conditioning period and the remaining 21 days as post-conditioning period. Active data recording were for 28 days with the initial 3 days of ethogram validation not included in the data analysis. Throughout the study, the captive dholes consistently spent a major portion of the day resting, followed by locomotive activities. Statistical analysis revealed significant ($p<0.05$) decrease in locomotive, submissive and reproductive behaviours during post-conditioning period and significant ($p<0.05$) increased frequency of the subjects to remain hidden from view. Simultaneously, an increase in average environmental temperature of 1.5°C and decrease in average relative humidity of 8.5% were significant enough to cause variations in behaviours observed during the post-conditioning period. The presence of free-ranging non-human primates, namely pig-tailed macaques (*Macaca nemestrina*), long-tailed macaques (*Macaca fascicularis*) and dusky leaf monkeys (*Trachypithecus obscurus*), near and within the outdoor enclosure caused significant ($p<0.05$) increase in the vocalisation repertoire of the captive dholes. The presence of the free-ranging non-human primates may be of either positive or negative consequences, which are a subject of interest that may require further studies under complementary physiological based research.

Keywords: captive dholes, *Cuon alpinus*, diurnal activity budge

FUNGAL FLORA IN THE NASOPHARYNX OF HORSES

Siti Aisah Abdul Rahman, [1]Nurul Hayah Khairuddin
[2]Abdul Rahim Mutalib & [3]John Tito Sapalo
[1]Department of Large and Exotic Animal Medicine and Surgery
[2]Department of Veterinary Laboratory Diagnosis
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Skuadron Istiadat Berkuda
Kem Sungai Buluh, 47000 Sungai Buluh, Selangor, Malaysia

ABSTRACT

Fungal infection of equine respiratory system, aspergillosis, is commonly caused by the *Aspergillus* species. Aspergillosis occurs in various compartments of the equine respiratory system including the guttural pouch, known as guttural pouch mycosis or the nasal cavity, known as mycotic nasal infection. Aetiological studies revealed that the most common agent for mycotic nasal infection is *Aspergillus fumigatus*. Fungi such as *Aspergillus*, *Scopulariopsis*, *Mucor*, and *Penicillium* spp. have been quoted as non-pathogenic and occur naturally in healthy guttural pouch. However, the characteristic of fungal population in the nasopharynx of horses is not well-understood. The fungi may be persistent in the environment or naturally occurring in the nasal cavity of horses. Hence, this study was carried out to identify the fungal flora in the nasopharynx of apparently healthy horses. The presence of fungal flora in nasopharynx was investigated through the use of nasopharyngeal lavage cultured onto sabourad dextrose agar with chloramphenicol and cycloheximide. These fungi were identified on the basis of their colony morphology and microscopic examination of the spores and hyphae. Lactophenol cotton blue staining technique was used for microscopic examination of the fungal hyphae. The culture of the nasopharyngeal lavage of 13 horses resulted in the isolation of *A. fumigatus* from 3 (30%) and *Penicillium* sp. from 8 (80%) horses and *Candida albicans* from one horse (10%). The data shows a predominance of *Penicillium* sp. and *A. fumigatus* in the nasopharynx of healthy horses, reiterating the importance of monitoring fungal population in the nasopharynx of horses.

Keywords: aspergillosis, fungal flora, nasopharynx, mycotic nasal infection

OCCURRENCE OF *SALMONELLA* AND *CAMPYLOBACTER* SPP. IN WILDBIRDS

Siti Sajidah Mustaffa, [1,3]Saleha Abdul Aziz & [2,3]Jalila Abu
[1]Department of Veterinary Pathology and Microbiology
[2]Department of Veterinary Clinical Studies
[3]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Salmonella and *Campylobacte*r spp. are known as important zoonotic foodborne pathogens. This study was undertaken to determine the occurrence of *Salmonella* and *Campylobacter* spp. in a population of wildbirds and to determine the antibiotic susceptibility of the isolates. Sixty-eight fresh faecal samples were collected from wildbirds in Bangi, Kepong, and two areas at Serdang, Selangor, Malaysia. One (1.47%) fresh faecal samples from a pigeon at Kepong were positive for *Salmonella* spp. The *Salmonella* spp. isolate was susceptible to 4 antibiotics which were ampicillin, ceftriaxone, ciprofloxacin, and gentamicin and resistant to erythromycin and tetracycline. Six (8.82%) fresh faecal samples were positive for *Campylobacter* spp. of which three (50%) were from pigeons collected from the Serdang area and another three (50%) were from pigeons from the Kepong area. All isolates were identified as *Campylobacter jejuni*. The *Campylobacter* spp. were resistant to trimethoprim-sulfamethoxazole (100%), followed by cefotaxime (83.3%), tetracycline (33.3%) and ampicillin (16.7%). The presence of *Salmonella* and *Campylobacter* spp. in wild birds poses a health risk because being reservoirs, they may transmit these enteric pathogens to humans or spread the organisms in the environment.

Keywords: wildbirds, *Salmonella* spp., *Campylobacter* spp., antibiotic susceptibility

OCCURRENCE OF *SALMONELLA* AND *CAMPYLOBACTER* SPP. IN PSITTACINE BIRDS

Soon Xue Qi, [1,3]Jalila Abu & [2,3]Saleha Abdul Aziz
[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
[3]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Nowadays, people commonly keep psittacine birds (parrots) as pet or as a family member and spend most of the time with them. However, the birds are recognised as carriers of a number of pathogens which may pose zoonotic risk. Therefore, this study was carried out to determine the presence and antibiotic resistance of *Salmonella* spp. and *Campylobacte*r spp. in psittacine birds. Fresh faecal samples and skin swabs were collected from 70 apparently healthy birds from 8 pet shops, one breeder and one owner located in Klang Valley, Selangor, Malaysia. *Salmonella* spp. was isolated from 3 fresh faecal samples (2.1%) out of 140 samples from 3 psittacine species which included African grey parrot, budgerigar, and blue and gold macaw. *Campylobacter jejuni* was isolated from 20 (28.6%) of 70 fresh faecal samples from the budgerigar. All *Salmonella* spp. isolates were resistant (100%) to erythromycin. *Campylobacter jejuni* isolates were resistant to ciprofloxacin (95%) and least resistant to clindamycin (15%). Multi-drug resistant *C.jejuni* was high, at 50% of the total isolates. Drug-resistant *Salmonella* spp. and *Campylobacter jejuni* pose serious threat to the public, as both are capable of causing severe gastroenteritis in humans.

Keywords: Psittacine birds, *Salmonella* spp., *Campylobacter* spp., occurrence, antibiotic resistance

EFFICACY OF INACTIVATED MALAYSIAN AVIAN PATHOGENIC *ESCHERICHIA COLI* ISOLATES FROM BROILER CHICKENS

Syamira-Syazuana Zaini, [1]Mohd Hair Bejo, [1]Siti Khairani Bejo & [1,2]Zunita, Zakaria

[1]Department of Veterinary Pathology and Microbiology
[2]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) infection is an important economic issue in the poultry industry, because it causes a number of diseases such as complicated chronic respiratory disease, colisepticaemia and colibacillosis. An isolate with ≥4 of 8 VAG is classified as an APEC. The pathogenicity of APEC can be determined by the presence of virulence-associated genes (VAG) in the isolate. Different number of genes in an isolate may show different pathogenicity of the organism and it is believed that an APEC isolate with more VAG should be used for vaccine development because it will provide better protection against the infection. The objective of this study was to determine the efficacy of two inactivated Malaysian APEC isolates against APEC infection in broiler chickens. Seventy-five day-old broiler chicks were divided into three groups. Groups A and B comprised 24 chicks per group, whilst Group C comprised 27 chicks. All chicks were fed commercial starter diet and water *ad libitum* throughout the trial. At one day old, three chicks from the Group C (Control) were sacrificed to represent healthy chicks. Then each chick in Groups A and B were inoculated subcutaneously with 0.1 mL of five (VAG-5) and six VAG (VAG-6) inactivated APEC (1×10^8 cfu/mL), respectively. Group C chicks were not inoculated. At 14 days of age, the chicks from the three groups were divided into two; the challenged and non-challenged groups. The chicks in the challenged groups were orally inoculated with 1.0 mL APEC (1×10^8 cfu/mL). Three chicks from each group were sacrificed at 7, 14, 15, 17 and 21 days of age. No significant clinical sign was observed in the non-challenged chicks throughout the trial. Among the challenged chicks: the Control and Group A showed moderate clinical signs including diarrhoea and ruffled feathers beginning at 15 until 21 days of age or 1 to 7 days post-challenge. However, the chicks from Group B only showed mild clinical signs. The body weight in the non-challenged and challenged groups increased gradually throughout the trial. At one day of age, *E. coli* was isolated from the cloaca (33%) and intestine (67%) of the control chicks. At 7 days of age, *E. coli* was isolated from the cloaca of all inoculated and control chicks. Then at 14 days of age, the intestine and cloaca of all inoculated and control chicks were positive for *E. coli*. The *E. coli* was isolated from the intestine of 67% of control, 100% of Group A and 33% of Group B chicks. At 15 days of age, the challenged and non-challenged

chicks from Group B showed lower *E. coli* isolation rate than the control and Group A chicks. At 17 and 21 days of age, the non-challenged and challenged from group B gave lower *E. coli* isolation percentage for all samples than the control and Group A chicks. Grossly the lesions were normal, however, histopathologically there were focal areas of necrosis in the liver of Groups A and C chicks at days 1, 3 and 7 post-challenged (pc). In the case of Group B chicks, focal areas of necrosis of the liver appeared at days 1 and 3 pc. Mild to moderate deciliation and tracheitis were seen in Groups A and C chicks at days 1, 3 and 7 and in Group B chicks at days 1 and 3 pc. Group B chicks showed normal liver and trachea histopathology at day 7 pc. The study showed that VAG-6 provided better protection against *E. coli* infection than VAG-5 inoculum.

Keywords: avian pathogenic *Escherichia coli* (APEC), inactivated APEC isolates, adjuvant, broiler chickens

IDENTIFICATION OF BACTERIA FROM GUT OF GROUPER (*EPINEPHELUS* SP.) AND THEIR RESISTANCE TO COMMONLY USED ANTIBIOTICS

Tan Chung Ren, [1]Mohamed Shariff Mohamed Din & [2]Sanjoy Banerjee
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
[2]Institute of Bioscience
Universiti Putra Malaysia, Selangor, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Marine fish culture in Malaysia has expanded rapidly and the occurrence of diseases has increased as a result of intensive farming. In order to control and prevent fish diseases effectively, a study on the presence of different bacteria and their resistance to commonly used antibiotics was conducted. In this study, bacteria was isolated from the gut of 10 groupers (*Epinephelus* sp.) purchased from a commercial fish farm in Pulau Ketam, Selangor, Malaysia. The isolated bacteria were identified by 16s rDNA and results revealed 17 different bacteria belonging to the families Vibrionaceae, Enterobacteriaceae, Shewanellaceae, Moraxellaceae, Aeromonadaceae, Enterococcaceae and Staphylococcaceae. The bacteria were then tested for their antibiotic sensitivity using the disc diffusion method. Antibiotic sensitivity tests showed that 58.8% of bacteria were resistant to ampicillin followed by compound sulphonamides at 47% (8/17), erythromycin 41.1% (7/17), oxolinic acid 29.4% (5/17), tetracycline 23.5% (4/17), trimethoprim / sulphamethoxazole 11.7% (2/17), doxycycline 5.8% (1/17) and chloramphenicol 5.8% (1/17). None of the isolated bacteria were resistant to norfloxacin and nitrofurantoin. Although this study showed that there was a relatively high percentage of resistant bacteria to several antibiotics tested, further larger scale studies are required to investigate the status of antibiotic resistance in the aquaculture industry in Malaysia.

Keywords: grouper (*Epinephelus* sp.), gut, antibiotic resistant bacteria

IMMUNOHISTOCHEMICAL EXPRESSION OF CD117 IN CANINE TESTICULAR TUMOURS

[1]Tan Yen Fhong, [1]Gayathri Thevi Selvarajah, [2]Rasedee Abdullah, [3,4]Mohd Zamri Saad & [5]Norhafizah Mohtaruddin

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Laboratory Diagnosis
[3]Department of Veterinary Pathology and Microbiology
[4]Ruminant Diseases Research Centre
Faculty of Veterinary Medicine
[5]Faculty of Medicine and Health Sciences
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Testicular neoplasia is common in old and intact dogs, similar to man. The major histopathology classification of testicular tumours in dogs is; seminoma, Sertoli cell tumour and Leydig cell tumour. Several biomarkers are currently investigated for diagnostic and prognostic purposes. One of the most common biomarker explored across canine neoplasia is CD117, also known as stem cell growth factor receptor (SCFR) or C-KIT. CD117 is a type-III tyrosine kinase receptor that was reported in human and canine seminoma. However, expression of CD117 has not been evaluated in other testicular tumours and pathologies. Therefore, the objectives of this study are: 1) to determine the expression of CD117 in canine testicular tumours and other pathologies using immunohistochemistry assay; and 2) to evaluate the association between the expressions of CD117 with clinical variables of dogs with testicular tumours. Twenty formalin fixed paraffin embedded canine testicular tissues of various pathologies and five histologically normal testes were retrieved from the Veterinary Histopathology Laboratory of the Faculty of Veterinary Medicine at Universiti Putra Malaysia. The protein expression of CD117 was evaluated using immunohistochemistry and microscopic examination. One Grade-3 canine mast cell tumour and one canine mammary gland lymphoma were included as control tissues for the assay. Testicular pathologies that were included are seminoma (n=6), Sertoli cell tumour (n=9), testicular lymphoma (n=1), atrophy (n=3) and orchitis (n=1). CD117 was expressed in the cytoplasm of 33.3% of seminoma (n=2) and testicular lymphoma 100% (n=1). CD117 expression was not present in all the other testicular pathologies and normal testis. The immunostaining intensity observed in the seminomas were relatively weaker compared to testicular lymphoma. Mast cell tumour and mammary gland lymphoma tissues demonstrated a higher intensity of CD117 expression compared to all the three positive testicular tumours. CD117 is expressed predominantly in canine seminoma and not in other testicular tumours. For the first time, this study also revealed that canine testicular lymphoma was highly positive for CD117. CD117 could contribute to the oncogenesis of a subset of canine

testicular tumours especially the seminoma and if this expression is associated with a specific histopathology variant warrants further investigation.

Keywords: canine, testicular tumour, seminoma, immunohistochemistry, CD117

OCCURRENCE OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN DAIRY CATTLE

Timothy Fong & [1,2]Saleha Abdul Aziz
[1]Department of Veterinary Pathology and Microbiology
[2]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Antibiotic usage in food animal production contributes to the emergence of antibiotic resistance in humans. The objectives of this study were to determine the occurrence of *Escherichia coli and Staphylococcus aureus* in dairy cattle and the resistance of isolates against 6 antibiotics, namely enrofloxacin, trimethoprim–sulphamethoxazole, penicillin G, tetracycline, ceftiofur and vancomycin using disk diffusion technique. Twenty-five dairy cows from 5 farms in Selangor, Malaysia were selected for the study. Seventy-five swab samples were collected from the tail, udder and rectum. The samples were cultured, isolates and identified. Forty-seven (62.7%) of *E. coli* and 32 (42.7%) of *S. aureus* were isolated. *E. coli* was mostly isolated from the rectum whereas *S. aureus* were frequently isolated from the tail. The highest resistant rate in isolated *E. coli* was towards penicillin G (91.5%) and least towards enrofloxacin and trimethoprim–sulfamethoxazole (2.1%). The highest resistant rate in the isolated *S. aureus* was towards ceftiofur (93.8%), and least to enrofloxacin (3.1%). 62.7% of *E. coli* 42.7% of *S. aureus* was resistant to one or more antibiotics. There is a need to monitor the use of antibiotics in food animal to control the occurrence of antibiotic resistance in the country.

Keywords: antibiotic resistant, dairy cattle, *Escherichia coli*, *Staphylococcus aureus*

EFFECT OF PREOPERATIVE TRAMADOL ON EARLY POSTOPERATIVE PAIN IN FEMALE DOGS UNDERGOING OVARIOHYSTERECTOMY

Trishul Prabhakar Vatakal & [1]Chen Hui Cheng
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Dogs presented for elective ovariohysterectomy (OHE), were randomly assigned to two groups. Dogs in Group 1 (tramadol, n=6), were premedicated with tramadol, 5 mg/kg body weight subcutaneously (SQ). Dogs in Group 2 (control, n=6), were given injections of sterile saline also SQ. Both groups were then anaesthetised with a combination of ketamine–xylazine, intravenously, at a dose rate of 11 and 0.5 mg/kg body weight respectively. Intra-operatively, systolic blood pressure (BP), heart rate (HR), respiration rate (RR) and haemoglobin saturation ($Sp0_2$) was monitored. Postoperatively, all dogs received ketoprofen, 2 mg/kg body weight intramuscularly. Following standing, pain scores were determined every hour for 8 hours, using the Glasgow Composite Pain Scale (Short Form). Intra-operatively, both BP and HR tended to be higher in the control group than the tramadol group. Increment in these parameters peaked during manipulation of the first ovarian pedicle and at skin closure. There was no treatment difference in RR and SpO2 between groups. Postoperatively, pain scores in the tramadol group were lower than the control group for the first 4 hours after standing recovery. Thus, it is concluded that tramadol administered preoperatively has a beneficial analgesic effect on early postoperative pain.

Keywords: dog, ketamine-xylazine, ketoprofen, ovariohysterectomy, pain, tramadol

PREVALENCE AND HAEMATOLOGICAL PROFILE OF CATTLE INFECTED WITH *THEILERIA* SPP. IN SELECTED FARMS IN SELANGOR

Ungku Syakira Syaza Ungku Ahmad, [1,3]Abd Wahid Haron, [1,3]Faez Firdaus Jesse Abdullah & [2,4]Shaik Mohamed Amin Babjee

[1]Department of Veterinary Clinical Studies
[2]Department of Veterinary Pathology and Microbiology
[3]Ruminant Diseases Research Centre
[4]Wildlife Research Centre
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

A cross-sectional study was carried out in 5 selected cattle farms in Selangor, Malaysia to determine the prevalence of *Theileria* spp., and to assess the parasitemia rate and its relationship with haematological profiles in cattle with *Theileria* spp. infection. Fifty animals were randomly selected based on different age groups comprising adult and young cattle. Blood was collected from the jugular veins using EDTA vacutainers for blood screening and parasite detection. *Theileria* spp. was then examined on Giemsa-stained thin blood films. Based on the survey, the prevalence for *Theileria* spp. was recorded at 70.0%. However, there was no significant ($p>0.05$) difference between the presence of parasite and age group. The haematological profiles in 35 cattle with theileriosis were divided into 4 subgroups with different parasitemia rates (<1, 1 to 3, 3 to 5 and >5%) and 10 healthy cattle served as controls. An independent T-test showed highly significant ($p<0.01$) differences in all erythrocyte parameters including erythrocyte count, haemoglobin concentration and packed cell volume between diseased and control cattle A significant ($p<0.01$) negative correlation between parasitemia rate and erythrocyte parameters was observed. However there was no correlation between parasitemia rate and leucocyte parameters. In conclusion, the prevalence of cattle infected with *Theileria* spp. recorded in the farms in Selangor, Malaysia was still considered high and the significant decline in erythrocyte parameters in the infected cattle with higher parasitemia rate coincided with the occurrence of anaemia.

Keyword: cattle, prevalence, Selangor, *Theileria* spp., thin blood film, parasitemia rate

EFFECT OF ACIDIFIERS ON PERFORMANCE OF WEANING PIGLETS

Yeow Wei Yang, [1] Peck Toung Ooi & [2]Mok Hsiao Li
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Bayer (Malaysia) Sdn Bhd, 46200 Petaling Jaya, Selangor, Malaysia

ABSTRACT

Acidifiers are feed additives comprising of organic or inorganic acids. Acidifiers facilitate the reduction of stomach pH and gastrointestinal tract pathogenic bacteria. Eighty weaning piglets were selected randomly from two farms (A and B) in Peninsular Malaysia. The piglets from each farm were divided into two groups; the treatment group was given normal feed with added acidifiers while the control was given normal feed. All piglets were weighed on days 0, 15, 30 and 45. Mean weight gain, average daily gain (ADG) and percentage weight gain were compared between treatment and control groups. Farm A showed no difference in body weight gain, ADG and percentage weight gain for either group. For Farm B, the treatment group showed better body weight gain, ADG and percentage weight gain. These differences piglet performance may be the result of the differences in farm husbandry and management practices. In general, Farm A practiced better husbandry using animal feed containing high amount of calcium and with high buffering capacity. These factors may have reduced the effectiveness of acidifiers. In conclusion, acidifiers do promote weight gain, ADG and percentage weight gain. Acidifier usage in weaning piglets can be an alternative to the use of antimicrobials in livestock production.

Keywords: weaning piglet, acidifier, average daily gain (ADG), body weight change, buffering capacity

ULTRASTRUCTURE OF SWIFTLET SALIVARY GLAND

Zainita Mohd Noor & [1,2]Tengku Azmi Tengku Ibrahim
[1]Department of Veterinary Preclinical Sciences
[2]Centre of Excellence on Swiftlet
Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

The salivary gland is an accessory gland of digestion. In the swiftlet this gland is well-developed during the breeding season to produce the highly priced and nutritious edible bird nest (EBN). This study examined the ultrastructure of the swiftlets salivary gland to provide an understanding of its structure in relation to the production of the bird nest. Well-developed salivary glands, obtained immediately following sacrifice of the birds were fixed and processed for examination under the transmission electron microscope. The salivary gland was surrounded by a very thin, transparent layer of connective tissue and unlike the salivary of other species the salivary gland was not divided into lobes and lobules. Instead each secretary cell was surrounded by, at the most, a single layer of connective tissue cells. The cytoplasm of each secretary cell was predominantly filled with secretory granules which accounted for the highly developed salivary gland. The prominent organelle in the cytoplasm of the secretory cell was the very extensive, highly elongated rough endoplasmic reticulum (rER). The ends of these rER appeared dilated and rER were observed to surround the secretory granules. A close search for the Golgi apparatus was made but none could be observed or identified with confidence. Absence of the Golgi complex could possibly indicate that in the highly efficient salivary gland production by the secretory granules does not require the Golgi apparatus. Dilatation at the ends of the rER itself could possibly assume the formation of the secretory granules.

Keywords: swiftlet, ultrastructure, edible bird nest, secretory granule, endoplasmic reticulum, golgi apparatus

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