11th Proceedings of the Seminar on VETERINARY SCIENCES

Faculty of Veterinary Medicine UPM
29 February - 2 March 2016

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Preface

The 2016 edition of the Proceedings of the Seminar on Veterinary Sciences is the eleventh of a series of final year Doctor of Veterinary Medicine, Universiti Putra Malaysia students' project reports. The Proceedings while archiving final project reports for reference also provides directions on future research undertakings. In the conduct of the final year projects, the students are guided by supervisors on the scientific practice of documenting hypotheses, experimental designs, response data on the treatments imposed, data analyses, interpretations of result, and research conclusions.

Challenges are aplenty confronting the veterinary professionals on a daily basis and with informed judgement, meaningful interventions could appropriately be instituted. We believe the experience gained from the final projects is training for our new veterinarians in the making of decisions to overcome challenges after taking into consideration all extenuating factors.

The editors would like to express our appreciation for the time and effort both the students and lecturers dispensed in preparing these abstracts. With a total of 104, the 11th Proceedings has the highest number of short and extended abstracts thus far. It is heartening to note that references were made of abstracts in our previous proceedings as there are made accessible via the internet.

Finally, the editors wish to thank the Dean and management of the Faculty of Veterinary Medicine for the continuous support in publishing the current Proceedings.

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ISOLATION AND IDENTIFICATION OF BACTERIA IN THE RESPIRATORY TRACT OF THE RED JUNGLEFOWL

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ABSTRACT

Respiratory diseases are the most important diseases affecting poultry and this has been observed in domesticated Red Junglefowl. The objectives of this present study were to isolate and identify bacteria from the sinus and lungs of Red Junglefowl and to determine the relationship between bacteria isolated with respiratory tract problems. Sixteen Red Junglefowl consisting of 12 clinically unhealthy and four apparently healthy chickens were obtained from a private farm. The chickens were slaughtered and sinus swabs and lung samples collected from each chicken. All samples were cultured on blood and chocolate agar for bacteria isolation. The bacteria isolates were identified using biochemical tests. Nineteen gram-positive (43.2%) and 25 gram-negative (56.8%) bacteria were isolated from clinically unhealthy Red Junglefowl. Five gram-positive (41.7%) and 7 gram-negative (58.3%) bacteria were isolated from apparently healthy Red Junglefowl. There was no significant ($p<0.05$) difference between the clinically unhealthy and apparently healthy Red Junglefowls in term of frequency of gram-positive and gram-negative bacteria isolated. The bacteria species isolated from clinically unhealthy chickens were *Staphylococcus species* (25%), *Corynebacterium species* (18.20%), *Avibacterium avium* (9.09%), *Neisseria species* (6.82%), *Gallibacterium anatis* bv. *haemolyticatis* (6.82%), *Escherichia coli* (6.82%), *Avibacterium gallinarum* (6.82%), *Proteus mirabilis* (4.55%), *Pasteurella multocida* subspecies *multocida* (4.55%), *Pasteurella* sp/A (4.55%), *Streptococcus species* (2.27%), *Pseudomonas aeruginosa* (2.27%), and *Aeromonas sp* (2.27%). The bacteria species isolated from apparently healthy chickens were *Staphylococcus sp* (16.67%), *Corynebacterium species* (16.67%), *Avibacterium avium* (16.67%), *Bacillus species* (8.33%), *Gallibacterium anatis* bv *haemolyticatis* (8.33%), *Escherichia coli* (8.33%), *Pseudomonas aeruginosa* (8.33%), *P. multocida* subspecies *multocida* (8.33%) and *Pasteurella haemolytica* (8.33%). In conclusion, domesticated Red Junglefowl harbour numerous opportunistic bacteria that might contribute to the respiratory problems in immunocompromised chickens.

Keywords: Red Junglefowl, respiratory problem, bacteria

INTRODUCTION

In poultry among common diseases are omphalitis, collibacillosis, salmonellosis, pasteurellosis, and infectious coryza. These diseases had direct negative impact on chickens including reduced weight gain and egg production, morbidity, and mortality. The negative impact in turn, caused major economic losses to the poultry industry.

Respiratory tract diseases are a significant component of the overall disease incidence in poultry (Glisson, 1998). The diseases do not just affect commercial chicken but also the Red Junglefowl, which is the wild ancestor of all domestic chickens. Diseases of the respiratory tract are often complex; with anatomy, management, environment and nutrition factors, all playing their roles in disease development and progression.

MATERIALS AND METHODS

Sixteen domesticated Red Junglefowl consisting of 12 clinically unhealthy and 4 apparently healthy chickens were selected from a farm in Dengkil, Selangor, Malaysia. The selected clinically unhealthy chickens were inactive, depressed, nasal discharge, exhibited gasping symptoms, and were blind. All the clinical signs shown were indication of respiratory problems. Post-mortem was done and sinus and lung samples collected from each chicken. The samples were aseptically inoculated onto blood and chocolate agars and incubated aerobically and anaerobically for 24 and 48 h, respectively at 37ºC. Following incubation, the growths on both agars were observed and the morphology of visible colonies recorded. Each colony from the blood and chocolate agars were subcultured into fresh blood and chocolate agars, aerobically and anaerobically, respectively for 24 h at 37ºC to obtain pure culture prior to gram staining. Colonies representing each bacterial species were identified and characterised using standard biochemical methods (Jang *et al.,* 2004). Descriptive statistical analysis of data obtained was performed using IBM SPSS software.

RESULTS AND DISCUSSION

Nineteen (43.2%) gram-positive and 25 (56.8%) gram-negative bacteria were isolated from clinically unhealthy Red Junglefowl lung and sinus samples. However, 5 (41.7%) gram-positive and 7 (58.3%) gram-negative bacteria were isolated from the lung and sinus samples of apparently healthy Red Junglefowl. Statistically, there was no significant ($p>0.05$) difference between the apparently healthy and clinically unhealthy chickens in terms of frequency bacteria isolated (Table 1).

The rate of occurence of the gram-positive and gram-negative bacteria in the lungs of clinically unhealthy chickens were 35.3 and 64.7% respectively whilst in in the sinus it was equal at 50% each. The rate of occurrence of the gram-positive

and gram-negative bacteria in the sinus of apparently healthy chickens were 48.1 and 59.1%, respectively and in the sinus of unhealthy chickens, 40 and 60%, respectively (Table 2). In contrast, the lower respiratory tract and sinuses are expected to be free from any bacteria (Sorum and Sunde, 2001). The findings suggest that Red Junglefowls may either be the reservoir for these bacteria or the chickens had subclinical infections. In the lungs and sinuses of clinically unhealthy chickens, the gram-negative bacteria predominated over the gram-positive bacteria. This finding was consistent with that of a previous study (Bisgaard, 1977) that reported shifting of gram-negative flora in chicken with respiratory infections. However, the gram-negative bacteria may be opportunistic, capable of causing disease in individuals under stress (Liau, 1997).

Table 1: Gram-positive and gram-negative bacteria in apparently healthy and clinically unhealthy Red Junglefowl.

Bacteria	Bacteria isolate Number (%)		Chi -square test (α=0.05)
	Apparently healthy	Clinically unhealthy	
Gram-positive	5 (41.7)	19 (43.2)	0.925
Gram-negative	7 (58.3)	25 (56.8)	
Total	**12 (100)**	**44 (100)**	

Table 2: Frequency of the bacteria isolated from the lungs and sinus of apparently healthy and clinically unhealthy Red Junglefowl

Bacteria	Bacteria isolate Number (%)			
	Sinus (p=0.73)		Lungs (p=1.00)	
	Apparently healthy	Clinically unhealthy	Apparently healthy	Clinically unhealthy
Gram-positive	4 (40)	13 (48.1)	1 (50)	6 (35.3)
Gram-negative	6 (60)	14 (51.9)	1 (50)	11 (64.7)
Total	**10 (100)**	**28 (14)**	**2 (100)**	**17 (100)**

Fifteen bacteria species were isolated from apparently healthy and clinically unhealthy Red Junglefowls (Table 3). The most important bacteria species isolated were *Escherichia coli* and *Pasteurella multocida* subspecies *multocida* that can cause respiratory disease in immunocompromised chicken. *E. coli* was isolated in both the apparently healthy and clinically unhealthy chicken. This is the main agent for the colibacilosis in fowls. In addition, as a normal flora of the upper respiratory tract, the bacteria is also an opportunistic pathogen. *E. coli* can flare up in immunocompromised chicken. In this present study, the change in weather could be the main factor for infection, since sampling was performed during the transition period from rainy to dry season.

Table 3: Bacteria isolates from sinuses and lungs of clinically unhealthy and apparently healthy Red Junglefowl.

Bacteria	Bacteria isolate Number (%)	
	Apparently healthy (n= 12)	Clinically unhealthy (n=44)
Staphylococcus species	2 (16.67)	11 (25)
Streptococcus species	0 (0)	1 (2.27
Bacillus cereus	1 (8.33)	0 (0)
Corynebacterium species	2 (16.67)	8 (18.18)
Neisseria species	0 (0)	3 (6.82)
Gallibacterium anatis bv. haemolyticatis	1 (8.33)	3 (6.82)
Pseudomonas aeruginosa	1 (8.33)	3 (6.82)
Avibacterium gallinarum	0 (0)	3 (6.82)
Proteus mirabilis	0 0	2 (4.55)
Avibacterium avium	2 (16.67)	4 (9.09)
Escherichia coli	1 (8.33)	1 (2.27)
Pasteurella multocida subsp. *multocida*	1 (8.33)	2 (4.55)
Pasteurella sp/A	0 (0)	2 (4.55)
Aeromonas species	0 (0)	1 (2.27)
Pasteurella haemolytica	1 (8.33)	0 (0)
Total	**12 (100)**	**44 (100)**

In the study, *P. multocida* was isolated from both the apparently healthy and clinically unhealthy Red Junglefowls. *P. multocida* is also an opportunistic pathogen and residents of the mucous membrane of upper respiratory tract and lower genital tract. The bacteria can cause subacute respiratory tract infection causing rales and mucopurulent nasal discharge (McVey *et al.,* 2013). Our result was consistent with the signs shown in the clinically unhealthy chicken. Healthy chickens that do not show clinical signs can become carriers of *P. multocida*.

Other opportunistic bacteria isolated in the study that may cause respiratory infection were *Staphylococcus sp*, *Neisseria sp, Avibacterium gallinarum, Proteus mirabilis* and *Pseudomonas aeruginosa.* Only *Staphylococcus sp* and *P. aeruginosa* were isolated from both the apparently healthy and clinically unhealthy groups. *Staphylococcus sp, P. mirabilis* and *P. aeruginosa* are ubiquitous in environment.

In conclusion, various bacteria species were successfully isolated from the lungs and sinuses of. All bacterial isolates from the lungs and sinuses of the Red Junglefowl were opportunistic bacteria that can causae respiratory problems in immunocompromised chicken.

REFERENCES

Bisgaard M (1977). Incidence of *Pasteurella haemolytica* in the respiratory tract of apparently healthy chickens and chickens with infectious bronchitis: characterisation of 213 strains. *Avian Pathology*, 6(4): 285-292.

Glisson JR (1998). Bacterial respiratory diseases of poultry. *Poultry Science*, 77(8):1139-1142.

Jang SS, Biberstein EL, Hirsh DC (2004). A Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology. University of California, Davis.

Liau CB (1997). The bacterial flora of the upper respiratoty tract in budgerigars and peaceful doves in captivity. Master thesis, Universiti Putra Malaysia. http://psasir.upm.edu.my/12201/ *(*Accessed on 28 September 2016).

McVey DS, Kennedy M, Chengappa MM (Editors) (2013). Veterinary Microbiology*:* Avian species, (3rd Edition)*.* Wiley-Blackwell.

Sorum H and Sunde M (2001). Resistance to antibiotics in the normal flora of animals. *Veterinary Research*, 32(3/4): 227-241.

MYCOBACTERIUM AVIUM SUBSPECIES *PARATUBERCULOSIS* INFECTION IN DAIRY CATTLE AT UNIVERSITY AGRICULTURE PARK, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) infection also known as Johne's disease is viewed as one of the most serious and chronic bacterial diseases among ruminants that indirectly cause economic losses to the farmers. This study was conducted to determine the presence of MAP organisms and antibodies in dairy cattle. One hundred and twenty-nine faecal samples and 43 serum samples were obtained from 43 dairy cattle at University Agriculture Park (TPU), Universiti Putra Malaysia (TPU) for the detection of antigen and antibodies using Ziehl-Neelsen acid-fast stain and complement fixation test (CFT), respectively. Twenty-three (17.8%) of 129 samples were positive for MAP but none of the cattle had anti-MAP antibody. Adult cattle tended to shed higher (p=0.029) number of MAP in faeces. The study showed that there is no active infection in the cattle herd at TPU.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), University Agriculture Park, Universiti Putra Malaysia. Ziehl-Neelsen acid fast stain

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) that causes paratuberculosis or Johne's disease among domestic and wild ruminants (cervids) was first recognised by Johne and Frothingham in 1895 (OIE, 2014). This disease is characterised by intestinal granulomatous lesions that develop into chronic or intermittent diarrheoa, emaciation, and death (Stabel, 1998; Manning and Collins, 2010). Infected dairy cattle show reduction in milk production and increase in the incidence of mastitis (Larsen and Kopecky, 1970).

This study was conduct to evaluate the health of the animals with regards to notifiable (Department of Veterinary Services) at the TPU.

MATERIALS AND METHODS

Sampling of were done from 12th to 22nd January 2016 at University Agriculture Park (TPU), Universiti Putra Malaysia. Forty-three dairy cattle comprising of 14 apparently healthy young calves and 29 adult cattle were recruited for the study. Faecal samples (n=129) were collected by scrapping the mucosal part of the rectum. All faecal and blood samples were subjected to Ziehl-Neelsen acid-fast staining and complement fixation test (CFT) for antibodies againsat MAP, respectively. Fisher's Exact Test was used to determine significance differences among groups.

RESULTS AND DISCUSSION

Ziehl-Neelsen acid-fast staining result showed that only 23 (17.8%) of samples were positive for MAP (Table 1). Adult cattle tended to shed higher (p=0.029) number of MAP in faeces. This is due to MAP being a slow growing bacteria and cattle infected with MAP would only develop clinical signs later at 2 to 5 years of age.

Table 1: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in faeces of cattle at Taman Universiti Putra Malaysia.

Cattle	MAP Number (%)	
	Positive	Negative
Young	3 (2.3)	39 (30.2)
Adult	20 (15.5)	67 (51.9)
Total	**23 (17.8)**	**106 (82.2)**

Basically, the Ziehl-Neelsen acid-fast staining is a quick and low cost technique that can be used in the preliminary diagnosis of MAP infections (Collins, 2013). However, the Ziehl-Neelsen staining does not provide species identification or differentiate mycobacteria species (OIE, 2014). Furthermore, the test can give a false-positive results. However, using this test it was shown that MAP was present in 49.3% of samples from clinically affected cattle and 19.3% from subclinical cases. Due to the poor detection rate using this test, the validity of the result must be ascertained by isolation and identification of MAP in faeces.

Anti-MAP antibody was not detected by the CFT. The failure to detect antibodies can be attributed to the stage of MAP infection. At stage I or II the concentration of antibodies is too low for detection. This is complicated by the fact that serology test is not sufficiently sensitive to detect the low circulating antibody titres (Rice *et al*., 1958). The study indicated that there was no active MAP

infection in TPU. Unfortunately, this result is inconclusive because of the insensitivity of the techniques used in the study. However, faecal culture can be used as complementary test in the detection of MAP, especially in subclinical animals.

REFERENCES

Collins MT (2013). Overview of paratuberculosis. http://www.merckvetmanual.com/mvm/generalized_conditions/paratuberculosis/overview_of_paratuberculosis.html (Accessed on 25 April 2016).

Larsen AB and Kopecky KE (1970). Mycobacterium paratuberculosis in reproductive organs and semen of bulls. *American Journal of Veterinary Research*, 31(2): 255-258.

Manning EJ and Collins MT (2010). Epidemiology of paratuberculosis. In: Paratuberculosis: organism, disease, control. Berhs MA, Collins DM, (Editors), 1st Edition, Cambridge: CAB International. p22.

Rice CE, Konst H, Smith AN (1958), Studies of Johne's disease in Canada III. Diagnostics complement-fixation tests. *Canadian Journal Comparative Medicine* 22(7): 249-254.

Stabel J (1998). Symposium: Biosecurity and Disease. *Journal of Dairy Science*, 81(1): 283-288.

A RETROSPECTIVE STUDY ON ANAEMIA IN CATS PRESENTED TO THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA IN 2015.

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ABSTRACT

The life-span of cat erythrocytes at approximately 73 days is shorter than of dogs. Thus, cats are more prone to develop anaemia than dogs. In fact, anaemia is one the most common condition among cats referred to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). However, no study has been done to characterise the type of anaemia in cats referred to UVH. Thus, the objective of this study is to determine the classification and aetiologies of feline anaemia cases in UVH, with respect to bone marrow response. Retrospective clinical records from year 2015 were obtained and analysed. There were 162 anaemic cats, of various ages, of which 55.56% (n=90) and 44.44% (n=72) had non-regenerative and regenerative anaemia, respectively. Among these cats, 65 (40.12%) were kept indoors, 59 (36.42%) were semi-roamers, and 38 (23.46%) were outdoor cats. Among vaccinated and dewormed cats, 13 (52%) cats had non-regenerative anaemia while 12 (48%) had regenerative anaemia. Among 52 cats infested with fleas, 69.2% were diagnosed with haemotropic mycoplasma. Of these 52 cats, 81 and 19% had regenerative anaemia and non-regenerative anaemia, respectively. The most common clinical signs observed in anaemic cats were dehydration, pallor, distended abdomen, and jaundice. The most common causes of anaemia in cats were infectious diseases, traumatic injury, and metabolic disorders. Sixty percent (n=18) of cats positive for either FeLV or FIV or both had non-regenerative anaemia while 40% (n=12) had regenerative anaemia. There was no significant ($p>0.05$) association between gender, vaccination, deworming, age, or management and the regenerative status of anaemia. However, there was a significant ($p<0.05$) positive association between flea infestation, FeLV and FIV infections and the regenerative status of anaemia in cats.

Keywords: anaemia, non-regenerative, regenerative, cats, aetiology, University Veterinary Hospital, Universiti Putra Malaysia

INTRODUCTION

Anaemia is the reduction to below normal in the total erythrocyte (RBC) count, packed cell volume (PCV), or haemoglobin (Hb) concentration that consequently decrease oxygen-carrying capacity and delivery to tissue (White and Reine, 2009). There are two types of anaemia, which are regenerative and non-regenerative anaemia. Regenerative anaemia is characterised by reticulocytosis typically in blood loss (haemorrhage) or destruction (haemolysis) of erythrocytes in the circulation. On the other hand, anaemia without reticulocytosis is referred to as non-regenerative anaemia due to diminished erythropoiesis in impaired bone marrow erythrocyte production from whatever cause (Tvedten, 2010).

At present, the classification and aetiologies of anaemia in cats referred to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM) is not well-described. Therefore, the objective of this study was to determine the classification and aetiologies of anaemia in terms of regeneration status in anaemic cats. The information gained would be useful for the clinicians to understand the common causes of anaemia and the typical laboratory findings of cats with anaemia to assist them in the treatment of feline diseases.

MATERIALS AND METHODS

Anaemic patients were identified retrospectively from the 2015 records, at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, UPM. These patients were categorised based on the bone marrow response. The medical records of these cases were obtained from the University Veterinary Hospital, UPM. Data such as the patient signalment, clinical signs, physical examination findings, FeLV and FIV test results and final diagnosis of anaemia were collected. The final diagnosis of anaemia were categorised into the DAMNITV classification (Korman *et al*., 2013) and analysed by SPSS 21.0. The association between the physical examination findings, patient signalment and aetiologies of anaemia with the bone marrow response were analysed using Pearson Chi-square Test.

RESULTS AND DISCUSSION

Descriptive data

One hundred and sixty-two anaemic cats were identified in the year 2015 of which 61 were female and 101 male cats. Among these cats, 44.44% (n=72) had regenerative while 55.56% (n=90) had non-regenerative anaemia. The cats were managed indoors (40.12%, n=65), as semi-roamers (36.42%, n=59), and outdoors (23.46%, n=38). Among the 162 anaemic cats, the highest frequency were cats aged 7 months to 2 years old.

Regenerative anaemia, clinical signs, and physical examination

Cats with non-regenerative anaemia mostly showed distended abdomen and dehydration while regenerative anaemia mostly jaundice and pallor. The clinical signs were compatible with the aetiology of anaemia. Distended abdomen was seen in cats with feline infectious peritonitis. Fifty-two cats were infested with ectoparasites, of which 10 had non-regenerative and 42 regenerative anaemia. The clinical signs associated with anaemia in the cat population in this study were vague and non-specific.

Diagnostic investigation

The frequency of anaemic cats with flea infestation and diagnosed/suspected with haemotropic mycoplasma infection was 36 (69.2%). Twenty-four cats were tested positive for either FeLV or FIV infection or both. Based on DAMNITV classification, the most common cause of anaemia in cats were infectious diseases, traumatic injury and metabolic disorders. Non-regenerative anaemia was most frequently caused by infectious diseases, followed in order by metabolic disorder and neoplasia while the most common causes of regenerative anaemia were traumatic injury and infectious diseases. There was no significant ($p>0.05$) association between gender, vaccination, deworming, age, or management and the regenerative status of anaemia. However, there was a significant ($p<0.05$) positive association between flea infestation, FeLV and FIV infections and the regenerative status of anaemia in cats. From the results, most FeLV- and/or FIV-positive cats had non-regenerative anaemia, confirming earlier findings (Stone and Freden 1990). Heavy flea infestation would cause haemorrhagic anaemia (Day and Kohn, 2012). In addition, flea infestations contribute to feline haemoplasma infections such as by *Mycoplasma haemofelis*, causing haemolytic anaemia (Woods *et al*., 2005).

REFERENCES

Day MJ and Kohn B (2012). *BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine*. 2nd Edition. British Small Animal Veterinary Association.

Korman RM, Hetzel N, Knowles TG, Harvey AM, Tasker S (2013). A retrospective study of 180 anaemic cats: features, aetiologies and survival data. *Journal of Feline Medicine and Surgery*, 5(2):81-90.

Stone M S and Freden GO (1990). Differentiation of anemia of inflammatory disease from anemia of iron deficiency. *The Compendium on Continuing Education for The Practicing Veterinarian,* 12(7): 963-966.

Tvedten H (2010). Laboratory and clinical diagnosis of anemia. WEISS, DJ; WARDROP, KJ Schalm's Veterinary Hematology. 6th Edition. Iowa: Blackwell Publishin. Pp152-161.

White C and Reine N (2009). Feline nonregenerative anemia: Pathophysiology and etiologies. *Compendium on Continuing Education for Practicing Veterinarian*, 31(7): E1-7

Woods JE, Brewer MM, Hawley JR, WisnewskiN and Lappin MR (2005).

Evaluation of experimental transmission of Candidatus *Mycoplasma haemominutum* and *Mycoplasma haemofelis* by *Ctenocephalides felis* to cats. *American Journal of Veterinary Research*, 66(6): 1008-1012.

TICKICIDAL PROPERTY OF TUBA ROOT WATER EXTRACT AND CITRONELLA OIL

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ABSTRACT

The Red Junglefowl (*Gallus gallus*) is susceptible to tick infestation. Tuba roots (*Derris elliptica)* and citronella plant (*Cymbopogon nardus)* have been used effectively as insecticides. This study was conducted to determine the effect of tuba root water extract and citronella oil on tick infestation in the Red Junglefowl. Tuba root was collected in Pahang, Malaysia, while citronella was obtained from the Institute of Bioscience, Universiti Putra Malaysia. The ticks, identified as of the *Haemaphysalis sp*., were collected from the ears and head of the Red Junglefowl a small reared in a free-ranging farm in Jenderam Ulu, Selangor, Malaysia. The tuba root suspension was boiled in water to obtain tuba root water extract. Fine chopped citronella plant was subjected to steam distillation to obtain the essential oil. In this study 100, 50 and 25% concentrations of the tuba root extract and citronella oil and combination of 1:1 ratio of extract to citronella oil at 100, 50 and 25% were used to determine their effect in causing 100% tick mortality. One millilitre of the extract or oil was added to the petri dish containing 10 live ticks. The time taken for 100% tick mortality was recorded. The results showed that both the tuba root water extract and citronella oil caused 100% tick mortality. However, there was no significant ($p>0.05$) difference in time taken for 100% tick mortality to occur between tuba root water extract and citronella oil treatments. There was also no significant ($p>0.05$) difference in efficacy between 100 and 50% of either the tuba root water extract or citronella oil. In conclusion, tuba root water extract and citronella oil alone and their combination have potential to be used as tickicides.

Keywords: tuba root water extract, citronella oil, tick, tickicide

INTRODUCTION

The Red Junglefowl (*Gallus gallus*) is susceptible to tick infestation. Ticks are parasitic arthropods of the class Arachnida, order of Acarina. *Haemaphysalis sp* that is commonly found in chicken comes from the family of Ixodidae. The medical and economic importance of ticks has long been recognised because of their ability to transmit diseases to humans and animals. Blood loss from heavy tick infestation causes anaemia and weight loss in animals. The saliva of the blood sucking arthropod has been identified as the source of toxins and allergens that can cause discomfort to the animal. Thus, controlling tick infestation in animal is one of the most important management procedures in farms to ensure animal health

Tuba roots (*Derris elliptica)* and citronella plant (*Cymbopogon nardus)* have been used effectively as insecticides. Tuba roots is poisonous to fishes and insects. The toxic properties of this plant are mainly due to rotenone (Evan, 2002), a selective, non-specific botanical insecticide with some acaricidal properties. The easiest method to extract rotenone is with water. Since rotenone is effective against the ticks, the plant extract can potentially be used as an acaricides to replace the current chemical-based acaricides.

Citronella oil is used in traditional medicine as an antiseptic, bactericidal, deodorant, tonic, weedicide, and acaricide. Citronella was shown to be an effective insect repellent (Ansari and Radzan, 1995). Thus, we believed that citronella can be used to control tick infestation in animals.

Therefore, this study was conducted to determine the effect of tuba root water extract and citronella oil and their combination on the ticks infesting the Red Junglefowl. It is hypothesised that treatments with tuba root water extract, citronella oil and their combinations cause fowl tick mortality.

MATERIALS AND METHODS

Sample collection

Tuba roots were collected from a district in Pahang whereas the citronella were obtained from the Institute of Bioscience, Universiti Putra Malaysia. The ticks were carefully collected from the comb, wattle, and inside the ears, using a fine forceps, of the Red Junglefowls reared in a small free-ranging farm in Jenderam Ulu, Selangor, Malaysia. Only ticks that were active with complete body structure were used in the study.

Sample preparation

Tuba roots (480 g) with some stem were used in the study. The roots and stems of the plant were thoroughly cleaned, cut into the smaller pieces, and mixed with 1 L distilled water. The suspension was heated to boiling for approximately 7 h to obtain the water extract. Stock tuba roots extract (100%) was the undiluted extract obtained after boiling the roots in water.

The citronella was subjected to steam distillation to obtain essential oil. The

citronella (2.3 kg) were thoroughly cleaned and cut into the smaller pieces. The chopped material consisting of leaves and the stem were placed in a flask and 6 L distilled water added. The suspension was boiled in a steam distiller for 7 h to produce approximately 15 mL of citronella oil. The stock citronella oil (100%) was the undiluted oil.

The concentration of tuba root extract and citronella oil used in this study were 100, 50, and 25%. To obtain the different concentrations, tuba root extract was diluted appropriately in distilled water and citronella oil in 30% ethanol. The tuba root water extract and citronella oil combination was obtained by mixing at 1:1 of either 100, 50 or 25% concentration of tuba root extract with citronella oil.

Experimental design

Ten live ticks were placed in each of 9 labelled petri dishes; 3 for tuba root extract, 3 for citronella oil and 3 for extract-oil combination treatments. One millilitre of either 100, 50, or 25% extract or oil or 100, 50, or 25% 1:1 extract-oil combination was added to the respective plates. The time taken for the ticks in each plate to reach 100% mortality was recorded.

RESULT AND DISCUSSION

The ticks used in the experiment were active and showed good motility before treatment with either tuba root extract or citronella oil. The motility of ticks gradually declined after treatment with the extract and oil and eventually all ticks died (Table 1).

Table 1. Time for 100% tick after treatment with tuba root extract and citronella oil.

Treatment	Concentration (%)			Mean± std. error
	100	50	25	
	Time for 100% mortality (hours)			
Tuba Root extract	4.7	5	11	$7.02^{a} \pm 0.70$
Citronella oil	1.2	0.89	1.4	$1.17^{b} \pm 0.05$
Tuba root extract-citronella oil Combination	1.2	1.1	1.3	$1.25^{b} \pm 0.07$

[a,b]Means within the same column with different superscripts are significantly different at $\alpha = 0.05$

There was no significant ($p>0.05$) difference between citronella oil and combination of tuba roots water extraction and citronella oil treatment in time taken to cause 100% tick mortality. With treatments, it took approximately 1 h for 100% tick mortality to occur. However, the ticks were comparatively more resistant to tuba root water extract alone compared with other treatments. In this case, it took 7 times longer for attainment of 100% tick mortality. Thus, this study showed that the most effective treatments to cause tick mortality are citronella oil alone and

combination citronella oil and tuba root water extract. A comparison between concentrations of the treatment versus 100% tick mortality was made (Table 2). The study showed that there was no significant ($p>0.05$) difference in time for 100% tick mortality between 100 and 50% concentrations of treatments. The time for attainment of 100% tick mortality after treatment with 100 and 50% treatment concentrations was approximately 2 h. Whereas it took approximately 4 h for 100% tick mortality to occur when they were treated with 25% concentration of extract, oil or their combination. Thus, the minimum effective concentration of tuba root water extract, citronella oil or their combination concentrations to cause 100% tick mortality was 50%.

Table 2. Relationship between times taken for ticks to reach 100% mortality among different concentration.

Concentration	Mean ± Std. error
100%	$2.41^{a} \pm 0.28$
50%	$2.34^{a} \pm 0.32$
25%	$4.70^{b} \pm 0.86$

[ab]Means in the same column with different superscripts and significantly different at $\alpha = 0.05$.

In our study, it was shown that the most effective treatment to cause 100% tick mortality are with citronella oil and tuba root extract-citronella oil combination. This study showed that tuba root water extract and citronella oil contain components that are effective tickicides. However, characterisation of the extract and oil was not done. Thus, it cannot be concluded as to which component of these compounds are responsible for causing tick mortality. Previous studies showed that the major component of *Derris elliptica* is rotenone (Sae-Yun *et al*., 2006). Rotenone is a respiratory enzyme inhibitor. Thus, it is possible the mortality of ticks caused by tuba root extract treatment is due to failure in this enzymatic reaction. The tickicidal effect of monoterpenes was in fact demonstrated in an earlier study on the southern cattle ticks, *Rhipicephalus (Boophilus) microplus* (Prates *et al.*, 1998) with citronellol be the most efficient compound, which is also one of the main components of citronella oil.

The components of tuba root extract and citronella oil, particularly rotenone, are quite toxic. Tuba roots can cause acute and chronic toxicity. Acute local exposure to tuba roots extract, among others, results in conjunctivitis, dermatitis, sore throat, and congestion. While, ingestion of rotenone produces effects ranging from mild gastric irritation to vomiting. Chronic toxicity of rotenone can manifest as growth retardation and vomiting. The toxic effect of Citronella, however, has not been conclusively demonstrated. Citronella has also been claimed to have carcinogenic properties through its methyleugenol content (Johnson *et al,* 2000). Others showed that large doses of citronella oil fed to rodents did not cause cancer

(National Pesticide Information Center). Thus, there is need for more extensive studies to determine the safety of citronella oil for consumption or therapeutic use in animals and humans.

CONCLUSION

The study showed tuba root water extract, citronella oil and the tuba root extract-citronella oil combination at concentrations of 100 and 50% cause 100% tick mortality. Thus tuba root water extract and citronella oil are effective in the control of tick infestation in Red Junglefowl.

REFERENCES

Ansari MA and Razdan RK (1995). Relative efficacy of various oils in repelling mosquitoes. *Indian Journal of Malariology*, *32*(3): 104-111.

Evans WC (2009) Trease and Evans Pharmacognosy, 16th Edition. London, WB Saunders. Pp510-511.

Johnson JD, Ryan MJ, Toft JD II, Graves SW, Hejtmancik MR, Cunningham ML and Abdo KM (2000). Two-year toxicity and carcinogenicity study of methyleugenol in F344/N rats and B6C3F(1) mice. *Journal of Agriculture and Food Chemistry*, 48(8): 3620-3632.

National Pesticide Information Center. Oil of citronella: General fact sheet. http://npic.orst.edu/factsheets/citronellagen.html. (Accessed on March 18, 2016).

Prates HT, Leite RC, Craveiro AA and Oliveira AB (1998). Identification of some chemical components of the essential oil from molasses grass (*Melinis minutiflora* Beauv.) and their activity against cattle-tick (*Boophilus microplus*). *Journal of the Brazilian Chemical Society*, 9(2): 193-197.

Sae-Yun A, Ovatlamporn C, Ithara, A, Wiwattanapatapee (2006). Extraction of rotenone from *Derris elliptica* and *Derris malaccensis* by pressurized liquid extraction compared with maceration. *Journal of Chromatograpy A*, 1125(2): 172-167.

PATHOGENICITY ASSESSMENT OF CELLULAR AND EXTRACELLULAR MEMBRANE PRODUCTS OF *AEROMONAS HYDROPHILIA* IN AFRICAN CATFISH *(CLARIAS GARIEPINUS)*

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ABSTRACT

African catfish is also a native fish species in African countries that has been introduced and commercially cultured in Malaysia This study aimed was to determine the pathogenicity of cellular (CMPs) and extracellular (ECPs) products of *Aeromonas hydrophilia* in the African catfish (*Clarias gariepinus)*. *A. hydrophilia* was cultured in Tryptic Soy Broth (TSB) and harvested by centrifugation to separate CMPs and ECPs. The CMPs and ECPs were injected into the fingerlings intraperitoneally, with PBS as control. Fish sera were collected on day 8 post-infection from the caudal peduncle vein and tested with agar gel precipitation test (AGPT) to detect antibodies against CMPs and ECPs. Lethargy, severe fin rot, corneal opacity, exophthalmia, eye-ball rupture and erosion of epidermis were noticed in fingerlings of the infected group. Based on cumulative mortality, CMPs were more virulent than ECPs. However, negative antibody-antigen reaction in the AGPT test indicated that the CMPs and ECPs did stimulate immunity in fish at day 8 post-infection. In conclusion, the CMPs and ECPs induce marked clinical signs and stimulate short-term immunity in the African catfish.

Keywords: *Aeromonas hydophilia*, cellular membrane products, extracellular membrane products, *Clarias gariepinus*, pathogenicity

INTRODUCTION

African catfish (*Clarias gariepinus*) is an important fish species in the aquaculture industry. The *Aeromonas* genus is a member of the *Aeromonadaceae* family and are gram-negative, motile, facultative anaerobe, non-spore forming and rod-shaped bacteria (Pridgeon and Klesius, 2011). *Aeromonas* strains are among the most important bacterial pathogens in aquatic animals (Shayo *et al*., 2012). This study aimed to obtain preliminary information on the virulence potential of *Aeromonas hydrophilia* in the African catfish by evaluating the clinical signs and host immune responses following incubation with CMPs and ECPs of the bacteria.

MATERIALS AND METHODS

Preparation of fish

One hundred and twenty of *C. gariepinus* fingerlings of lengths ranging from 3 to 8 inches were obtained from a commercial farm in Seri Kembangan, Selangor, Malaysia. The fingerlings were divided into three group; control, group A (CMPs treatment), and group B (ECPs treatment). Groups A and B each consisted of 5 tanks and with 5 fingerlings/tank with duplicates. The control group consisted of 2 tanks representing controls for groups A and B CMPs and ECPs treatment, respectively was done on 5 fingerlings/tank with duplicates. The fish was fed daily with 3% body weight commercial feed. The temperature, pH and salinity were kept constant. The fingerlings were bathed in 0.2% NaCl and acclimatized for 1 week prior to experimentation.

Preparation of inoculum

Tryptic soy broth (TSB)-cultured *A. hydrophilia* of optical density of 1.972 at absorbance of 600 nm was used to infect the African catfish. The broth culture was centrifuged at 1800 × *g* for 15 min to obtain the bacterial pellet or cellular (CMPs) and supernatant or extracellular (ECPs) bacteria products, which were finally serially diluted 10-fold that used for the treatments.

Experimental infection

Control group fingerlings were injected with 0.1 mL 0.85% normal saline intra peritoneally (i.p.). Group A and B fingerlings were treated with i.p injections of 0.1 mL ECPs and CMPs, respectively.

Blood sample collection

The blood was taken on day 8 post-inoculation via the caudal vertebral vein to obtain serum.

RESULTS

Clinical signs that appeared in the fingerlings 24 h following inoculation of bacterial products were lethargy, fin rot, reduced feed intake and reddening of skin. In morbid fish showed severe fin rot, barbell necrosis, ruptured orbit-induced blindness and corneal opacity due to exophthalmia in one or both eyes. Dermal lesions with focal haemorrhage and inflammation were also present in the bacterial product-treated fingerlings. However, there was no difference in the severity of signs in the fingerlings across treatments with various dilutions of CMPs and ECPs.

The percentage of survivability in the treatment groups were high. However, there was no significant ($p>0.05$) difference in fingerling survivability across treatments with various dilutions of ECPs and CMPs. The mortality pattern for treated fingerlings were random and not significant. The overall total mortality and survivability of fingerlings treated with CMPs were insignificantly ($p>0.05$) higher than those treated with ECPs. Hence, it could be concluded that the CMPs and ECPs causes similar pathogenic effect in the *C. gariepinus*.

The AGPT were negative for antigen-antibody reaction showing that at day 8 post-treatment with the bacterial products no immune response was mounted in the into the African catfish.

DISCUSSION

The fingerlings treated with CMPs and ECPs showed various clinical signs resembling the natural infection that began at 24 hour post-inoculation. This finding is in agreement with those observed by previous researchers (Bach, 1978; Huizinga, 1979). The experimental infection mimics the incubation period of the disease and ranged between 8 to 48 hours post-treatment with the bacteria. However, the incubation period of the disease varies among fish species and with state of disease resistance, environmental conditions, and season (Yardimci and Aydin, 2011). Total mortality of the fingerlings treated with CMPs was higher than those treated with ECPs. The result suggests that CMPs were more virulent than ECP because *A. hydrophilia* organism are more pathogenic to the African catfish than ECPs is toxic (Pridgeon and Klesius, 2011). The growth of *A. hydrophilia* is influenced by the environmental temperature and this may also affect the expression profile of the ECPs such as aerolysin (heat-labile haemolysin) (Deen *et al*., 2014). Thus, the bacterial products used in this study may not be pathogenic enough to induce mortality in the catfish fingerlings. The source environment of fingerlings could also cause variations in the effect observed while host resistance could also vary according to the immune status and species of fish. The method of bacteria culture in the experiment may most likely be different to the *in vivo* situation. Bacteria while in the host can modulate virulence factors in such a way that allows the pathogen to evade the defence mechanism of the fish. Furthermore, the strain of the *Aeromonas*, the infective dose and route of administration could also influence the pathogenicity of the bacteria (Anyanwu *et al*., 2015). Mortality rates in fingerlings can also be influenced by light intensity because it could affect water temperature. Low water temperature could decrease the immunoglobulin production fishes causing mortality since optimal immune response is dependent on fish body temperature, and this vary with species (Kreutz *et al*., 2014). In the present study, the immunogenicity test did not show antibody and antigen reaction in the treated fish at day 8 post-inoculation. Thus it is possible that the reaction of the fish immune response to the bacterial products was poor.

CONCLUSION

Typical clinical signs were observed in fingerlings inoculated with the CMPs and ECPs of *A. hydrophilia*. The study shows that CMPs is more virulent than ECPs to the African catsfish. The bacterial products were capable to induce clinical signs in fish but did not cause mortality. The mortality rates of fingerlings treated with bacterial products are highly associated with the stress-inducing factors such as temperature, water quality and handling.

REFERENCES

Anyanwu MU, Chah KF, Shoyinka VS (2015). Evaluation of pathogenicity of motile Aeromonas species in African catfish. *International Journal of Fisheries and Aquatic Studies*, 2(3): 93-98.

Yardimci B and Aydin, Y (2011). Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia *(Oreochromis niloticus). Ankara Âœniversitesi Veteriner Fakültesi Dergisi,* 58(1): 47-54. http://dergiler.ankara.edu.tr/dergiler/11/1446/16247.pdf

Deen AEN, Dorgham SM, Hassan HM, Hakim AS (2014). Studies on *Aeromonas hydrophila* in cultured *Oreochromis niloticus* at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. *World Journal of Fish and Marine Sciences*, 6(3): 233-240.

Kreutz LC, Pavan TR, Alves AG, Correia AG, Barriquel B, dos Santos ED, Barcellos LJG (2014). Increased immunoglobulin production in silver catfish (*Rhamdia quelen*) exposed to agrichemicals. *Brazilian Journal of Medical and Biological Research*, 47(6): 499-504.

Pridgeon J and Klesius P (2011). Virulence of *Aeromonas hydrophila* to channel catfish *Ictaluras punctatus* fingerlings in the presence and absence of bacterial extracellular products. *Diseases of Aquatic Organisms*, 95(3): 209-215.

Shayo SD, Mwita CJ, Hosea KM (2012). Virulence of *Pseudomonas* and *Aeromonas* bacteria recovered from *Oreochromis niloticus* (Perege) from Mtera hydropower Dam; Tanzania. *Annals of Biological Research*, 3(11): 5157-5161.

PREVALENCE OF *ASPERGILLUS* SP. CONTAMINATION IN COMMERCIAL POULTRY FEED AND POULTRY FEED INGREDIENTS

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ABSTRACT

Mycotoxins produced by fungus can occur naturally in feedstuffs and may endanger the health of livestock and man. Mycotoxins are converted to toxic metabolites when temperature and humidity are optimum for the growth of certain fungi on feedstuff while in the field, at transport and storage, or during processing and manufacturing. This study was conducted to determine the occurrence of *Aspergillus* sp. the producer of aflatoxin, contamination in poultry feed. Four brands of commercial broiler feed consisted of broiler starter and grower-finisher feeds and 10 feed ingredients were bought from several petshops in Bangi, Kajang and Serdang, Selangor, Malaysia. The fungal counts for all commercial brands and all feed ingredients were conducted and the results for the four feedstuff compared. Fungi were isolated from the all feed samples by culturing on sabouraud dextrose agar and identified macroscopically and microscopically after incubation for 1 week at room temperature. The results showed that *Aspergillus* sp. isolated from feed ingredients was highest in grade A corn (1.9×10^2 CFU/g) and soybean meal (0.7×10^2 CFU/g). Among commercial diets, Brand A had the highest fungal count (2.1×10^2 CFU/g) while Brand D broiler starter feed was negative for fungal count. For grower-finisher complete feed, Brand D had the highest fungal count (3.1×10^2 CFU/g) and Brand A had the lowest fungal count (0.3×10^2 CFU/g). The *Aspergillus* sp. contamination in commercial feeds ranged from 6 to 30% with an average of 21.2%; however *Aspergillus sp.*contamination in feed ingredients ranged from 12 to 30% with a mean value of 20.6%. The main *Aspergillus sp.*identified from the commercial feeds were *A. terreus* (30.3%) and *A. flavus* (27.3%). Among single feed ingredients, the *Aspergillus* sp. identified were *A. terreus* (29.4%) and *A. flavus* (23.4%). The results suggested that almost all feeds in this study had some degree of *Aspergillus* sp. contamination and this is a health hazards concern. Poultry feeds should be periodically examined for contamination to ensure safety for consumption and optimal growth and production performance.

Keyword: contamination, commercial diet, feed ingredient, *Aspergillus*, poultry

INTRODUCTION

Poultry sector is the biggest component of livestock industry in Malaysia supplying about 81% of total meat and almost 111% egg of domestic market demand. These poultry products area also exported mainly to Singapore, Brunei, Hong Kong and Japan (Malaysian Livestock Breeding Policy 2013). Poultry health must be well maintained to prevent outbreak of disease that may cause dire consequences towards production and human health. Feed with adequate amounts of the necessary nutrients are important for poultry consumption (Chiba, 2014). Commercial feeds from reliable companies and retail stores contain balanced feeds with nutrients in right proportions for optimal poultry growth and production.

Poultry feed may serve as carriers for a wide variety of animal and human pathogens. Poultry feed component of plants and animal origin are often contaminated with microorganisms, mostly bacteria and fungi. The number and types of contaminating microorganisms vary according to function of materials, origin, climatic conditions, harvesting, processing method, storage transport technologies employed and packaging materials (D'Mello, 2006).

Some *Aspergillus* develops and sporulates easily in contaminated feedstuffs under poor quality bedding and environment, and poor hygiene of indoor farm environments. Inadequate ventilation and dusty conditions increase risk of bird exposure to aerosolised spores (Arné *et al*, 2011). Contamination of poultry feed with this toxigenic fungus under favourable conditions may lead to mycotoxin build-up in feeds and feed ingredients, reaching injurious levels for farm animal and human health (Saleemi, 2010).

This study focused on the determination of quality of commercial poultry feeds and feed ingredients obtained from several pet shops in Malaysia by determining the level of *Aspergillus sp.* contamination.

MATERIALS AND METHODS

Preparation of poultry feed

Four brands of commercial broiler feed and 10 single feed ingredients were purchased from several pet shops in Serdang, Bangi, and Kajang, Selangor, Malaysia. The commercial feeds for starters, growers, and finishers were stored in a dry room with unregulated temperature and humidity for several days before commencement of the project.

Analysis of poultry feed

Sampling was performed manually from the bags of feed by collecting from different parts of the bags. One kg of feed samples was thoroughly mixed from which 10 g of feed sample was taken for analysis and identification of *Aspergillus* sp. The feed samples were added into 90 mL of sterile distilled water in a stomacher bag and mixed thoroughly with the stomacher for 120 second 0.1 mL of each mixture was inoculated into a labelled plate containing sabouraud dextrose

agar, a selective medium for growth of *Aspergillus* sp. The inoculated plates were then incubated at room temperature for at least one week (Habib *et al*., 2015)

Identification of Aspergillus sp.
The macroscopic morphology of the fungal growth was identified from the plate (Habib *et al*., 2015). A small suspected fungal colony was picked from the medium and placed on a slide using a sterilised platinum inoculating pin. One to two drops of lactophenol cotton blue was placed on the clean glass slide and covered with coverslip. The coverslip was slightly pressed with the tip of the finger to expel any air bubble. The slides were observed under ×10 magnification for morphological assessment and identification of the fungus. Total fungal counts were compared among 4 commercial feeds and feed ingredients.

RESULTS AND DISCUSSION

The fungus identified from the commercial feeds were *Penicillium sp., Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergilllus terreus, and Mucor* sp. This study showed 63.6% of the fungus in commercial feeds were *Aspergillus* sp. The total *Aspergillus sp.* fungal count in starter commercial feed was the highest in Brand A with 2.1×10^2 CFU/g while no fungus was isolate from Brand D starter feed. The total *Aspergillus sp.* fungal count in grower finisher commercial feed was the highest in Brand D with 3×10^2 CFU/g and lowest in Brand A grower-finisher feed with 0.3×10^2 CFU/g.

Table 1: Fungus isolated in commercial poultry feed

Fungus	%
Penicillium sp.	33.3
Aspergillus terreus	30.3
Aspergillus flavus	27.3
Aspergillus sp.	6
Mucor sp.	6
Total	**100**

The high fungal counts in broiler starter and broiler grower finisher feed samples confirms the findings of Salanitro *et al*., (1974). Among feed ingredients, the rate of *Aspergillus* sp. Isolation was 82.3%. The highest fungal counts were for *Aspergillus terreus* (29.4%) and *Aspergillus flavus* (23.5%). Grade A corn had the highest *Aspergillus* sp. count of 1.9×10^2 CFU/g, while wheat was negative for *Aspergillus* sp. Soybean meal had the highest *Aspergillus sp.* at 0.7×10^2 CFU/g, while fishmeal and palm kernel cake were negative for *Aspergillus sp.*

Table 2: Fungal isolated from feed ingredients.

Fungus	%
Aspergillus terreus	29.4
Aspergillus flavus	23.5
Aspergillus niger	17.6
Aspergillus fumigatus	11.8
Penicillium sp.	11.8
Mucor sp.	5.9
Total	**100**

Microbial contamination of poultry feeds of plant and animal origin may be due to climatic conditions, harvesting, processing, storage and transport technologies employed (D' Mello, 2006). In this study, a large proportion of samples were contaminated with fungi. Most *Aspergillus sp.* isolated from the feed are similar to that shown by others (Arotupin *et al*., 2007; Saleemi, 2010; Habib *et al.,* 2015). For feed ingredients, *Aspergillus sp.* isolation was the most in corn and soybean meal. Negative isolation of *Aspergillus sp.* in feed ingredients is suggested to be due to small sample size.

To overcome *Aspergillus sp.* contamination, effective prevention and control measures should be undertaken to include appropriate hygiene and strict biosecurity programmes. To ensure reliability of the results of this kind of study the sample size should be larger and study should include determination of aflatoxin in the feed samples.

CONCLUSIONS

The study suggests that most of the feed ingredients are contaminated with *Aspergillus* sp. The broiler grower finisher feed contains twice the *Aspergillus* sp. count of the starter feed. It is recommended that all feedstuff should be periodically examined for ensure safety and meets the need for optimal performance in poultry production.

REFERENCES

Arotupin DJ, Kayode RMO, Awojobi KO (2007). Microbiological and physicochemical qualities of selected commercial poultry feed in Akure, Nigeria. *Journal of Biological Sciences,* 7(6): 981-984.

D'Mello JPF (2006). Microbiology of animal feeds. http://www.fao.org/docrep/article/agrippa/556_en.htm (Accessed on 7 June 2016).

Habib MA, Abdu P, Kwanashie CN, Kabir J, Negedu A. (2015). Isolation and identification of *Aspergillus* species from poultry feeds in Kaduna State,

Nigeria. *Net Journals,* 3(2):27-32.
Chiba LI (2014). Poultry nutrition and feeding. Animal nutrition handbook. Pp410-425. http://www.ag.auburn.edu/~chibale/an12poultryfeeding.pdf (Accessed on 7 June 2016).
Malaysian Livestock Breeding Policy 2013. Department of Veterinary Service, Malaysia, 42 pages. http://www.dvs.gov.my/dvs/resources/user_1/DVS%20pdf/Livestock_Breeding_Policy.pdf (Accessed on 7 June 2016).
Saleemi MK (2010). Mycobiota of Poultry Feed, Feed Ingredients and Pathological Effects of *Aspergillus* Fungi in Chicken Embryos. PhD dissertation, University of Agriculture, Faisalabad, Pakistan. http://prr.hec.gov.pk/Thesis/626S.pdf (Accessed on 7 June 2016).
Salanitro JP, Fairchilds IG, Zgornicki YD (1974). Isolation, culture characteristic and identification of anaerobic bacteria from chicken cecum. *Applied Microbiology,* 27(4): 678-687.
Arné P, Thierry S, Wang D, Deville M, Le Loc'h G, Desoutter A, Féménia F, Nieguitsila A, Huang W, Chermette R, Guillot J (2011). *Aspergillus fumigatus* in Poultry. *International Journal of Microbiology*, 2011: Article ID 746356: 14 pages.

BLOOD FATTY ACID IN CAPTIVE ESTUARINE CROCODILE (*CROCODYLUS POROSUS*)

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ABSTRACT

The state of fatty acid metabolism in crocodiles may reflect its nutrition management status. In this study, 5 male estuarine crocodiles, *Crocodylus porosus,* feed 80 kg of commercial chicken meat twice a week were selected based on their similarity in management. The fatty acid profiles of these crocodiles were analysed based on their dietary management especially on essential fatty acids. Heparinised blood samples were collected from ventral caudal vein and centrifuged to obtain plasma. The fatty acids determinations in plasma and feed samples were by total lipid extraction, fatty acid methyl esters (FAME) preparation, and gas chromatography. Polyunsaturated fatty acids (PUFAs) was the dominant fatty acid followed by saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). Linoleic acid (18:2 n-6) composition in the plasma of *C. porosus* may be influenced by chicken meat diet that had a high composition of linoleic acid (18:2 n-6). The arachidonic acid (20:4 n-6) composition in the plasma was 3.81% and feed was 0.73%. It is suggested that fatty acid profile in the plasma of *C. porosus* in this study was influenced by several factors, to include the diet and body fatty acid metabolism.

Keywords: estuarine crocodile, dietary management, fatty acid profiles, polyunsaturated fatty acids (PUFAs)

INTRODUCTION

In Malaysia, estuarine crocodiles (*Crocodylus porosus*) farming is primarily for production of superior leather. However, diet management of crocodiles kept in captive is problematic. In the last 50 years, few studies were done on crocodile nutrition and lipid composition and metabolism (Morpurgo *et al*., 1993). In Malaysia, the common diet of captive crocodiles is commercial chicken meat given once or twice a week.

According to Garnett (1985), in young *C. porosus,* docosapentaenoic acid (20:5

n-3) and docosahexanoic acid (22:6 n-3) may be the essential fatty acids. That study showed that the requirements and metabolism of fatty acids in reptiles is different from that of mammals. Research on nutrition of crocodiles is becoming more important with the increasing number of farmed crocodilian either for conservation or commercial purposes.

In this study, plasma fatty acid composition of adult *C. porosus* was analysed to determine the plasma fatty acid profile of captive estuarine crocodile fed commercial chicken meat.

MATERIALS AND METHODS

Animal husbandry

The estuarine crocodiles of the Sarang Buaya, Pasir Gudang, Johor, Malaysia (latitude 1.44249°N, longitude 103.99933°E) were used in this study. Five male crocodiles housed together with 7 females were chosen based on their similarity in management. The crocodiles were fed a total 80 kg of commercial chicken meat fed twice in a week.

Blood collection

Blood samples were collected from the ventral caudal vein using 18G spinal needle fitted to a 10 mL plastic syringe. The skin area for blood collection was first swabbed with 70% alcohol. About 10 mL of blood was collected and transferred to 6 mL lithium heparin tubes, centrifuged, and plasma collected for fatty acid analysis.

Plasma fatty acid profile determination

Fatty acid profile determination consisted of 3 parts. First was total lipid extraction that comprised of extraction of total fatty acids from plasma and feed using chloroform and methanol. Second, transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) were carried out using 20% methanolic boron trifluoride (BF3). Third, identification of fatty acid profiles done by gas liquid chromatography by comparing relative FAME peak retention times to standards (Sigma, USA).

RESULTS AND DISCUSSION

The mean total fatty acids in plasma of *C. porosus* is shown in Table 1. High composition of ω-6 PUFA in the *C. porosus* is mainly due to the high commercial chicken diet. Similar results also were demonstrated in another study on captive *C. niloticus* (Osthoff *et al*., 2010). The high ω-6 PUFA is believed to be due to linoleic acid (18:2 n-6) from high chicken diet. Arachidonic acid (20:4 n-6) was found to be at 3.81% in the plasma of the crocodile, while its chicken meat diet composition was 0.73%. Garnett (1985) similarly showed that starved *C. porosus* had a high

7.31% plasma arachidonic acid. The high composition of arachidonic acid (20:4 n-6) in *C. porosus* plasma compared to that of the commercial chicken meat diet is an intriguing issue. This phenomenon suggests that *C. porosus* has the ability to synthesise its own arachidonic acid (20:4 n-6) from the precursor linoleic acid (18:2 n-6). It seems that arachidonic acid (20:4 n-6), a long-chain PUFA, was further synthesised in crocodiles suggesting that they have the ability to convert short-chain to long-chain PUFAs.

Table 1. Mean total fatty acids in plasma of *C. porosus*

Fatty acids	Mean (%)
Myristic acid (14:0)	1.31
Palmitic acid (16:0)	24.86
Palmitoleic acid (16:1 n-7)	1.16
Stearic acid (18:0)	9.51
Oleic acid (18:1 n-9)	12.00
Linoleic acid (18:2 n-6)	10.13
α-Linolenic acid (18:3 n-3)	2.59
Arachidonic acid (20:4 n-6)	3.81
Eicosopentaenoic acid (20:5 n-3)	1.42
Docosapentaenoic acid (22:5 n-3)	3.11
Docosapentaenoic acid (22:5 n-6)	1.45
Docosahexanoic acid (22:6 n-3)	27.70
Total Saturated Fatty Acid	36.59
Total Unsaturated Fatty Acid	63.40
Total MUFA	13.16
Total PUFA n-3	34.83
Total PUFA n-6	15.41
Ratio n-6:n-3	0.45
Ratio UFA : SFA	1.75
Ratio PUFA : SFA	1.39

The omega-3 polyunsaturated fatty acid (ω-3 PUFA) composition in the plasma of *C. porosus* in this study was 34.83%. The contributing fatty acid was docosahexanoic acid (22:6 n-3), which was at 27.70%. However, the concentration of α-Linolenic acid (18:3 n-3), the precursor for docosahexanoic acid, was 2.59%. It is suggested that plasma composition of docosahexanoic acid (22:6 n-3) in estuarine crocodile is governed by species and genetic factors.

CONCLUSION

In conclusion, the dominant fatty acid in the plasma of *C. porosus* fed commercial chicken meat is polyunsaturated fatty acid. Commercial chicken meat diet influences the composition of linoleic acid in the plasma of *C. porosus*. The *C. porosus* seems to have the ability to convert short-chain to long-chain polyunsaturated fatty acids.

REFERENCES

Garnett S (1985). Fatty acid nutrition of the estuarine crocodile, *Crocodylus porosus*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. 81(4):1033-1035.

Morpurgo B, Robinzon B, Lance, VA, Gelman A (1993). Plasma fatty acidcomposition in wild and captive Nile crocodile, *Crocodylus niloticus*. *Comparative Biochemistry and Physiology Part A: Physiology,* 104(2): 373-376.

Osthoff G, Hugo A, Bouwman H, Buss P, Govender D, Joubert CC, Swarts JC. (2010). Comparison of the lipid properties of captive, healthy wild and pansteatitis-affected wild Nile crocodiles (*Crocodylus niloticus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 155(1):64-69.

PATHOGENICITY OF EXTRACELLULAR AND CELLULAR MEMBRANE PROTEINS OF *STREPTOCOCCUS AGALACTIAE* AND THEIR IMMUNOMODULATORY EFFECTS IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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ABSTRACT

Cellular membrane proteins (CMPs) are bacterial surface proteins that could be a source of immunogens while the extracellular protein (ECPs) are bacterial secretory products that can activate host immune response. Currently, there is a dearth of information on the immune response of catfish towards ECPs and CMPs. The objective of this study was to evaluate the *in vivo* effect of the ECPs and CMPs of *Streptococcus agalactiae* on the immune response of African catfish (*Clarias gariepinus*) fingerlings. The ECPs and CMPs of *S. agalactiae* were intraperitoneally (i.p.) injected into the African catfish fingerlings. Pure culture bacteria were harvested and the ECPs and CMPs were obtained by centrifugation. The ECPs and CMPs were diluted serial to concentrations of 10^{-1} to 10^{-4}. The morbidity, cumulative mortality, and percentage survivability of the fish were recorded within 7 days post-inoculation. Pooled serum samples from fingerlings of each dilution of ECP and CMP as well as control group were taken via the caudal peduncle vein at 8 spi. Clinicals signs observed include anorexia, lethargy, erratic swimming, corneal opacity and exopthalmia. The sera were tested for antibody by agar gel precipitation test. The results based on mortality showed that CMPs were more virulent than ECPs. However, statistically there was no significance ($p>0.05$) difference in the effect of ECPs and CMPs on the fingerlings. There was also no significant ($p>0.05$) difference between the dilutions of the inocula and the percentage survivability of fingerlings treated with ECPs and CMPs. ECPs and CMPs did form antigen-antibody complex at all dilutions.

Keywords: *Streptococcus agalactiae*, Extracellular Proteins (ECPs), Cellular Membrane Proteins (CMPs), African catfish (*Clarias gariepinus*), immunity

INTRODUCTION

Freshwater catfish production has the highest production compared to other freshwater fish species and it is a highly demanded freshwater food fish and

cultivar species in Malaysia. Therefore, infectious diseases caused by pathogenic organisms such as *Streptococcus agalactiae* are an important issue that has caused a lot of financial loss to the aquaculture industry (Smith *et al.*, 2003). *S. agalactiae* was reported to cause clinical signs such as erratic swimming, loss of appetite, exophthalmia and visceral cavity distension in infected fish. According to Song *et al*. (2013), the development of vaccines is one of the solutions for sustainable prevention and control this emerging disease. Extracellular proteins (ECPs) are extracellular secretory proteins which can activate the host's immune response since they are secreted from cells and are easily in contact with the host (Zhang *et al*., 2012). Cellular Membrane Proteins (CMPs) are surface proteins that are expressed by many *Streptococcus* strains and serve as targets for protective antibodies (Lancefield *et al*., 1975). Studies have been done on outer membrane protein as a source of immune protective immunogens, and recently there is increasing interests in extracellular secretory proteins since it easily activates host immune response (Zhang *et al*., 2012). Thus, this study was conducted to study the pathogenicity of the bacterial products and its effect as an immunomodulator in African catfish.

MATERIALS AND METHODS

This study used 120 fingerlings of *Clarias gariepinus*, 3 to 8 inches long, in duplicates. The fingerlings were acclimatised for one week prior to the experiment. Bacteria were recovered from stock agar and streak on tryptic soy agar (TSA) and incubated at 28°C for 24 hours. Purified and fresh bacteria colony was isolated for morphological characterisation using gram staining and were identified as *S. agalactiae* group B using commercial identification kits (BBL Crystal GP ID kit). The bacteria were inoculated into 10 mL tryptic soy broth (TSB) and incubated at 28°C in an incubator shaker (70 rpm) for 24 hours. Bacteria cultures were harvested from TSB by centrifuging at 1800 × *g* for 15 minutes. The supernatant contains ECP while the pellet contains the CMP.

The cell pellets were washed twice in phosphate buffered saline (PBS) by centrifugation at 1000 × *g* for 10 minutes. To obtain CMPs, the bacteria cells were boiled at 100°C for 10 minutes to kill the bacteria and break the cells. After cooling, the CMPs were pelleted again using centrifugation at 1000 × *g* for 10 minutes. Ten-fold serial dilutions were made to give 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} diluents. The supernatant was filter-sterilised using a 0.45 µm membrane filter. The ECPs were also diluted with a ten-fold serial dilution to give 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} diluents.

The fish were divided into three groups. Groups A and B of 50 fishes are the treatment groups while the control group had 20 fishes. The control group fingerlings were mock injected with 0.1 mL 0.85% normal saline intraperitoneally (i.p) while Group A fingerlings were treated intraperitoneally (i.p) with 0.1 mL of ECPs of *S. agalactiae*. Group B fingerlings were treated i.p with CMPs of the bacteria. The morbidity, mortality and percentage survivability of the fish were recorded for 7 days.

Blood serum samples were collected at day 8 post-inoculation from the caudal peduncle vein from the control and challenged fish. The immunogenicity of CMPs and ECPs antigen and the serum dilution from fish were determined using agar gel precipitation test (AGPT) following the Ouchterlony technique. The agar was then incubated in a moist container at room temperature for 24 hours. Precipitin lines were observed after 24 hours

Statistical analysis of the experimental data was performed using Kruskal-Wallis test (IBM SPSS version 20.0) and data comparison between ECPs and CMPs on mortality was analyzed using Chi-square test at a significance difference level of $p<0.05$.

RESULTS AND DISCUSSION

Clinical signs such as lethargy inappetence, erratic swimming, corneal opacity, exopthalmia, and caudal fin rot were observed in fish treated with ECPs and CMPs. These findings were in agreement with a previous study done by Abuseliana *et al.* (2011). The accumulated mortality was at 8.33% in CMPs group fingerlings compared to ECPs group fingerlings with a 6.67% accumulated mortality. Mortality was observed after 48 hours post-inoculation in the CMPs group while in the ECPs group mortality it occurred after 72 hour. However, statistically there was no significance difference between different bacteria products on mortality.

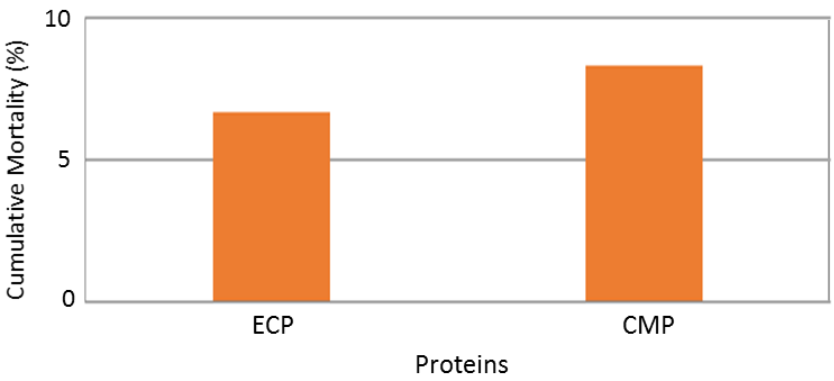

Figure 1: Graph shows relationship between ECPs and CMPs on cumulative mortality of fingerlings

There was variation in the pattern of mortality for both ECPs- and CMPs-treated ingerlings. Statistically there was no significance ($p>0.05$) difference between various concentrations of ECP and CMPs on survivability of the fish. This mortality pattern could be due to variation in responsed among fish response towards bacteria toxins. In this present study, the ECPs and CMPs may cause lethal toxicity in some fish. There is possibility that variation in light intensity that cause variation in water temperature may affect mortality pattern. In fact, a study done by

Avtalion *et al.* (1973) reported that primary antibody response is suppressed at low temperatures.

No AGPT precipitation lines were observed in our study suggesting that the antibody titres were low in the fingerlings. Antibody concentration and persistence in serum can differ according to species, age, sexual maturity and physiological events (Takahashi *et al*., 2012). It is also possible that the doses of bacteria used were too low to induce detectable immune response in the fish (Amrullah *et al.,* 2014).

CONCLUSION

In conclusion, clinical signs were observed in ECPs- and CMPs-treated fish similar to naturally infected fish but low mortality was recorded. Based on this study, the ECPs is a better immunostimulant than CMPs. Water temperature seems to also affect survivability and antibody production of the fish. No antibodies were detected in ECPs- and CMPs-treated fish by AGPT and this could be due to the low antigen dose and short duration of the study.

REFERENCES

Abuseliana A, Daud HM, Abdul Aziz S, Bejo S, Alsaid M (2011). Pathogenicity of *Streptococcus agalactiae* isolated from a fish farm in Selangor to juvenile Red Tilapia. *Journal of Animal and Veterinary Advances*, 10(7), 914-919.

Amrullah, Sukenda, Harris E, Alimuddin, Lusiastuti AM (2014). Immunogenicity of the 89 kDa Toxin Protein from Extracellular Products of Streptococcus in Oreochromis niloticus. *Journal of Fisheries and Aquatic Science*, 9(4), 176-186.

Avtalion RR, Wojdani A, Malik Z, Shahrabani R, Duczyminer M Lefler E, Katz E (1973). Influence of environmental temperature on the immune response in fish. *Current Topics in Microbiology and Immunology*, 61; 1-35

Lancefield RC, McCarty M, Everly WN (1975) Multiple mouse-protective antibodies directed against group B streptococci. Special reference to antibodies effective against protein antigens. *Journal of Experimental Medicine* 142(1): 165-179.

Smith VJ, Brown JH, Hauton C (2003). Immunostimulation in crustaceans: does it really protect against infection? *Fish & Shellfish Immunology* 15(1): 71-90.

Song M, Xie J, Peng X, Li H (2013). Identification of protective immunogens from extracellular secretome of *Edwardsiella tarda*. *Fish & Shellfish Immunology*, 35: 1932-1936.

Zhang M, Wu H, Li X, Yang M, Chen T, Wang Q, Liu Q, Zhang Y (2012). *Edwardsiella tarda* flagellar protein FlgD: A protective immunogen against edwardsiellosis. *Vaccine* 30(26): 3849-3856.

CUSTOMER SATISFACTION ON SERVICE QUALITY OF UNIVERSITY VETERINARY HOSPITAL FELINE SECTION, UNIVERSITI PUTRA MALAYSIA: APPLICATION OF SERVQUAL MODEL

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ABSTRACT

Service quality is an assessment of how well a delivered service fit to the customers' expectations. It is important to measure service quality to ensure delivered service meets customer satisfaction. Service quality can be measured using the SERVQUAL model. No study had been done to determine the quality of service offered by veterinary hospitals. This study was conducted to determine the service quality in the Feline section of University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM) and to identify areas that can be improved. This study was conducted at the Feline section over 4 weeks. Two sets of questionnaires based on the SERVQUAL model were given to the customers. The first set of questionnaire is on customers' expectations of veterinary hospitals, while the second set of questionnaire is on customers' perceptions of UVH. The SERVQUAL model measures service quality based on gap score (GS) of five dimensions which are tangibles, reliability, responsiveness, assurance and empathy. A gap score on each dimensions was computed based on the following formula; GS = Perception (P) – Expectation (E). Eighty-nine respondents participated in this study. Customers' perceptions do not meet customers' expectations in all five dimensions. There was a significant ($p < 0.05$) difference in mean of expectations and mean of perceptions in all dimensions except for empathy. The lowest gap scores were tangible (-0.176) and empathy (-0.140) dimensions. The highest gap scores were reliability (-0.494) and responsiveness (-0.431) dimensions. Although assurance dimension had a gap score of -0.281, service provided by UVH closely met the customers' high expectations in this dimension. The UVH should improve its reliability and responsiveness dimensions for better performances in service quality.

Keywords: service quality, customer satisfaction, SERVQUAL, cat

INTRODUCTION

The number of veterinary clinics in Malaysia are on the increase. To be competitive, veterinary clinics, like other customer-based services such as banking, retailing, hospitality, education, and healthcare must satisfy their customers. Service quality has been increasingly identified as the main factor in distinguishing between services and building competitive advantages (Wan Rashid and Jusoff, 2009). This could increase demand of the service provided and enable the business to grow. The most commonly used model to measure service quality is the SERVQUAL model developed in the eighties and aimed at measuring the scale of quality in the marketing service. In 1995, SERVQUAL was adapted to healthcare (Babakus and Mangold, 1992). Since then, many studies that had been done that adapt the SERVQUAL model in measuring service quality in hospitals and clinics.

MATERIALS AND METHODS

Sampling and data collection

A survey was conducted to measure service quality in the Feline Section of the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). This survey was conducted over a period of 4 weeks using a modified version of the SERVQUAL questionnaire that has been adapted for the marketing perspective of the hospital environment (Parasuraman *et al*., 1988; Babakus and Mangold, 1992). Two sets of questionnaires, customers' expectation and customers' perception, were given to the clients of the Feline Section of UVH. The first set of questionnaire determines customers' expectations of general service quality of UVH after registration of the case. The second set of questionnaire determines customers' perceptions on the quality of service provided after billing. Each questionnaire contained 15 pairs of statements representing the five SERVQUAL dimensions of service quality: tangibles, reliability, responsiveness, assurance, and empathy (Parasuraman *et al*., 1988).

Data Analysis

Data were prepared and edited in Excel® (Microsoft Corporation, Redmond). SERVQUAL measures service quality as the gap score between a customer's expectations for a service offered and the customers' perceptions of the service received. The gap score was computed using the following formula:

SERVQUAL gap score = perception score – expectation score.

All statistical analyses were performed using IBM SPSS statistics version 22. Shapiro-Wilk test was used to ascertain the normality of the dataset. Mann-Whitney U test was used to identify the mean difference between customers' expectations and customers' perceptions. Pearson correlation was used to determine relationship between continuous variables. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Eighty-nine respondents participated in this survey. The majority of respondents were females of Malay race. More than half of the respondents were employed and were categorised as high income per month (>RM5000).

Table 1: Mean expectations and perceptions and gap score by SERVQUAL dimensions.

Dimension	Expectations	Perceptions	Gap scores	Gap scores by dimensions
Tangibles	4.44	4.26	-0.18	- 0.176
Reliability	4.57	4.07	-0.49	- 0.494
Responsiveness	4.59	4.15	-0.43	-0.431
Assurance	4.69	4.44	-0.25	- 0.281
Empathy	4.56	4.43	-0.14	- 0.140

All values are means of 89 responses.

In the SERVQUAL dimensions, the larger the gap scores, the more the dissatisfied the respondent. Customers' perceptions do not meet customers' expectations on all dimensions as demonstrated by negative gap scores (Table 1). Negative gap scores indicate the customers were not satisfied with the service provided by UVH. Empathy and tangibles dimensions exhibited the smallest gap scores. However, these two dimensions had the lowest customers' expectations. This showed that, UVH closely met customers' expectations on the appearance of physical facilities, equipment, personnel and communication materials and effort by UVH to understand customers.

The largest gap scores were on reliability and responsiveness dimensions. These two dimensions represented the second and third highest customers' expectations. However, customers' perceptions were way below customers' expectations as shown by the large gap scores. This shows that UVH was under-performing in these two dimensions by not providing the promised service dependably and accurately and by not willing to provide all assistance and prompt service to customers. The hospital performed well in assurance dimension by achieving the highest perceptions and exhibited a small gap score. Thus, this dimension is UVH's strengths, showing that the employees were knowledgeable, courteous, and trustworthy.

CONCLUSION

This study revealed that customers' perceptions do not meet in all SERVQUAL dimensions as shown by negative gap scores. However, UVH performed well in the assurance dimension showing that UVH employees were knowledgeable, courteous and trustworthy. The hospital did not perform well in reliability and responsiveness

dimensions. Therefore, UVH should make all efforts to improve these dimensions of service to ensure client satisfactions.

REFERENCES

Babakus E and Mangold WG (1992): Adapting the SERVQUAL scale to hospital services: an empirical investigation. *Health Service Research,* 26(6): 767-786.

Anderson EA (1995): Measuring service quality in a university health clinic. *International Journal of Health Care Quality Assurance,* 8(2): 32-37.

Parasuraman A, Zeithaml VA, Berry LL (1988): SERVQUAL: A multiple- item scale for measuring consumer perceptions of service quality. *Journal of Retailing,* 64(1): 12-40.

Wan Rashid WE and Jusoff K (2009): Service quality in healthcare setting. *International Journal of Health Care Quality Assurance,* 22(5): 471-482.

CUSTOMER SATISFACTION ON SERVICE QUALITY OF UNIVERSITY VETERINARY HOSPITAL CANINE SECTION, UNIVERSITI PUTRA MALAYSIA: APPLICATION OF SERVQUAL MODEL

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ABSTRACT

Service quality is important in planning the market strategy and financial performance of a business. SERVQUAL instrument is commonly used to measure service quality by comparing the gap between customer expectations and perceptions across five dimensions of service quality: tangibles, reliability, responsiveness, assurance and empathy. This gap score indicates how well the service is performing towards fulfilling customer satisfaction. The aim of this study was to identify customers' expectations, perceptions, and satisfaction level on the quality of service provided by the University Veterinary Hospital (UVH) Canine Section. This study will enable UVH to identify areas requiring improvement for better quality service. Two sets of questionnaire containing 15 questions each; the first set concerns customers' expectations towards veterinary hospitals in general, and the second set concerns customers' perceptions of the service received in UVH, were distributed among clients of the Canine Section during a four-week period. Gap scores were computed based on the following formula: Gap Score (GS) = Perception (P) – Expectation (E). Seventy clients responded and the majority were working Chinese females with high income. Perceptions were significantly ($p < 0.05$) lower than expectations for all dimensions indicated by negative gap scores. The reliability dimension exhibited the biggest gap score of -0.66 while tangible and empathy dimensions had the lowest gap scores of -0.38 and -0.39, respectively.

Keywords: service quality, veterinary, SERVQUAL, customer satisfaction, dog

INTRODUCTION

The increase in pet companionship leads to increase in growth of pet-care services such as animal clinics and hospitals, pet shops, and pet-grooming centres (Euromonitor International, 2015). The increase in numbers of veterinary clinics in Malaysia shows that pet-owners are willing to spend money to ensure their companion animals are well-cared and get proper medical health care. Thus, these pet-owners tend to patronise the best healthcare services with high expertise in diagnosing problems faced by their pets while expecting quality service in veterinary clinics.

In order to match the expectation of good quality service, service businesses such as veterinary clinics and hospitals need to gauge the quality of services they are providing. This will enable them to identify the state of the business, whether it meets the customer needs and expectations or not. This will also allow them to determine where they stand among veterinary service providers in Malaysia, besides knowing the areas required for improvement (Anderson, 1995).

MATERIALS AND METHODS

Data collection

Data collection was done over a period of 4 weeks at the University Veterinary Hospital (UVH) Canine section, Universiti Putra Malaysia (UPM). Samples were collected via purposive sampling through customer interviews based on questionnaires, after their registration of case and after paying their consultation bills. Questionnaires used for the interview were both in English and Bahasa Malaysia. Two sets of questionnaires containing 15 statements each; with the first set containing statements to guage customers' expectation of a veterinary hospital in general and the second set containing statements to guage customers' perception of service received at UVH, based on the five dimensions of SERVQUAL. Gap scores define the customers' satisfaction levels and were computed based on the following formula: Gap Score (GS) = Perception (P) – Expectation (E).

Data analysis

Microsoft Excel® 2013 (Microsoft Corporation, Redmond) was used to collate and plotting of data. Data were analyzed using the IBM Statistical Package of Social Science (SPSS). Mann-Whitney test was used to compare the mean differences between perceptions and expectations, and Pearson's correlation was used to identify the relationship between two continuous variables. A $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Seventy clients responded and the majority were females of Chinese descendants. The clients aged between 20 to 70 years and more than 50% were married. The majority were working, either under employment or self-employed, and mostly with high incomes of >RM5,000/month. Approximately 50% of respondents were with high education and their expectations of the services provided by the hospital was presumed to be high.

The survey showed that among five dimensions of SERVQUAL, perceptions do not meet expectations as suggested by the Gap scores. Satisfaction level is inversely related to gap scores.

Reliability dimension exhibited the biggest gap score, responsiveness had the second biggest gap score and followed by assurance, while empathy and tangible exhibited the smallest gap scores (Table 1). Both male and female respondents showed similar trends across the five dimensions, however, females were less satisfied compared to males based on their gap scores.

There was no relationship between gap scores and the likelihood of the clients returning for UVH services. However, a trend showed by the small gap scores that clients tend to return to UVH for consultations for the dogs. Although perceptions do not meet expectations in the five dimensions of SERVQUAL, the majority of the customers thought that the UVH Canine Section provided good services. Since majority of clients reside in the Selangor area, UVH is their preferred professional veterinary service provider. The availability of veterinarians with practice specialties at UVH encourages repeat clients.

CONCLUSION

In conclusion, UVH do not generally meet customers' expectations. This is especially apparent in the reliability dimension.

REFERENCE

Anderson E (1995). Measuring service quality at a university health clinic. *International Journal of Health Care Quality Assurance*, 8:32-37.

Euromonitor International: Pet Care in Malaysia (August, 2015). http://www.euromonitor.com/pet-care-in-malaysia/report (Accessed on 20 February 2016).

SEMINAL CHARACTERISTICS OF GENETICALLY IMPROVED FARMED TILAPIA STRAIN TREATED WITH THE SPAWNING AGENT, OVAPRIM®

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ABSTRACT

Genetically Improved Farm Tilapia (GIFT) strain was developed through selective breeding of Nile tilapia and cultured for its ability to tolerate a wide range of water conditions. There are very few studies on the effects of spawning agent on seminal characteristics of the GIFT strain. This study was carried out to determine the effects of the spawning agent, Ovaprim®, on seminal characteristics of GIFT strain. The study also determined the optimum dose of Ovaprim® to be used for the GIFT strain. The fish, with body weights between 200 and 360 g, was divided into 4 treatment groups; Control (no treatment), and three groups treated with either 0.1, 0.3 or 0.5 mL/kg body weight of Ovaprim® intramuscularly. Volume of milt and sperm density, motility, viability and morphometry were evaluated. Fish treated with 0.5 mL/kg of Ovaprim® had the highest mean sperm density, motility, and viability showing that this Ovaprim® dose is optimum among doses tested for spawning and induction in GIFT.

Keywords: GIFT strain, spawning agent, Ovaprim®, milt, optimum dose

INTRODUCTION

Tilapia is a freshwater species of the family *Cichlidae* and is cultured for its hardiness and ability to tolerate a wide range of temperature and salinity levels. Nile tilapia was chosen for the breeding programme of Genetically Improved Farmed Tilapia (GIFT) strain (Asian Development Bank, 2005). In two recent studies, African catfish has shown success in increasing its sperm quality and quantity when spawning is induced with the synthetic agent, Ovaprim® (Kasi *et al.,* 2015; Gbemisola and Adebayo, 2014). In addition, Ovaprim® was also effective in inducing final maturation, increasing milt production and improving the fecundity of African catfish (Kasi *et al.,* 2015).

The objectives of the study are to compare the seminal characteristics of GIFT

strain treated with Ovaprim® and to establish the optimum Ovaprim® dose to be used in the GIFT strain.

MATERIALS AND METHODS

Fish management
Thirty GIFTs with body weights between 200 to 360g were procured from the Puchong Hatchery Unit, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The study was conducted at the Faculty of Veterinary Medicine, UPM. The fish were fed twice daily with commercial fish pellets. The Each fish was maintained in a 10-L tank and acclimatised for 2 days before experimentation.

Hormonal injection technique and milt collection
The fish were divided into four (4) groups according to treatment; Control (no treatment), three groups each treated with either 0.1, 0.3, and 0.5 mL/kg body weight of Ovaprim®. One dose of Ovaprim® was administered intramuscularly at the dorsal muscle of the fish above the lateral line but below the anterior part of the dorsal fin (Pao *et al.,* 1999), with a fixed latency period of 12 h. The fish were immobilized with 200 mg/L Tricaine Methane Sulfonate (MS-222) (Musa, 2010) prior to milt collection. The abdomen and urogenital papilla were dried and the abdomen pressed gently and massaged, milt collected into a sterile test tube, and stored in crushed ice at 4 °C (Musa, 2010; Muchlisin *et al.*, 2015).

Sperm parameters
Sperm density was determine using a haemocytometer. The sperms were activated by adding normal saline or freshwater in the ratio of 1:4 (Navarro *et al.,* 2014). To determine sperm motility, diluted sample (1:100) was pipetted onto a glass slide and motility scored. Sperm viability was determined by using modified eosin-nigrosin staining method (Memon *et al.,* 2012). The average survival percentage was obtained by counting a minimum of 100 sperms per slide (Noor-Hidayati *et al.,* 2014). Sperm morphology and morphometry were determined by image analysis (Moticam Pro 285A).

RESULTS AND DISCUSSION

Sperm parameters
Fish treated with 0.1 mL Ovaprimm/kg body weight yielded the highest mean milt volume after 12 h of latency period (Figure 1). However, 0.5 mL Ovaprim®/kg body weight gave the highest mean sperm density (Figure 2). There was no significant difference in milt volume and sperm density among Ovaprim® doses. This could be due to the 'thinning effects', in which the first injection of Ovaprim® induces a transient elevation of seminal plasma production with a much smaller increase in spermatozoa production (Clemens and Grant, 1965).

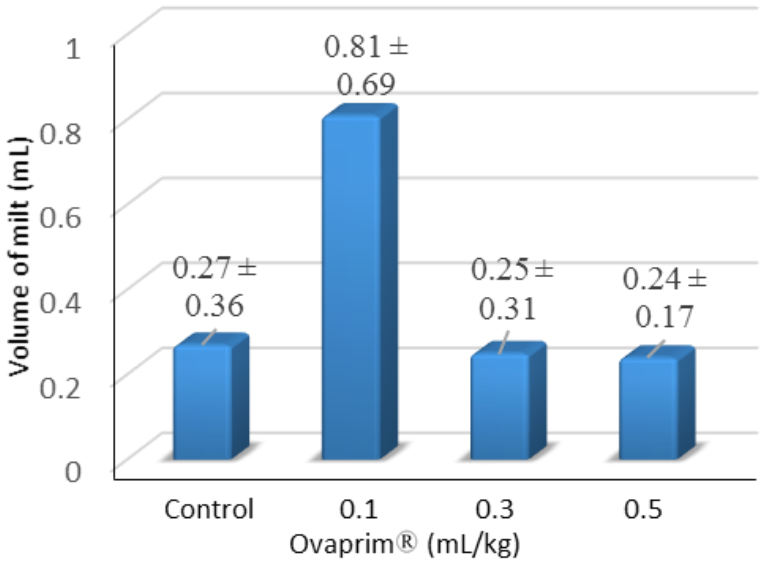

Figure 1: Effect of ovaprim® on milt volume of genetically improved farm tilapia.

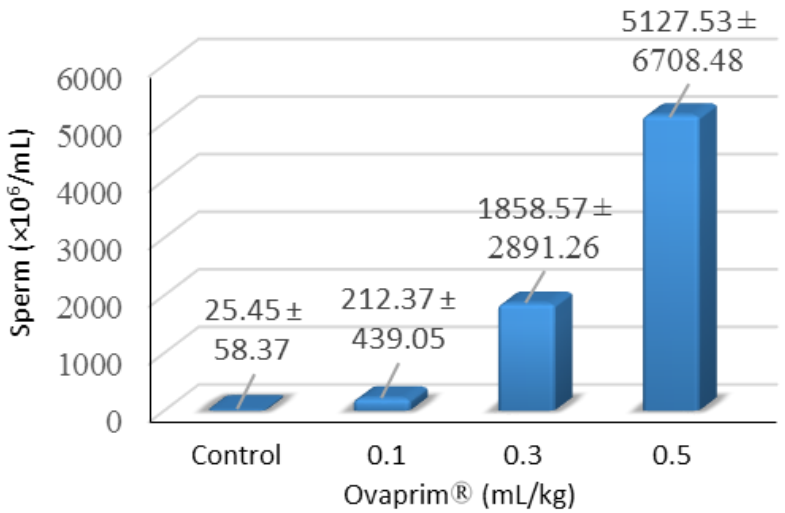

Figure 2: Effect of ovaprim® on sperm density of genetically improved farm tilapia.

Sperm parameters (sperm density, motility and viability) are tabulated in Table 1. Treatment group 0.5 mL/kg showed the highest mean sperm motility and viability compared with the other groups. However, there were no significance differences among the four treatment groups for sperm viability but there was significant difference in sperm motility. The sperm motility of GIFT strain was very short and lasted less than one minute. This finding was supported by Cosson *et al.* (1999), where motility decreased progressively. The optimum or recommended dose of Ovaprim® is 0.5 mL/kg because it gives the highest mean sperm density, motility and viability. Based on the study carried out by Mohd. Yusof (2008), the female tilapia was induced successfully with Ovaprim® at the dose of 1.0 mL/kg and males were given half the dose of females.

Table 1: Mean sperm motility and viability of GIFT injected with Ovaprim®

Parameters	Ovaprim® (mL/kg)			
	Control	0.1	0.3	0.5
Motility (%)	33.33 ± 27.87	35.83 ± 29.23	41.67 ± 31.89	75.50 ± 15.60
Viability (%)	55.47 ± 6.74	60.96 ± 15.28	69.67 ± 15.76	74.89 ± 13.10

Sperm morphology showed that the sperms had rounded heads with midpieces and tails. For sperm morphometry, there was no significant difference in sperm length among treatment groups. There were several sperm abnormalities such as tailless, coiled tail, and macrocephaly. However, the usage of normal saline gradually causes the tail of sperm to coil. Normal saline (osmolality < 160) causes the tail of sperm to gradually coil due to the swelling of tail plasma membrane. Then, it causes the flagellum to shorten with the occurrence of tight bending at the distal part of the flagellum. This leads to the formation of a full loop sperm and eventually causing the sperm velocity to decrease and stop when the flagellum is totally coiled (Perchec *et al.,* 1996). This process is known as osmotic trauma (Fauvel *et al.,* 2010).

REFERENCES

Asian Development Bank (2005). An impact evaluation of the development of genetically improved farmed tilapia and their dissemination in selected countries. Operations Evaluation Department, ADB, Manila, Philippines. https://www.adb.org/sites/default/files/publication/29623/ies-tilapia-dissemination.pdf (Accessed on 1 september 2016)

Clemens HP and Grant FB (1965). The seminal thinning response of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) after injections of pituitary extracts. *Copeia*, 2:174-177.

Cosson J, Billard R, Gibert C, Dreanno C, Suquet M (1999). Ionic factors

regulating the motility of fish sperm. In: The Male Gamete: From Basic Science to Clinical Applications. 1st Edition, Gagnon, C. (Editor), Cache River Press, Vienna. Pp 161–186.

Gbemisola OB and Adebayo OT (2014). Sperm quality and reproductive performance of male *Clarias gariepinus* induced with synthetic hormones (Ovatide and Ovaprim®). *International Journal of Fisheries and Aquaculture*, 6(1): 9-15.

Kasi M, Nirmell S, Aminur Rahman M, Arshad A, Gokul Raj M, Arockiaraj J (2015). Induced ovulation and spawning of African catfish *Clarias gariepinus* (Bloch) using Ovaprim®. *Journal of Environment & Biotechnology Research*, 1(1): 2-9.

Memon AA, Wahid H, Rosnina Y, Goh YM, Ebrahimi M, Nadia, FM. (2012). Effect of antioxidants on post thaw microscopic, oxidative stress parameters and fertility of Boer goat spermatozoa in Tris egg yolk glycerol extender. *Animal Reproduction Science*, 136(1-2): 55-60.

Mohd. Yusof MF (2008). Effects of Ovaprim® stimulation on egg production, hatching rate and fry survival rate in red tilapia (*Oreochromis niloticus*) (Doctoral dissertation, University of Malaya).
http://dspace.fsktm.um.edu.my/xmlui/handle/1812/491
(Accessed on 1 September 2016).

Muchlisin ZA, Nadiah WN, Nadiya N, Fadli N, Hendri A, Khalil M, Siti-Azizah MN. (2015). Exploration of natural cryoprotectants for cryopreservation of African catfish, *Clarias gariepinus*, Burchell 1822 (Pisces: Claridae) spermatozoa. *Czech Journal of Animal Science*, 60(1): 10-15.

Musa N (2010). Sperm activation in Nile tilapia (*Oreochromis niloticus)* and the effects of environmentally relevant pollutants on sperm fitness. PhD Thesis, Unversity of Stirling, UK.
http://dspace.stir.ac.uk/handle/1893/2310#.V-y_1ck8Z0B
(Accessed on 1 September 2016).

Navarro RD, Navarro FKSP, Felizardo VO, Murgas LDS, Pereira, MM. (2014). Cryopreservation of semen of Thailand tilapia (*Oreochromis spp*.) fed diet with different oil sources. *Acta Scientiarum Technology*, 36(3): 399-404.

Noor-Hidayati AB, Shahreza, MS, Abol-Munafi AB, Ikhwanuddin M (2014). Sperm quality assessment of Banana shrimp *Fenneropenaeus merguiensis* (De Man, 1888) from ultraviolet irradiation for initial development of gynogenesis application. *Journal of Fisheries and Aquatic Science,* 9(4): 187-196.

Pao, X, Kuanhong M, Jian Z, Jianxin, W, Yongseng G (1999). Comparative Studies on Spawning-Inducing Using Ovaprim® and Other Hormones. Freshwater Fisheries Research Center, Chinese Academy of Fishery Science, Wuxi, China.
http://www.syndelasia.com/pdf/resources/Comparative%20Studies%20on%20Spawning-inducing%20using%20Ovaprim%20and%20other%20hormones.pdf
(Accessed on 1 September 2016).

Perchec G, Cosson MP, Cosson J, Jeulin C, Billard R (1996). Morphological and Kinetic Changes of Carp (*Cyprinus carpio*) Spermatozoa after Initiation of MotiIity in DistiIled Water. *Cell Motility and Cytoskeleton*, 35(2): 113-120.

EFFECT OF *STREPTOCOCCUS INIAE* INOCULATION ON RED HYBRID TILAPIA

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ABSTRACT

Tilapia production is one of the most important and expanding aquaculture industries in Malaysia. The production of the fish can be adversely effected by environmental factors and infections. This study was aimed to describe the lesions and immunolocalisation of antigens in the organs of experimentally infected Red Hybrid Tilapia in presence or absence of stress factors. Fifteen Red Hybrid Tilapia fish in duplicates were inoculated intraperitoneally with 10^9 CFU/mL *Streptococcus iniae* diluted in PBS while another 15 served as controls. Clinical signs were recorded and samples from gills, brain, eyes and kidney collected. Each sample was subjected to bacterial culture and isolation and histopathology. Immunohistochemistry (IHC) and polymerase chain reaction (PCR) were done to detect antigen. There were no obvious sign or macroscopic finding in the fish during the 24-hour period post-*S. iniae* challenge. Intense IHC staining in blood vessel lumen and wall, macrophages in choroid, focal haemorrhages in the renal interstitium and meninges were among the lesions observed. In conclusion, stress Red Hybrid Tilapias are more likely to develop severe signs of *S. iniae* infection than those not stressed.

Keywords: Red Hybrid Tilapia, *Streptococcus iniae*, intraperitoneal, immunolocalisation, immunochemistry, polymerase chain reaction.

INTRODUCTION

Streptococcus iniae was first discovered on freshwater dolphin in Amazon (Pier and Madin, 1976), hence, the name '*iniae'* that was derived from the "Inia", a genus of the river dolphin in South America. Phylogenetically, *S. iniae* is closely related to *S. parauberis*, both being clustered with *S. agalactiae, S. dysgalactiae*, and *S. pyogenes* according to sequence similarity in their 16S rRNA gene (Facklam, 2002). Streptococcosis is a general name for a variety of diseases caused by Streptococcus. Some "strep" organisms may normally live on the body of humans and animals without causing disease. Initially, tilapias were regarded to be more resistant towards bacterial, fungal, viral and parasitic infections than other cultured

fish species (Amal *et al.*, 2011). However, recently tilapias have been found to be susceptible to these infections. In this study, the objectives were to determine the effect of *S. iniae* infection on the brain, eyes and kidneys of stressed Red Hybrid Tilapias.

MATERIALS AND METHODS

Fish and Fish Culture

Forty-five apparently healthy Red Hybrid Tilapia (*Oreochromis* sp.) weighing 10 ± 3 g were obtained from the Aquaculture Extension Centre (AEC), Department of Fisheries Malaysia, Bukit Tinggi, Pahang, Malaysia.

Bacterial Culture

S. iniae in nutrient agar was subcultured onto the blood agar and then onto the brain heart infusion broth (BHIB). The final concentration of live *S. iniae* used for inoculation was adjusted to 10^9 CFU/mL.

Experimental Design

The experiment was conducted over a period of 24 hours by challenging the fish with 50 µL of 10^9 CFU/mL *S. iniae*. The fish (n=45) were divided into 3 groups. Group A (n=15), was subjected to heat stress at 31 °C before the challenge. Group B (n=15), was challenged without stress and water was maintained at room temperature. Group C (n=15) served as a control group without challenge. Tissue samples were subjected to bacterial culture were collected every 6 hours for 24 hours and subjected to polymerase chain reaction, histopathology and immunohistochemical staining.

Polymerase Chain Reaction

Wizard Genomic DNA Purification Kit (Promega, USA) was used to confirm S. *iniae* by extracting the total cellular DNA according to manufacturer's protocol.

Histology

Specimens were sacrificed with pitting technique, where the gills, eye, brain, and kidney tissue samples were collected for every 6 hours post-challenge, then fixed in 10% formaldehyde, and processed using a standard histological technique. The scoring of the lesions was ranked based on severity; 0 to 3 as no lesion to most severe lesion.

Immunohistochemistry

Serial 5 µm thick sections were cut from the wax blocks onto silane-coated glass slides and processed using conventional immunohistochemistry technique.

Statistical Analysis

Statistical analyses were performed using MedCalc for Windows, version 12.2.1.0

(MedCalc Software, Mariakerke, Belgium) and tested at 5% level of significance. The differences in the data of lesion scoring were analysed using Kruskal-Wallis test with post-hoc analysis tests if significant is applied.

RESULTS

Clinical Signs and Macroscopic Findings
All the fish appeared to be completely healthy.

Bacterial Isolation and Identification
A small whitish umbonate colony approximately 1 mm in diameter with opaque border and centre and translucent growth ring was observed on blood agar media. The bacteria stained gram-positive, and encapsulated cocci in appearance.

Polymerase Chain Reaction
The PCR amplification and sequencing was positive for all the samples from the positive bacteria cultures.

Immunohistochemistry
Immunolocalisation can be observed in the brain and kidneys as early as 6 hours post-challenge. On the other hand, immunolocalisation in the eyes occured at 12 hpc for group A and 18 hours post-challenge for group B (Figure 1). Organs of control fish were negative at IHC staining.

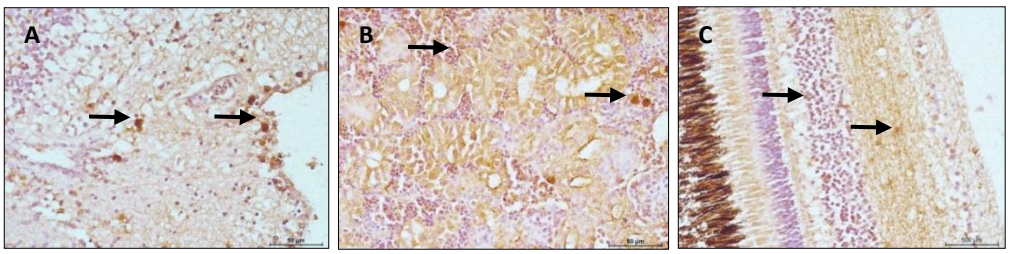

Figure 1: Coccus shaped bacteria with brown colour staining (arrows) with a diffuse distribution of *S. iniae* in (A) brain, (B) kidney, and (C) eye.

Histology
Some of the common histological findings observed in the eyes were detachment of blood vessel walls, inflammatory cell infiltration, fibrin deposition. In the brain, lesions such as congestion, inflammatory cell infiltration and vacuolation were observed in the cerebellum and telencephalon. In the gills, lesions such as oedema, secondary lamellae hyperplasia, fusion or clubbing, congestion, and inflammatory cells infiltration were observed. In the kidney, there were glomerulus atrophy, tubular cell swelling, hemosiderin deposition, inflammatory cell infiltration, congestion and degeneration of the structures were observed. Among groups, the lesions were more severe in heat stressed *S. iniae*-infected fish.

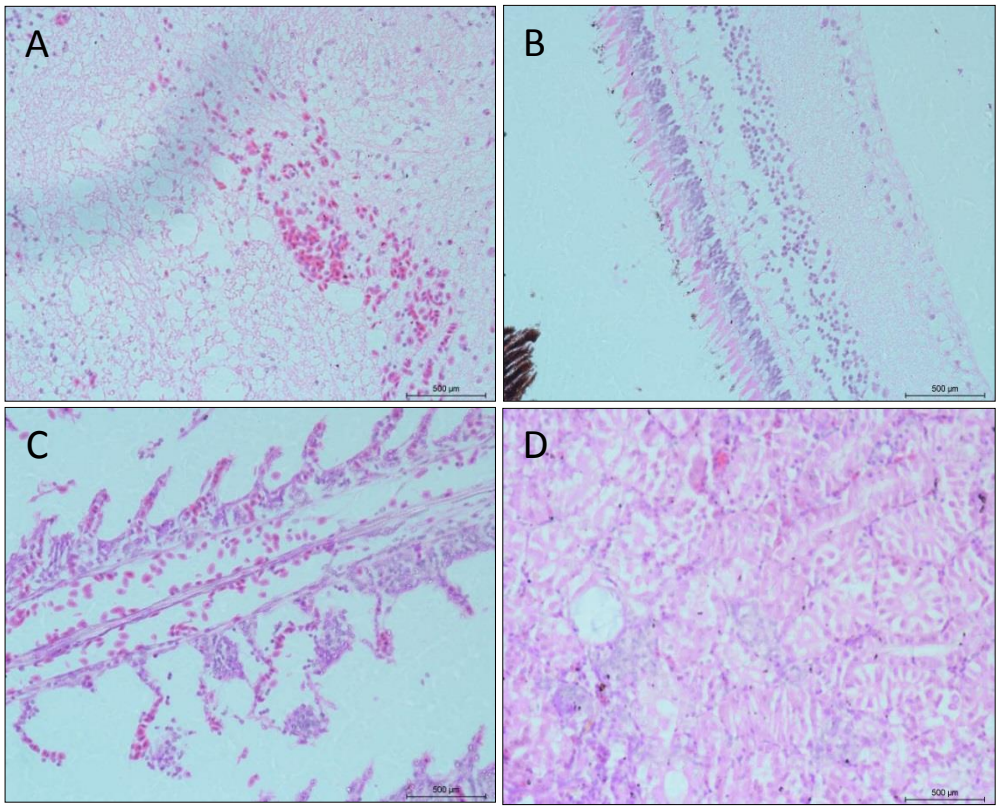

Figure 2: Organ tissues of heat-stressed Red Hybrid Tilapia experimentally infected with *S. iniae* (A) Brain with meninges showing neuroglia cell degeneration and inflammatory cell infiltration and vacuolation, (B) Eye showing pigment epithelium detachment from photoreceptor layer, (C) gill showing epithelium degeneration, necrosis, and numerous rodlet cells, (D) kidney showing collecting duct dilatation.

Statistical Analysis

Table 1 shows the semi-quantitative scoring of the brain, eye, kidney, and gill tissues. MedCalc for Windows, version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium) was used and tested at 5% level of significance.

Table 1: Lesion scoring in organ so heat stressed red hybrid tilapia and experimentally treated with *Streptococcus iniae*.

Organ	Lesion	Lesion Score											
		A				B				C			
		6h	12h	18h	24h	6h	12h	18h	24h	6h	12h	18h	24h
Brain	Infiltration by inflammatory cells, congestion, vacuolation	0,1,1	1,0,0	2,1,1	3,3,2	0,1,1	1,0,0	1,1,2	2,2,2	0,0,0	0,0,0	0,0,0	0,0,0
Eye	Haemorrhage, infiltration, thickening of layers, detachment of layers	0,2,0	0,1,0	0,0,2	0,0,2	0,0,1	1,1,0	0,1,1	1,0,2	0,0,0	0,0,0	0,0,0	0,0,0
Kidney	Glomerulus atrophy, tubular cell swelling, hemosiderin, infiltration, congestion, degeneration	0,1,1	1,1,2	1,1,2	1,2,2	1,1,1	1,1,1	1,0,2	1,1,2	0,0,0	0,0,0	0,0,0	0,0,0
Gills	Oedema, hyperplasia, fusion or clubbing, congestion, infiltration	0,1,1	0,0,1	1,1,2	2,3,3	0,1,1	0,1,1	1,2,1	1,2,2	0,0,0	0,0,0	0,0,0	0,0,0

A=heat stressed at 31 °C before challenge with *S. iniae*; B=Challenged with *S. iniae* only; C=Not heat-stressed, not challenged. h=hour.
0=None, 1=Mild, 2=Moderate, 3=Severe

DISCUSSION

There were no mortality or gross lesion in the fish of this study. The tilapia can control natural *S. iniae* infections more effectively than other fish, thus lesions due to the infection are not normally seen (Chen *et al*., 2007). However, in our study, heat-stressed Red Hybrid Tilapia subjected to *S. iniae* challenge showed mild to severe pathological changes and lesions in organs, except the eyes, with severity increasing with time. The mortality and clinical signs were observed only after 24 hours post-challenge. It seems that the larger the fish, the more susceptible to infections. Besides, juvenile fish are more susceptible to bacterial diseases than older ones (Hernandez *et al.,* 2009).

In this experiment, there were differences of lesions in the brain, kidney and gills among treatments but not among sampling time. Heat-stressed fish challenged with *S. iniae* showed more severe lesion. These findings confirm a previous observation that stress increases bacteria virulence (Ali Farag *et al.,* 2011). Gross lesions were not observed in the experimental fish in this study. However, immunochemistry showed that bacteria were present in the tissues. The bacteria antigen was detected early at 6 hours hpc in the brain and kidneys of fish challenged with *S. iniae.* In conclusion, although *S. iniae* infections progressed slowly and it is not as pathogenic as other streptococcal species, the introduction of stress such heat increases the pathogenicity of the bacteria.

REFERENCES

Ali Farag A, Mohd. Daud H, Abdul Aziz S, Bejo SK, Alsaid M (2011). Pathogenicity of *Streptococcus agalactiae* isolated from a fish farm in Selangor to juvenile Red Tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances*, 10(7): 914-919.

Amal MNA and Zamri-Saad M (2011). Streptococcosis in tilapia (*Oreochromis niloticus*): a review. *Pertanika Journal of Tropical Agricultural Science*, 34(2): 195-206.

Chen C-Y, Chao C-B, Bowser, P (2007). Comparative histopathology of *Streptococcus iniae* and *Streptococcus agala*ctiae-infected tilapia. *Bulletin-European Association of Fish Pathologists*, 27(1): 2-8.

Facklam R (2002). What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clinical Microbiology Reviews*, 15(4): 613-630.

Hernandez E, Figueroa J, Iregui C (2009). Streptococcosis on a red tilapia, *Oreochromis* sp., farm: A case study. *Journal of Fish Diseases*, 32(3): 247-252.

Pier GB and Madin SH (1976). *Streptococcus iniae* sp. nov., a beta-hemolytic streptococcus isolated from an amazon freshwater dolphin, *Inia geoffrensis*. *International Journal of Systematic Bacteriology*, 26(4): 545–553.

COMPOSITION OF FAECAL CONTENT AND FEED OF BROILER DUCKS AND VILLAGE CHICKENS

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ABSTRACT

Poultry manures have been long recognized as one of the most desirable for fertiliser because of high nitrogen content. Compositions of faecal contents and feed of broiler ducks and village chickens were investigated. Faecal sample were obtained from commercial Cherry Valley duck and a small scale village chicken farm, managed under the open-house free-range system and close-house system, respectively. Twenty-five gram of faecal sample was collected from each adult broiler ducks and village chickens. Proximate analysis was carried out to determine the moisture, dry matter, ash and crude protein content in the samples using the official method of AOAC. There was no significance ($p>0.05$) difference in digestibility of dry matter and crude protein content between village chicken and Cherry Valley duck. The faecal samples showed that the ash content in village chicken (1.06±0.12%) was significantly ($p<0.05$) lower than in Cherry Valley duck (3.133±0.49%). However, there was no significance ($p>0.05$) difference between village chicken and Cherry Valley duck in moisture (75.56±1.31 and 72.4±1.91%, respectively), dry matter (24.43±1.13 and 27.6±1.91%, respectively), ash (4.43±0.63 and 5.23±0.83%, respectively), and crude protein (24.43±1.13 and 27.6±1.91%, respectively) contents of their feeds.

Keywords: Cherry Valley ducks, village chickens, proximate analysis, digestibility

INTRODUCTION

The nutritional value in products from ducks can enhance the nutritional standard of human food (Pingel, 2004). However, the conversion of dietary nitrogen to animal products is relatively inefficient with only 50 to 80% of the nitrogen excreted (Arogo *et al.*, 2001). The aim of this study was to compare the moisture, dry matter, ash, and crude protein contents of faeces of village chickens and Cherry Valley ducks and determine the digestibility of their feeds.

MATERIALS AND METHODS

Pool sampling of faeces was done at a commercial Cherry Valley duck and a small-scale village chicken farm, managed under the open-house free-range system and close-house system, respectively. The faecal samples and feed samples were analysed for moisture, dry matter, ash and crude protein by the standard procedures of AOAC (1995). Data collected were analysed using independent sample *t*-test (SPSS 20.0).

RESULTS

The dry matter was 90.2±0 and 90.8±0%, ash was 4.43±0.63 and 5.23±0.83%, and crude protein was 21.73±0.81 and 19.92±1.22% in the chicken broiler starter crumble mixed with corn and duck broiler finisher pellet, respectively. The digestibility of dry matter was 72.91±1.25% and 69.6±2.1%, and crude protein was 65.64±0.39% and 56.77±4.37% in village chicken and Cherry Valley ducks, respectively. There was no significance ($p>0.05$) difference in digestibility of dry matter and crude protein content between village chicken and Cherry Valley duck. The proximate analysis of faecal samples showed significance ($p<0.05$) difference in percentage of ash contents between village chicken (1.06±0.12%) and in Cherry Valley duck (3.133±0.49%) faeces. However, there was no significance ($p>0.05$) different between village chicken and Cherry Valley duck in moisture (75.56±1.31 and 72.4±1.91%, respectively), dry matter (24.43±1.13 and 27.6±1.91%, respectively), ash (4.43±0.63 and 5.23±0.83%) and crude protein (24.43±1.13 and 27.6±1.91%, respectively) contents of their feeds.

DISCUSSION

The nutrient composition of poultry manure varies with the type of bird, feed ration and proportion, proportion of litter to droppings, type of litter, and manure handling system. According to Short *et al.* (1999), the total nitrogen content of chicken and ducks faeces does not differ. However, the moisture and dry matter content can be greatly influenced by several factors and types of animal, management system, climate, temperature, humidity, manure storage, and handling (Burton and Turner, 2003). Current methods to reduce manure losses include the use of a lots of bedding materials to absorb liquid manure, storage of manure in an area with water-tight bottom, providing protection from weather, and addition of phosphate to manure pile to trap nitrogen.

CONCLUSION

The key determinants of manure fertiliser quality are the ratio of nitrogen, phosphorus, and potassium. The study showed that the crude protein and dry matter content in the faeces of village chicken and broiler ducks did not differ. However, the ash content in village chicken was lower than in Cherry Valley duck faeces. The study showed that both the village chickens and broiler ducks faeces are suitable to be used as manure.

REFERENCES

Arogo J, Westerman PW, Heber AJ, Robarge WP, Classen JJ (2001). Ammonia in animal production – A review. Paper number 01-4089, 2001 presented at the ASAE Annual Meeting July 30- August 1, 2001, Sacramento, USA. American Society of Agricultural and Biological Engineers. http://www.manuremanagement.cornell.edu/Pages/General_Docs/Other/Arogo_Ammonia_in_Animal_Production_a_Review.PDF (Accessed on 1 September 2016).

Burton C and Turner C (Editors) (2003). Manure management: treatment strategies for sustainable agriculture, (2nd Edition). Bedford, UK, Silsoe Research Institute.

Short FJ, Wiseman J, Booman KN (1999). Application of a method to determine ileal digestibility in broilers of amino acids in wheat. *Animal Feed Science and Technology*, 79(3): 195-209.

EFFECT OF REPRODUCTIVE STAGE ON THE PREVALENCE OF NORMAL FLORA IN FEMALE BOER GOATS

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ABSTRACT

The genital tract of animals is known to harbour non-specific bacteria known as normal flora, but under certain circumstances they can be opportunistic pathogens. This study was designed to isolate and identify and to determine the prevalence of normal bacteria in the vaginal of pregnant and non-pregnant Boer goats at various stages of reproduction. Fourteen sterile vaginal swabs were collected from non-pregnant and pregnant does. The swabs were cultured and incubated for 24 hours and morphology of colonies recorded. Subculture was done to obtain the pure colonies followed by gram-staining and biochemical testing. More bacteria were isolated from the vagina of pregnant (55.6%) than non-pregnant (44.4%) does. The most dominant bacteria were *Staphylococcus* (58.33%), followed by *Streptococcus* and *Enterococcus* (8.33%). This study that the stage of reproduction influences vaginal bacterial population.

Keywords: pregnant, non-pregnant, normal bacterial flora, vaginal swabs, Boer doe

INTRODUCTION

Mucosal sites, such as the lower female reproductive tract, should be capable of discriminating between commensal and the pathogenic organisms in order to generate protective immune responses (Entrican and Wheelhouse, 2006). Hormones, notably oestrogen, progesterone, and PGE2, play important roles in regulating local immunity in the genital tract. Fluctuations these hormone levels during the reproductive cycle and pregnancy can influence immune surveillance and disease susceptibility and poor immune response can cause commensal bacteria to cause disease (Entrican and Wheelhouse, 2006). Thus, this study was designed to determine the prevalence of normal bacteria in the vaginal environment of pregnant and non-pregnant Boer goats.

MATERIALS AND METHODS

Selection of animals and sampling
Seven pregnant and 7 non-pregnant Boer goats were selected for the study. The pregnancy status was determined by the failure to return to oestrus, ultrasound examination, and physical appearance that included increase in abdominal size, udder development, and vulva swelling. Vaginal swabs collected for the bacteriology was processed immediately.

Isolation and identification of bacteria
Swabs were cultured on blood agar and incubated at 37^{o}C under aerobic conditions for 24 hours. The colonies were described based on size, elevation, outline, colour, and effect on the medium. Smears of swab samples were fixed and gram-stained. The identity the gram-positive bacteria were confirmed by triple sulphate iron agar, urease, sulphate indole motility agar (SIM), citrate, and oxidase tests, while gram-positive bacteria by catalase, coagulase, haemolysin, and sugar tests. The combination of colonial morphology, growth conditions, bacterial morphology and reaction to gram stain were used for presumptive identification of the bacteria.

RESULTS

From non-pregnant goats, the dominant bacteria isolated were *Staphylococcus intermedius* (37.5%) while from pregnant goats they were *S. intermedius* (35%) and *S. aureus* (30%). Other bacteria species were also isolated but at a relatively lower rates. Total bacteria isolated from the vagina of non-pregnant was 44.44% compared 55.56% from pregnant goats. Higher percentage of bacteria was isolated from the vagina of the pregnant than non-pregnant goats. There were differences in type of bacteria isolated from non-pregnant and pregnant goats (Table 1).

DISCUSSION

More bacteria were isolated from the vagina of pregnant than non-pregnant goats. The bacteria flora in the environment of the vagina is influence by several factors including pH changes during oestrus or pregnancy. Previous study had showed that hormonal changes, especially high progesterone level during oestrus cycle can also effect on vaginal bacterial population (Entrican and Wheelhouse, 2006).

E. coli, S. aureus and *Klebsiella* spp. were the most common genital bacterial isolates in ewes associated with genital infections (Sokkar *et al*., 1980). However, these bacteria may not cause infection. In fact, these bacteria play a protective role in the vagina of the goats. In present study, female reproductive tract micoorganisms may be saprophytic or opportunistic when conditions of stress (Bukar *et al.,* 2007). These vaginal and uterine infections may cause reproductive failure in ewes and other domestic ruminants (Levinson and Jawetz, 1994). During

peripartum period the bacteria may gain access to the uterus causing metritis and endometritis and subsequently compromising the reproductive capacities of these animals.

The does in this study did not show any reproductive tract abnormality. Their reproductive tracts were grossly normal and bacteriological findings should reflect the "normal" flora of the animals are not stressed. Thus, keeping does under non-stressful condition is important in prevention of infection of reproductive tract due to opportunistic bacteria.

Table 1: Bacteria isolated from vagina of non-pregnant and pregnant Boer goats.

Bacterial isolates	Frequency of occurrence			
	Non-pregnant		Pregnant	
	Number	%	Number	%
Gram-positive bacteria:				
Staphylococcus aureus	2	12.5	6	30
Staphylococcus intermedius	6	37.5	7	35
Streptococcus spp.	2	12.5	1	5
Enterococcus spp.	2	12.5	1	5
Enterococcus faecalis	-	-	1	5
Gram-negative bacteria:				
Escherichia coli	-	-	1	5
Proteus mirabilis	1	6.25	1	5
Pasteurella aeromonas	1	6.25	-	-
Mannheimia haemolytica	1	6.25	-	-
Klebsiella aerogenes	1	6.25	1	5
Acitenobacter	-	-	1	5
Total	**16**	**44.44**	**20**	**55.56**

CONCLUSION

From our results, *Staphylococcus, Streptococcus,* and *Enterococcus* spp. were the most dominant microorganism in the vagina of healthy Boer goats. More bacteria were isolated nonpregnant than pregnant goats.

REFERENCES

Bukar KYM, Amin, JD, Zaria LT (2007). Bacteria flora of the anterior genitalia of the Sahelian doe in Maiduguri-borno state, Nigeria. *Nigerian Veterinary Journal,* 28(2): 60-62.

Entrican G and Wheelhouse NM (2006). Immunity in the female sheep reproductive tract. *Veterinary Research,* 37(3): 295–309.

Levinson WE (Editor) (2010). Medical microbiology and immunology. 11th Edition. Lange Basic Science, McGraw-Hill.

Sokkar SM, Kubba A, Al-Augaidy F (1980). Studies on natural and experimental endometritis in ewes. *Veterinary Pathology,* 17(6): 693- 698.

RETROSPECTIVE STUDY ON THE PREVALENCE OF DIABETES MELLITUS IN CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FROM YEAR 2010 TO 2015

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ABSTRACT

Diabetes mellitus (DM) is one of the most common endocrinopathies in cats and the prevalence is about 1% in the United States of America and Australia. In Malaysia, there is lack of documentation on the prevalence of feline DM. Therefore, a retrospective study was carried out at the University Veterinary Hospital (UVH), Universiti Putra Malaysia from year 2010 to 2015. The objectives of the study were to determine the prevalence of feline DM based on cases presented to UVH, to describe clinical signs, and to determine whether the prognosis of cats with DM can be determined from clinicopathological findings. In the study, 25 cats were reported to be diabetic during the sampling period. Sixteen of these cats that received insulin treatment were categorised as either survived or dead cats. The results showed that the prevalence of DM in cats presented to UVH was between 0.03 and 0.17%. Diabetic cats showed polyuria and polydipsia. From clinicopathological findings, diabetic cats showed inflammatory leukogram, hyperglycaemia, azotaemia, increased serum alanine aminotransferase concentration, hyperproteinaemia, glucosuria, and haematuria. Urinalysis showed that diabetic cats commonly showed glucosuria, proteinuria, and high urine specific gravity. The prognosis of diabetic cats with insulin treatment was significantly (P=0.036) better than those that did not receive insulin treatment. However, only serum creatinine concentration was significantly (P=0.05) lower in diabetic cats that survived from those that eventually died.

Keywords: feline, diabetes mellitus, glucosuria, hyperglycaemia, insulin

INTRODUCTION

Feline diabetes mellitus (DM) is one of the most common endocrinopathies in cats (Rand and Marshall; 2004; Little and Miller, 2014). The disease is characterised by chronic hyperglycaemia resulting from defect in insulin secretion and/or action.

According to Rand and Marshall (2004), 80 to 95% of feline DM is analogous to human type 2 DM, which is caused by abnormal insulin secretion in conjunction with peripheral insulin resistance. Diabetes mellitus can affect all cats. The disease often occurs in older (>7 years old) and obese cats (Prahl *et al*., 2007; Huang, 2012). Male cats are more commonly afflicted with DM than female cats. According to Lederer *et al*., (2003), DM in Burmese cat is more likely a multifactorial disease. Chronic pancreatitis and hormonal diseases are potential environmental causes of impaired insulin secretion in cats.

In Malaysia, there is lack of documentation on feline DM. Therefore, a retrospective study on feline DM at University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM) from year 2010 to 2015 was carried out with objectives to determine prevalence of feline DM; to describe patient signalment, clinical findings, clinicopathological findings and treatment outcomes in diabetic cats; and to identify the prognosis of diabetic cats based on clinicopathological findings.

MATERIALS AND METHODS

A retrospective study was performed based on the records available at the Haematology and Clinical Biochemistry Laboratory, Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, UPM, from year 2010 to 2015. The reports were screened to obtain blood and urinalysis profiles of cats suggestive with diabetes mellitus. Then, the case numbers of the representative cases were obtained and patient medical records were reviews from the archives of UVH, UPM. From the patient medical records, patient signalment inclusive of age, breed, gender and neuter status, clinical findings, diagnostic investigations, therapeutic plan, and clinical outcomes were recorded for diabetic cats. In the study, 25 cats were reported to be diabetic during the sampling period. Sixteen of these cats that had received insulin treatment, were categorised into 2 groups diabetic cats; survived and dead. The clinicopathological findings of the two groups were analysed using Shapiro-Wilk test, Mann-Whitney test, independent t-test, and Chi-square analysis. Statistical significance at value was determined at α=0.05.

RESULTS AND DISCUSSION

Prevalence

During the years 2010 to 2015, cases of feline DM with the lowest prevalence was 0.03% and the highest was 0.17%, a range that was lower than that reported in the United States of America and Australia (Huang, 2012). However, since the study was conducted in cases presented to UVH, UPM, these prevalence values are not representative of the total incidence in the cat population of Malaysia.

Age

The mean age of cats at the time diagnosed with DM was 8.6 years old in this study.

According to Prahl *et al*. (2007), cats that aged >7 years old were at highest risk. It is because older cats cannot adequately respond to increased insulin requirement.

Sex

Among the cats in the present study, 76% were male while 24% female. Male were at higher risk of getting DM compared to female cats, due to their high body weight gain and low insulin sensitivity (Appleton *et al*., 2001). Among the diabetic cats, 60% were neutered. It seemed that the glucose tolerance test results were not significantly ($p>0.05$) different among cats before and after neutering (Hoenig and Ferguson, 2002; Thiess *et al*., 2004).

Breed

According to breed, 84% of diabetic cats were Domestic Short Hair cat breed. However, this finding may be biased since this breed is indigenous to Malaysia.

Clinical Sign

Based on clinical findings, 72% of diabetic cats were shown to have history of polyuria and polydipsia. These symptoms are due to hyperglycaemia and glucosuria that led to excessive urination and increased water consumption by the cats (Reusch, 2010). Diabetic cats often have good appetite but tend to lose weight due to diabetic polyphagia. Weight loss is also a result of calorie lost in the glucose-laden urine (Greco). Our study showed that 36% of diabetic cats had weight loss while 16% had polyphagia. Among the diabetic cats, 44% were dull and depressed upon presentation, which may be early signs of diabetic ketoacidosis. As metabolic condition continues to deteriorate, clinical signs of anorexia, vomiting and dehydration may have developed (Reusch, 2010). The study also showed that 36% of diabetic cats were anorexia, 28% dehydrated, and 8% had history of vomiting.

Haematology

From the clinicopathological findings, it was shown that 55% of diabetic cats showed inflammatory leukogram, typically neutrophilia with left shift. Among the diabetic cats, 40% also showed monocytosis and 55% showed hyperglobulinaemia. The haematological findings are suggestive of concurrent infection or inflammation (Rios and Ward, 2008).

Biochemistry

All diabetic cats show hyperglycaemia with mean blood glucose concentration of 26 mmol/L. The study also showed that 55% of diabetic cats had increased serum ALT concentration with a mean value of 172 U/L. This finding is similar to those of Reusch (2010). The increase in serum ALT activity is suggested to be the result of reactive hepatopathy and hepatic lipidosis often seen in diabetic cats. On the other hand, 60% of diabetic cats showed azotaemia. Elevation of tissue polypol concentration as a result of hyperglycaemia may have contributed to renal dysfunction. This renal disease is typically expressed by thickening of glomerular basement membranes and glomerular hypertension. The azotaemia is suggested to

present a late consequence of diabetic nephropathy. Among the diabetic cats, 82% also had hyperproteinaemia. This is a manifestation of haemoconcentration often seen in DM. In DM, persistent hyperglycaemia also causes increased binding of plasma protein to glomerular basement membrane (Greco).

Urinalysis

Urinalysis showed that 91% of diabetic cats had glucosuria, while 64% had proteinuria. The proteinuria may be indicative of bacterial infection or damage to glomerular membrane secondary because of DM (Rios and Ward, 2008). Reusch, (2010) also showed that urine specific gravity in diabetic cat is more than 1.020 and sporadic ketone bodies may be found. In our study, 91% of diabetic cats had urine specific gravity of >1.020, which could possibly be caused by glucosuria (Rios and Ward, 2008). Ketonuria that occurred in 4 diabetic cats in this study is suggested to be due to fat mobilisation in DM.

Survival

In term of survivability, diabetic cats received insulin treatment had better prognosis compared to those without insulin treatment ($p<0.05$). Insulin-treated diabetic cats that survived also had lower creatinine concentration than those that died.

CONCLUSION

In conclusion, this retrospective study shows DM in cats presented to UVH, UPM from 2010 to 2015 was of low prevalence. Feline DM is a multifactorial disease where genetic and environmental factors are involved in the pathogenesis. Diabetic cats showed polyuria and polydipsia. From clinicopathological findings, diabetic cats showed inflammatory leukogram, hyperglycaemia, azotaemia, increased serum alanine aminotransferase concentration, hyperproteinaemia, glucosuria, and haematuria. On the other hand, prognosis of diabetic cat with insulin treatment is significantly better compared to diabetic cats without insulin treatment. With good blood glucose control, it may facilitate diabetic cats to undergo clinical remission.

REFERENCES

Appleton DJ, Rand JS, Sunvold GD (2001). Insulin sensitivity decreases with obesity, and lean cats with low insulin sensitivity are at greatest risk of glucose intolerance with weight gain. *Journal of Feline Medicine and Surgery*, 3(4): 211-228.

Greco DS. Managing diabetics. http://www.delawarevalleyacademyvm.org/pdfs/jun11/4monitordiabetes.pdf (Accessed on 29 September 2016).

Hoenig M and Ferguson DC (2002). Effects of neutering on hormonal concentrations and energy requirements in male and female cats. *American*

Journal of Veterinary Research, 63(5): 634-639.
Huang A (2012). Feline diabetes mellitus. http://www.cliniciansbrief.com/sites/default/files/attachments/Feline%20Diabetes%20Mellitus.pdf (Accessed on 30 September 2016).
Lederer R, Rand J, Hughes IP, Fleeman LM (2003). Chronic or recurring medical problems, dental disease, repeated corticosteroid treatment, and lower physical activity are associated with diabetes in Burmese cats. *Journal of Veterinary Internal Medicine*, 17(3): 433.
Little S and Miller L (2014). Diabetes mellitus in the Cat. *Winn Feline Foundation:* For the Health and Well-being of All Cats. http://www.winnfelinefoundation.org/docs/default-source/cat-health-library-educational-articles/diabetes-mellitus-in-the-cat.pdf?sfvrsn=2. (Accessed on 9 February 2016).
Prahl A, Guptill L, Glickman NW, Tetrick M, Glickman LT (2007). Time trends and risk factors for diabetes mellitus in cats presented to Veterinary teaching hospitals. *Journal of Feline Medicine and Surgery,* 9: 351-358.
Rand J and Marshall R (2004). Feline diabetes mellitus. BSAVA Manual of Canine and Feline Endocrinology. 3rd Edition.
Reusch C (2010). Feline diabetes mellitus. In: Ettinger, S.J, Feldman, E.C. *Textbook of Veterinary Internal Medicine.* 7th Edition. St. Louis; Elsevier.
Rios L and Ward C (2008). Feline diabetes mellitus: Diagnosis, treatment, and monitoring. *Compendium,* 626-640.
Thiess S, Becskei C, Tomsa K, Lutz TA, Wanner M (2004). Effects of high carbohydrate and high fat diet on plasma metabolite levels and on i.v. glucose tolerance test in intact and neutered male cats. *Journal of Feline Medicine and Surgery*, 6: 207-218.

A POLYMERASE CHAIN REACTION TECHNIQUE FOR THE DETECTION OF *MYCOPLASMA HYOPNEUMONIAE* AND PSEUDORABIES VIRUS IN PORCINE CLINICAL SAMPLES

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ABSTRACT

With recent advancement in molecular biology, molecular detection has become an important diagnostic technique. Since Aujeszky's disease and enzootic pneumonia cause great economic losses to the swine livestock industry, this study aimed to determine suitable primers sets for pseudorabies virus (PRV) and *Mycoplasma hyopneumoniae* (Mhyo) detection in clinical samples using polymerase chain reaction (PCR) assay. Fifteen pigs aged approximately 2 month showing clinical signs of respiratory distress were sampled. Commercial vaccine and positive clinical samples were used as positive controls. Each lung and tonsil tissue samples were subjected to conventional PCR assay using 3 different sets of primers designed to target conserved regions of genomic DNA of *M. hyopneumoniae* and PRV respectively. Based on PCR assay on lung tissue samples, 3 out of 15 pigs were positive for Mhyo using the 3 primers sets whereas for tonsil tissue, all 15 pigs were negative for PRV. To further optimize the protocol, larger sample size should be used and gradient PCR assay performed. In conclusion, all the primers sets chosen were suitable for Mhyo and PRV detection using PCR.

Keywords: porcine, PCR, primer, Pseudorabies virus, *Mycoplasma hyopneumoniae*

INTRODUCTION

Mycoplasma hyopneumoniae is the aetiological agent of swine enzootic pneumonia (SEP) and also contributes to porcine respiratory disease complex (PRDC) by interacting with respiratory viral pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and pseudorabies virus (PRV) (Sibila *et al*., 2009; Villarreal, 2010; Zimmerman *et al*., 2012). Both SEP and PRDC cause significant economic losses to the swine production industry. On the other hand, PRV, which belongs to the genus

Varicellovirus of the subfamily *Alphaherpesviriae* and family *Herpesviridae,* causes Aujeszky's disease and high mortality in piglets and abortion in pregnant sows.

Traditional bacterial culture and viral isolation are time-consuming and labour-intensive and molecular detection method such as polymerase chain reaction (PCR) technique can provide faster diagnosis. Therefore, the objective of this study is to determine suitable primers set for the detection of *M. hyopneumoniae* and pseudorabies virus in porcine clinical samples using the PCR technique.

MATERIALS AND METHODS

Samples Collection

Fifteen pigs with respiratory distress were sampled from 4 local pig farms located in Perak and Selangor, Malaysia, using the convenience sampling method. The pigs were humanely euthanised and necropsied *in situ* and lung and tonsil tissue samples collected.

DNA Extraction

The DNA was extracted from the lung and tonsil tissue samples using a commercial DNA extraction kit (DNeasy® Blood &Tissue Kit 250, Qiagen®, Germany). Positive control was the commercial vaccine (Ingelvac® Aujeszky MLV, Germany) for PVR detection and *M. hyopneumoniae*-positive clinical samples for *M. hyopneumoniae* detection.

Measurement of DNA concentration

The DNA concentration was estimated via spectrophotometry (BioPhotometer Plus photometer, Eppendorf, Germany).

Primer selection

Three sets of primers were selected for each of *M. hyopneumoniae* and PRV detection based published articles (Tables 1 and 2).

RESULTS AND DISCUSSION

From the results, 3 out of 15 pigs were positive for *M. hyopneumoniae* with all primers sets used in this study. None of the porcine clinical sample was positive for PRV by the PCR assay.

CONCLUSION

Although the PCR assay only detected *M. hyopneumoniae* not PRV in the clinical samples in this study, with the use of appropriate primers, it is still a useful and fast

diagnostic tool for the detection of these infections.

Table 1: Primers set for detection of *Mycoplasma hyopneumoniae.*

Primer Set		Nucleotide Sequence 5' → 3'	Expected product (bp)	Reference
1	Forward	5'- GAGCCTTCAAGCTTCACCAAGA-3'	649	Cai *et al.* (2007)
	Reverse	5'- TGTGTTAGTGACTTTTGCCACC-3'		
2	Forward	5'- TAGAAATGACTGGCAGACAA -3'	853	Baumeister *et al.* (1998)
	Reverse	5'- GAGGCTTGATTTTGGAGTC -3'		
3	Forward	5'- GGGCCGATGAAACCTATTAAAATAGCT -3'	948	Caron *et al.* (2000)
	Reverse	5'- GCCGCGAAATTAAATATTTTTAATTGCATCCTG-3'		

Table 2: Primers set for detection of pseudorabies virus**.**

Primer Set		Nucleotide Sequence 5' → 3'	Expected product (bp)	Reference
1	Forward	5'- GGTGGACCGGCTGCTGAACGA -3'	455	Perez &Arce (2009)
	Reverse	5'- GCTGCTGGTAGAACGGCGTCA-3'		
2	Forward	5'- ATGGCCATCTCGCGGTGC – 3'	334	Ayala *et al.* (2012)
	Reverse	5'- ACTCGCGGTCCTCCAGCA – 3'		
3	Forward	5'- GTTTCCTGATTCACGCCCACGC – 3'	788	Fonseca Jr. *et al.* (2010)
	Reverse	5'- GAAGGGCTCACCGAAGAGGAC – 3'		

REFERENCES

Ayala MA, Escatel GS, Salinas LE, Salas ME, Castaneda EC (2012). Polymerase chain reaction for diagnosis of Aujeszky disease in Mexico. *Journal of Animal and Veterinary Advances*, 11(22): 4217-4220.

Baumeister AK, Runge M, Ganter M, Feenstra AA, Delbeck F (1998) Detection of *Mycoplasma hyopneumoniae* in bronchoalveolar lavage fluids of pigs by PCR. *Journal Clinical Microbiology,* 36(7): 1984-1988.

Cai HY, Van DT, McEwen B, Hornby G, Bell RP, McRaild P, Josephson G, Maxie G (2007). Application and field validation of a PCR assay for the detection of *Mycoplasma hyopneumoniae* from swine lung tissue samples. *Journal of Veterinary Diagnostic Investigation,* 19(1): 91-95.

Caron J, Ouardani M, Dea S (2000). Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. *Journal of Clinical Microbiology,* 38(4):1390-1396.

Fonseca Jr, Camargos MF, de Oliveira AM, Ciacci-Zanella JR, Patrício MA, Braga AC, Cunha ES, D'Ambros R, Heinemann MB, Leite RC, dos Reis JK. (2010). Molecular epidemiology of Brazilian pseudorabies viral isolates. *Veterinary Microbiology,* 141(3-4): 238-245.

Pérez LJ and Arce, HD (2009). Development of a polymerase chain reaction assay for the detection of pseudorabies virus in clinical samples. *Brazilian Journal of Microbiology*, 40(3): 433-438.

Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW (Editors) (2012). Diseases of Swine, 10th Edition. Wiley-Blackwell.

EFFICACY OF INACTIVATED *PASTEURELLA MULTOCIDA* IN THE PROTECTION OF BROILER CHICKEN AGAINST THE BACTERIA INFECTION

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ABSTRACT

Pasteurella multocida infection in chickens results in fowl cholera, associated with high morbidity and mortality leading to economic losses to the poultry industry. This study determined the efficacy of inactivated *P. multocida*, either as single or combination of serogroups in the protection of broiler chickens against the bacterial infection in broiler chickens. Eighty-four day-old boiler chickens were divided into seven groups. The chickens were inoculated with 0.1 mL of 1×10^{11} CFU/mL inactivated *P. multocida* serogroups A and D, subcutaneously. Groups 1 and 4 received serogroup A, Groups 2 and 5 received serogroup D, whilst Groups 3 and 6 received combination serogroups A and D. Groups 4, 5 and 6 received a booster inactivated *P. multocida* on day 14 post-challenge (p.c). One chicken each from groups 1 and 7 died s on days 1 and 2 p.c. and *P. multocida* was isolated. The dead chicken showed severe generalised congestion of the liver and visceral organs. Histologically, there were severe hepatitis with degeneration and necrosis of hepatocytes and severe pneumonia with cellular degeneration and necrosis. The chickens that survived were sacrificed on day 8 p.c. These chickens showed normal to mild hepatitis and pneumonia. In conclusion, combined inactivated *P. multocida* serogroups A and D treatment provided better protection against *P. multocida* infections in chickens than treatment with either serogroup A or D alone.

Keywords: efficacy, inactivated *Pasteurella multocida*, serogroups A and D, broiler chicken

INTRODUCTION

Fowl cholera is a contagious bacterial disease of domesticated and wild avian species caused by *Pasteurella multocida* (Anun, 2001). The infection usually appears as septicaemic disease associated with high morbidity and mortality, often with chronic and benign conditions (Glisson *et al.*, 1991). The infected chickens usually show fever, anorexia, depression, mucus discharge from mouth, diarrhoea, ruffled feathers, drop in egg production, small eggs, increased respiratory rate and cyanosis before death (Carter, 1967).

Excessive usage of the drugs in food-producing animals to control fowl cholera had caused antibiotic resistance and residues that are harmful to consumers. Therefore, this disease should be controlled with vaccines instead of antimicrobial drugs. The objective of this study was to determine the efficacy of inactivated *P. multocida* serogroups A and D either as single or in combination treatments of against the bacterial infection in broiler chickens.

MATERIALS AND METHODS

P. multocida serogroup A isolate (UPM 1231) and serogroup D isolate (UPM 1387) were used in the study. The bacterial suspension was prepared by serial dilution using McFarland standards. Formalin (2.5%) was added to the suspension to inactivate the bacteria (Ievy *et al*., 2013) and aluminum potassium sulphate alum added to the bacteria suspension at the ratio of 1:10. The bacterial suspension were either singly as serogroups A or D, or combined in 1:1 ratio were stored at 4 °C.

Eighty-four day-old boiler chicken were divided equally into seven groups. Groups 1 and 4 were inoculated with inactivated serogroup A, Goups 2 and 5 with serogroup D, Groups 3 and 6 with combination of serogroup A and D, and Group 7 was not inoculated and served as the control group. All the chickens from group 1 to 6 were inoculated subcutaneously with 0.1 mL of 1×10^{11} CFU/mL of inactivated *P. multocida*. Booster doses was given to Groups 4, 5 and 6 on day 14. On day 28, the chickens were divided equally into three groups of non-challenged, and challenged either intramuscular or intranasal with 1×10^{8} CFU/mL in 0.1 mL of inactivated *P. multocida* suspension. All chickens were observed twice daily. The chickens were sacrificed on day 8 post-challenge. At necropsy, gross lesions were recorded and liver and lung tissue samples collected for *P. multocida* isolation and histopathology examination.

RESULTS

Clinical signs

No significant clinical sign was recorded among the chickens from all groups 1 to 7 throughout the trial. The chickens were healthy with good appetite and water intake and normal body condition. In contrast, one chicken from group 1 (inoculated with inactivated serogroup A) and group 7 (control group) died on days 1 and 2 post challenged via intramuscular route, respectively.

Necropsy

On necropsy, severe generalized congestion of liver, lungs and kidneys were observed in dead chickens. However, no significant lesion was recorded in all the sacrificed chickens.

Histopathology findings

Normal to mild hepatitis and pneumonia with mild cellular degeneration and necrosis were evident in the sacrificed chickens. In contrast, severe hepatitis with severe degeneration and necrosis of hepatocytes and severe pneumonia with severe cellular degeneration, necrosis, and congestion of the lungs were recorded in dead chickens.

DISCUSSION

The study demonstrated that inoculation with combination inactivated *P. multocida* serogroups A and D with booster dose provide better protection against infection compared to single intramuscular dose. The higher mortality rate in chickens challenged with intramuscular route *P. multocida* is proposed to be due to respiratory failure from pneumonia and congestion of the lungs or massive bacteraemia and endotoxic shock (Powel, 1987; Rimler and Rhoades, 1987). *P. multocida* rapidly enters the blood circulation and multiply in liver and spleen before spreading to the whole body and causing death in chickens (Tsuji and Matsumoto, 1989; Kubatzky, 2012). In our study, no mortality was seen in chickens inoculated intranasally with the bacteria, if they were first vaccinated with combination inactivated *P. multocida* of serogroups A and D. This shows that the defense mechanism of the chickens at mucosal layer prevents invasion of the agent in the host. No mortality was recorded even at day post-challenge showing that elimination of the invading organs by mucosal IgA became effective from day three post-infection (Tamura and Kurata, 2004). Thus, it can be concluded that inactivated *P. multocida* combination of serogroups A and D could provide good protection against *P. multocida* serogroup A infection.

REFERENCES

Anun M (2001). Molecular characterization and pathogenicity of *Pasteurella multocida* isolated in chickens with fowl cholera. MVSc. thesis, Universiti Putra Malaysia. http://psasir.upm.edu.my/11810/1/FPV_2001_8_A.pdf (Accessed on 9 September 2016)

Carter GR (1967). Pasteurellosis: *Pasteurella multocida* and *Pasteurella hemolytica*. *Advances in Veterinary Science*, 11: 321-379.

Glisson JR and Cheng HN (1991). *In vivo* antigen expressing by *Pasteurella multocida*. *Avian Disease*, 35(2): 392-396.

Ievy S, Rahman Khan MF, Islam MA, Rahman MB (2013). Isolation and identification of *Pasteurella multocida* from chicken for the preparation of oil adjuvante vaccine. *Microbes and Health*, 2(1): 1-4.

Kubatzky KF (2012). *Pasteurella multocida* and immune cells. *Microbiology and Immunology*, 361: 53-72.

Powel PC (1987). Immune mechanisms in disease of poultry. *Veterinary*

Immunology and Immunopathology, 15: 87-113.
Rhoades KR (1964). The microscopic lesions of acute fowl cholera in mature chickens. *Avian Disease*, 8: 658-665.
Rimler RB and Rhoades KR (1987). Serogroups F, a new capsule serogroup of *Pasteurella multocida*. *Journal of Clinical Microbiology*, 25: 615-618.
Tamura S and Kurata T (2004). Defense mechanisms against influenza virus infection in the respiratory tract mucosa. *Japanese Journal of Infectious Diseases*, 57: 236-247.
Tsuji M and Matsumoto M (1989). Pathogenesis of fowl cholera: Influences of encapsulation on the fate of *Pasteurella multocida* after intravenous inoculation in turkeys. *Avian Disease*, 33: 238-247.

IDENTIFICATION OF A SUPEROXIDE DISMUTASE-1 GENE MUTATION IN CLIENT-OWNED DOGS AT THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Superoxide dismutase 1 mutation (*SOD1*:c.118G>A) has been recognised as a major risk factor for a canine neurodegenerative condition, degenerative myelopathy (DM). Affected dogs typically show chronic progressive onset of paraparesis that ultimately progresses to tetraplegia and death. Recent studies have linked homozygosity of the mutant alleles to higher risk of developing DM. The distribution of canine DM appears to be global however its occurrence in Malaysia is currently unknown. A genotyping analysis against the point mutation would provide preliminary genotypic distribution that could aid identifying potential DM cases in Malaysia. Therefore, the aim of this research is to investigate the genotypic distribution of the *SOD1* mutation in client-owned dogs registered to University Veterinary Hospital. Genomic DNA was extracted from 30 blood samples and subjected to conventional polymerase chain reaction (PCR) assay to amplify the region containing the mutation site. Restriction enzyme digestion of the PCR products was performed and were subsequently analysed by agarose gel electrophoresis. The mean age of dogs was 5.1 years while the breeds were German Shepherd Dog (GSD), Labrador, and Malinois with ratio of 2 females to 13 males. The *SOD1* genotypic distribution among all was 20% wild type (G/G), 57% heterozygous (G/A) and 23% homozygous (A/A). Five of the subjects had some form of neurological deficits. The findings show that the *SOD1* mutation is present in the dog population in this study. The data justify prospective follow-up researches to link the mutation to development of clinical disease.

Keywords: canine, superoxide dismutase 1 gene, *SOD1*

INTRODUCTION

The superoxide dismutase 1 (*SOD1*) gene is a highly conserved, ubiquitous encodes *SOD1* protein, which acts as a major antioxidant enzyme. This enzyme confers defense against oxygen toxicity by metabolising superoxide radicals to

molecular oxygen and hydrogen peroxide (Niwa *et al*., 2007). Mutations in the *SOD1* gene have been identified to be the cause of amyotrophic lateral sclerosis (ALS), which is a neurodegenerative disorder in humans characterised by the progressive loss of upper and lower motor neuron function (Kiernan *et al*., 2011). Canine degenerative myelopathy (CDM) is a similar condition in dogs characterised by gradual and progressive pelvic limb ataxia and paraparesis that ultimately develops into tetraplegia and eventually cause death. The CDM was identified to also caused by a mutation in the *SOD1* gene (Coates and Wininger, 2010). A recent genetic study had identified a mutation in the *SOD1* gene at amino acid position 118 of the *SOD1* protein in affected dogs (Awano *et al*., 2009). This mutation currently serves as a genetic biomarker for CDM and the genotyping protocol for this biomarker has been developed. There is currently no study on the prevalence of this disease and presence of the *SOD1* mutation in Malaysia. The neurological disease is underdiagnosed in Malaysia because of lack of awareness and limitations of diagnostic facilities. Therefore, this study was undertaken to improve the genotyping protocol for detection of specific *SOD1* mutation and to determine the genotypic distribution of a superoxide dismutase 1 mutation (*SOD1* 118G>A) in dogs referred to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM).

MATERIALS AND METHODS

Blood samples were obtained from 30 dogs of various breeds and age presented to UVH, UPM. Genomic DNA(gDNA) was then extracted using a commercial kit (DNeasy® Blood and Tissue, Qiagen, UK). Quantification of gDNA was performed by spectrophotometry (Eppendorf, EU). Polymerase chain reaction (PCR) was carried out on the extracted gDNA to amplify the *SOD1* gene. Forward and reverse primers (Eurofins, Germany) and PCR reaction mix (TopTaq® DNA polymerase, Qiagen, UK) were used. Purification of PCR products was by QIAquick® PCR Purification Kit (Qiagen, UK). Polymerase chain reaction and purified PCR products were visualised using agarose gel with 100bp mass DNA ladder to ascertain DNA quality (Quick-Load, New England Biolabs, UK). Digestion of purified PCR products was then performed with *Hpy*AV restriction endonuclease (New England Biolabs, UK) and viewed on 2.0% agarose gel. An undigested control was included for comparison.

RESULTS AND DISCUSSION

The samples were classified as either being of wild type (G/G), heterozygous (A/G) or homozygous for the mutation (A/A). Samples of wild type show a single band of 204bp while for those homozygous for the mutation show a single band of 236bp that is the same as the undigested control. Samples heterozygous for the mutation show up as two bands with sizes of 204 and 236bp at ideally equal intensity. In this

study, the undigested control was shown to have result consistent with homozygosity for the mutation in multiple digestions, it was chosen for comparison with other samples.

Twenty-three percent (n=6) had a wild-type profile with a single band at 204bp, whereas majority of the samples (57%; n=17) were heterozygous for the mutation. The remaining samples (20%; n=7) were homozygous for the mutation (Table 1).

Table 1: Distribution of wild type, heterozygous and homozygous *SOD1* gene mutants in dogs.

Genotype	Number of samples
Wild Type (G/G)	6 (23%)
Homozygous mutant (A/A)	7 (20%)
Heterozygous (G/A)	17 (57%)
Total	30

The genotypic ratio of wild type (WT), heterozygous (Het) and homozygous mutants (Homo) in GSDs is 3WT:10Het:4Homo with high heterozygous frequency while for the Labradors it was 3WT:5Het:3Homo. The Malinois dogs were heterozygous in mutation. In studies by others, the distribution of wild type (G/G) in was reported to range from 9 to 49% while for those homozygous in mutation (A/A) ranged 8 to 48.4%. The variation in mutants could be attributed to breed differences. The heterozygous mutants ranged from 27 to 45% (Awano *et al*., 2009; Chang *et al*, 2013 Holder *et al*., 2014; Zeng *et al*., 2014). The frequency of heterozygosity in mutation in our study was inconsistent, with values being on the high side (57%). We did not know the heritage of the subjects used in this study, thus it could possibly be due to the inheritance of the mutant gene from their parents. In our study, homozygote mutants did not express any clinical sign consistent with DM. The average of onset of clinical signs of DM is 8 years. Since the subjects in our study were young dogs with average age of 4.3 years, DM clinical signs were yet to develop (Coates and Winnger, 2010).

CONCLUSION

The genotyping protocol had detected *SOD1*:c.118G>A mutation in the dogs referred to UVH, UPM. In this study, we successfully determined the genotypic distribution of the mutation in the dogs. It showed be noted that none of the homozygous mutant dogs developed clinical signs consistent with DM. Thus, follow-up studies on these dogs are required to determine if they develop the disease when they are older.

REFERENCES

Awano T, Johnson GS, Wade CM, Katz ML, Johnson GC, Taylor JF, Perioski M, Biagi T, Baranowska I, Long S, March PA, Olby NJ, Shelton GD, Khan S, O'Brien DP, Lindblad-Toh K, Coates JR (2009). Genome-wide association analysis reveals a *SOD1* mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences*, 106(8): 2794-2799.

Chang HS, Kamishina H, Mizukami K, Momoi Y, Katayama M, Rahman MM, Uddin MM, Yabuki A, Kohyama M, Yamato O (2013). Genotyping assays for the canine degenerative myelopathy-associated c. 118G> A (p. E40K) mutation of the *SOD1* gene using conventional and real-time PCR methods: a high prevalence in the Pembroke Welsh Corgi breed in Japan. *Journal of Veterinary Medical Science*, *75*(6): 795-798.

Coates JR and Winnger FA. (2010). Canine degenerative myelopathy. *Veterinary Clinics of North America Small Animal Practice*, 40(5): 929-950.

Holder A, Price J, Adams J, Volk H, Catchpole B. (2014). A retrospective study of the prevalence of the canine degenerative myelopathy associated superoxide dismutase 1 mutation (*SOD1*:c.118G > A) in a referral population of German Shepherd dogs from the UK. *Canine Genetics and Epidemiology*, 1(1): 10.

Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrel JR, Zoing MC (2011). Amyotrophic lateral sclerosis. *The Lancet*,*377* (9769): 942-955.

Niwa J, Yamada S, Ishigaki S, Sone J, Takahashi M, Katsuno M, Tanaka F, Doyu M, Sobue G (2007). Disulfide bond mediates aggregation, toxicity, and ubiquitylation of familial amyotrophic lateral sclerosis-linked mutant SOD1. *Journal of Biological Chemistry*, *282*(38): 28087-28095.

Zeng R, Coates JR, Johnson GC, Hansen L, Awano T, Kolicheski A, Ivansson E, Perloski M, Lindblad-Toh K, O'Brien DP, Guo J, Katz ML, Johnson GS (2014). Breed Distribution of *SOD1* Alleles Previously Associated with Canine Degenerative Myelopathy. *Journal of Veterinary Internal Medicine*, *28*(2): 515-521.

JOINT SURGICAL PROCEDURES IN ASSOCIATION WITH RACE PERFORMANCE CONDUCTED ON THOROUGHBRED HORSES AT PERAK TURF CLUB VETERINARY HOSPITAL FROM YEAR 2008 TO 2015

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ABSTRACT

Locomotion problems and joint injuries are the main cause of early retirement in athletic horses. A retrospective study on cases of equine joint surgeries at Perak Turf Club (PRTC) from year 2008 to 2015 was carried out to determine the occurrence and frequency rates in relation to prognosis post-operative return to races. Relationships between duration to first start and number of races after surgery, and frequency of lifespan races with its contributing factors were determined. Records were acquired from equine surgery log book of Perak Turf Club Veterinary Hospital, Malaysia and race performances of the chosen horses obtained from the Malayan Racing Association website. Twenty-five percent, 218 joint surgeries out of all 858 surgery cases, were identified. The highest occurrence of joint surgeries was arthroscopic surgery at 83% (181/218 cases) with the rate done on the right carpal joint at 34% (74/218 cases). Carpal joint arthroscopic surgery contributed 81% (177/218 cases) while fetlock joint only 18% (39/218). One hundred and thirty-five horses had at least one start after the surgery with the median number of days to the start of 180 days. Eighty-eight horses were identified to have successfully raced with a median of 6 races, and 3 horses recorded more than 30 races after surgery. The average race life-span was 15 years and 54 horses (25 %) participated in 16 races. There was a relationship between age group with joint surgeries with horses aged 5 and 6 years undergoing the highest number of arthroscopic surgeries among age groups. In conclusion, horses that underwent arthroscopic surgery have good prognoses and with good restoration of post-operative racing performance. Thus, arthroscopic surgery is suggested to be the best treatment for joint injuries for race horses.

Keywords: arthroscopic, arthrotomy, racing performance, prognosis

INTRODUCTION

Diseases of locomotion system and joint injuries are the most important cause of early retirement in athletic horses (Reed, *et al.*, 2010). These abnormalities had led

to lameness and become the primary reason of lost of training days for racehorses. Most joint injuries involve the carpal, metacarpophalangeal (MCP) and metatarsophalangeal (MTP) sustained from repetitive impact loads.

In the performance of surgery in athletic horse, the optimal objective is to return the horses to athletic soundness. The recovery to athletic soundness can only occur if the joint is not too seriously damaged by injury or as a result of surgery. The decision to invade the joint surgically, involve the selection of the surgical techniques. Currently, equine surgical techniques for joint problems have gone through considerable revolution with the greatest advances in orthopedic surgery being internal fixation of fractures and arthroscopic surgery (Mcllwraith and Bramlage, 1996).

This project was conducted to determine the predisposition of anatomical locations of horses to skeletal or joint related problems due to intensive training. Hence, the objectives of this study were to identify the equine joint surgical procedures at the Perak Turf Club and determine post-operative race performance of horses.

MATERIALS AND METHODS

Hospital surgical records and life-time race records were obtained for Thoroughbred horses that underwent joint surgery for locomotion disturbances and lameness at Perak Turf Club Veterinary Hospital, Malaysia between year 2008 and 2015. From Malaysian Racing Association (MRA) website, the data collected were frequency of races in the lifetime to date, number of races after the surgical procedure, and duration from surgery to first start. There were 3 different surgical approaches for the different joint locations or lesions. The arthroscopic and arthrotomic surgeries were classed based on the joint anatomical locations and arthroscopic-guided internal lag screw fixation according to lesions. Each surgical technique was then determined for significance association with duration to the first race, number of post-operative races, and number of lifespan races using the Kruskal Wallis and *post hoc* Mann-Whitney tests.

RESULTS AND DISCUSSION

From 858 surgical cases performed in Perak Turf Club Veterinary Hospital (VH), 218 were related to the joint area. The results showed that arthroscopic surgery was the most frequent surgery done at 181 cases (83.0%), followed by arthroscopic-guided internal lag screw fixation at 19 cases (9.0%) and arthrotomy at 18 cases (8.0%).

In arthroscopic surgery, the frequency of the carpal joint surgery was the highest with 147 cases (8%), followed by fetlock joint with 33 cases (18%) and stifle joint at 2 cases (1%). Most carpal joint injuries involved abnormalities of the subchondral bone or articular margin. Usually carpal joint appearred most

vulnerable to chronic subchondral bone pathology (Reed *et al.*, 2010).

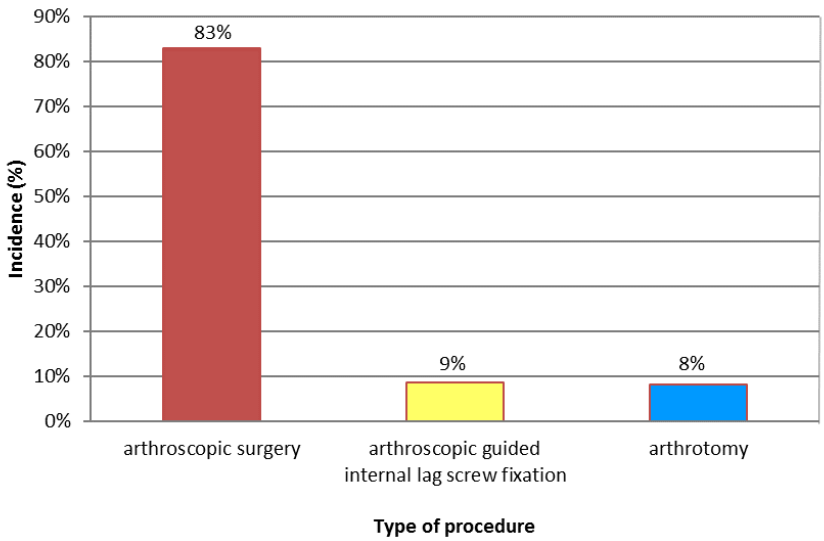

Figure 1: Frequency of joint surgical procedures at Perak Turf Club Veterinary Hospital from year 2008 to 2015.

Out of 19 arthroscopic-guided internal lag screw fixation, the third carpal slab fracture was the most frequent injury requiring surgical treatment with 11 cases (58%), followed by left forelimb medial sesamoid fracture with 3 cases (16%), right forelimb medial sesamoid fracture with 2 cases (11%), and the least of all, the third carpal sagittal fracture, lateral condylar and left forelimb distal cannon fracture with each cases reported occurring only once throughout the study. The high frequency of median sesamoid bone injury suggests that during intense activity, the suspensory and distal sesamoidean ligament exerts tensile forces on the proximal sesamoid bones.

Eighteen cases requiring artrotomic surgeries were recorded and the highest lesion was found at the left forelimb fetlock joint with 7 cases (38.9%), followed by equal frequency of right forelimb and right hind limb fetlock joint surgeries at 4 cases (22.2%) each, the right carpal joint with 2 cases (11.1 %), and finally the left carpal joint with one case (5.6 %). The fetlock joint surgery-requiring surgeries were in horse aged between 5 to 9 years. This finding is attributed to the accumulated microdamage from joint impacts in the older horses (Reed *et al.*, 2010)

The median age of horse the underwent arthroscopic and arthroscopic-guided surgeries was 5 years old and for arthrotomic 6 years old. The fastest return to racing (first start) was 120 days in horses subjected to arthroscopic surgery while the slowest was 510 days in horses that underwent arthroscopic-guided internal lag

screw fixation. The slow return to racing in the horses after arthroscopic surgery was similarly reported by Mcllwraith, *et al.*, 1987. The median number of post-operative race for after the surgical procedures was 6 races with the highest at 72 races after arthroscopic surgery.

In conclusion, horses aged from 5 to 9 years showed the highest frequency of joint injuries that required surgery.

REFERENCES

Reed SR, Jackson BF, Mcllwraith CW, Wright IM, Pilsworth R, Knapp S, Wood JL, Verheyen KL (2010). Descriptive epidemiology of joint injuries in Thoroughbred racehorses in training. *Equine Veterinary Journal*, 44(1): 13-19.

Mcllwraith CW and Bramlage LR (1996). Surgical treatment of joint injury. In: McIlWraith, CW and Trotter, GW (Editors), *Joint disease in the horse*, 1st Edition, Philadelphia, Saunders. Pp292-316.

Mcllwraith CW, Yovich JV, Martin GS (1987). Arthroscopic surgery for the treatment of osteochondral chip fractures in the equine carpus. *Journal of the American Veterinary Medicine Association*, 191(5): 531-540.

A RETROSPECTIVE STUDY ON EQUINE SKIN DISEASE CASES REFERRED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FROM YEAR 2011 TO 2015

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ABSTRACT

A retrospective study on cases of equine skin diseases referred to University Veterinary Hospita (UVH), Universiti Putra Malaysia from the year 2011 to 2015 was conducted to determine the incidence and prevalence of skin diseases in horses and to determine the relationship between disease occurrences with contributing factors. Records were obtained from equine case log books at UVH. Six hundred and sixty-six skin cases were referred to the hospital during the five years. There were 133 cases of equine cutaneous diseases with the prevalence rate of 25, 24, 21, 17 and 22% in 2011, 2012, 2013, 2013, 2014, and 2015, respectively. The highest incidence of equine skin diseases was caused by traumatic injury, followed by dermatophilosis, cutaneous myiasis, insect bite hypersensitivity, and dermatophytosis. There was no significant ($p>0.05$) relationship between gender and breed with disease distribution. Horses used for leisure riding showed the highest number of skin problems. The area of the body most affected by skin diseases were the mane, neck, shoulder, and thoraco-abdominal regions.

Keywords: retrospective study, prevalence, incidence, cutaneous, equine

INTRODUCTION

Cutaneous diseases are the second most commonly reported clinical case at University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). Although almost all equine skin diseases and conditions are not serious and are merely a cosmetic blemish that can be left untreated, some problems can affect other body systems or worsen underlying illnesses. Skin diseases of horses found in a herd are usually caused by either infection, nutritional deficiency or poisoning (Inokuma *et al*., 2003; Cafarch *et al.,* 2013; Yu, 2015;). In Malaysia, although equine skin diseases are reported all the time, few studies has been conducted. Therefore, this study was undertaken to determine skin disease-causing factors and

preventive measures to be institute to avoid disease from recurring and becoming a herd problem.

MATERIALS AND METHODS

This retrospectively study was conducted on cases related to equine skin diseases referred to the Large Animal Ward, UVH, UPM. The cases during the period of January 2011 to December 2015 were divided into two categories: infectious and non-infectious diseases. All cases that were taken into account were only from primary visit/complaint and data obtained were case number, owner/place involved, patient signalment (such as age, sex and breed), months, lesion distribution on body regions, and type of work/purpose of the horse.

RESULTS AND DISCUSSION

From 3060 equine cases that have been referred to UVH from the year of 2011 until 2015, only 666 cases were due to skin diseases and injuries. The average number of cases of equine skin diseases was 133 per year, with the highest number frequency in 2011 at 163 (25.19%) and the lowest in 2014 at 117 (17.41%) (Figure 1). The cases showed a decreasing pattern during the five period. The trend is suggested to be attributed to horse owners becoming more aware of the disease and had taken greater precaution by improving stable management, hygiene, and pest control.

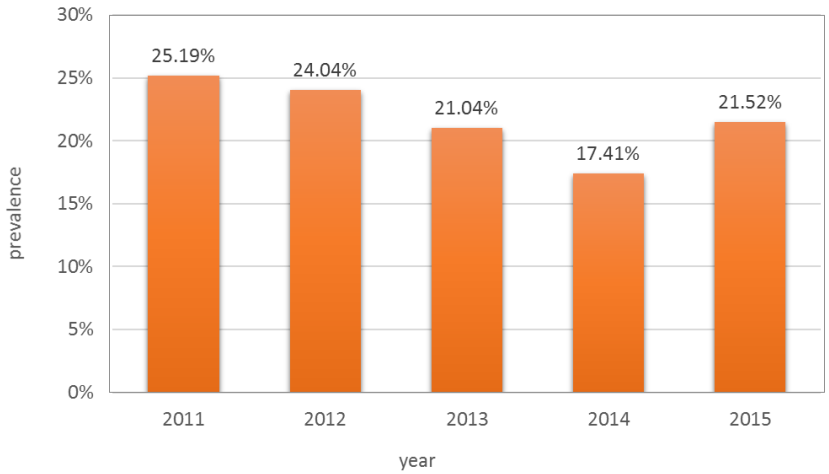

Figure 1: Prevalence of equine skin diseases referred to University Veterinary Hospital, Universiti Putra Malaysia from year 2011 to 2015.

The was no relationship between frequency of skin diseases and sex/gender of the horses. There were 358 (53.75%) and 240 (36.04%) cases in geldings and mares, respectively. In comparison, the number of cases reported for stallions was only 38 (5.71%). These figures reflect that horse owners in Malaysia geld their stallions based on their behaviour and stallions are only raised for breeding purposes.

The highest incidence of equine skin diseases was in horses used for leisure riding with 349 (52.40%) cases. This indicates that these horse were kept under poor stable management, nutrition, and hygiene. Traumatic injuries were of highest incidence in horses, regardless of their purpose.

Bacterial and fungal skin diseases cases were of highest frequencies between October and December each year. In contrast, parasitic infestation in horses were more often in May with 7 cases compared to other months. Out of 20 cases of cutaneous habronemiasis reported to UVH in the 5-year period, 12 were during the dry season, from March and July. In the non-infectious group, skin allergy/hypersensitivity reported the highest number of cases in driest (March and April) and wettest (November) months. This is suggestive to be associated with proliferation of culicoides biting midges in the wet months and allergenic pollen in the dry months.

The highest incidence of bacterial cases, particularly dermatophilosis were around the mane, neck and shoulder region at 31.3%, followed by thoraco-abdominal area at 25.0%, and brisket region, tail base, back and rump each area at 12.5%. Similarly, in cases of dermatophytosis the most common area to be affected were the mane, neck, shoulder, and thoraco-abdominal regions, which was at 28.6%, followed by tail base, back and rump at 14.3%, facial area at 9.5% and chest at 4.8%. Facial area, particularly periocular region showed the highest distribution of parasitic infestation especially due cutaneous habronemiasis at 58.3%. The mane, neck and shoulder region were mostly affected by mange at 16.7% while the thoraco-abdominal, chest and limbs region by tick infestation and cutaneous habronemiasis at 8.3%. Only 4 cases were reported with equine viral papillomas with 40% of lesion distribution around the tail base and facial region. Skin allergy or hypersensitivity was highest incidence on the mane, neck and shoulder region at 37.5%, followed by along the dorsal midline, back and rump at 25.0%. Among the skin allergies/hypersensitivities, 20.8% were generalized. Equine cutaneous neoplasia, melanoma, was highest lesion distribution around the genital area and tail base at 23.5 and 11.8%, respectively.

REFERENCES

Yu A (2015). Equine Crusting Dermatosis. *AAEP Resort Symposium.* http://files.eventsential.org/b6a3b65a-f39c-4146-ae3a-c5737f59fefb/event-338/64072229-Yu%20-%20Equine%20Crusting%20Dermatosis.pdf (Accessed on 13 September 2016).

Inokuma H, Kanaya N, Fujii K, Anzai T, Maeda K, Okuda M, Onishi T (2003). Equine pyoderma associated with malnutrition and unhygienic conditions due

to neglect in a herd. *Journal of Veterinary Medicine Science,* 65(4): 527- 529.
Cafarchia C, Figueredo LA, Otranto D (2013). Fungal diseases of horses. *Veterinary Microbiology,* 167: 215- 234.

COLISTIN SUSCEPTIBILITY PATTERN OF MULTIDRUG- RESISTANT *ESCHERICHIA COLI* FROM POULTRY FARMS IN MALAYSIA

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ABSTRACT

Colistin, a critically important antibiotic in human medicine is being used in the poultry industry to control bacterial infections. this was undertaken to describe the colistin susceptibility pattern of multidrug-resistant (MDR) *Escherichia coli* in selected poultry farms in Malaysia. Data for 69 antimicrobial susceptibility profile of multidrug-resistant *E. coli* that were subjected to antibiotic sensitivity testing using Kirby-Bauer disk diffusion method for the period of 1st January 2014 to 1 December 2015 was retrieved from a private laboratory. The results showed MDR *E. coli* showed highest susceptibility to colistin at 90%, followed by fosfomycin at 57%. The MDR *E. coli* showed total non-susceptibility to amoxicillin/clavulanic acid, amoxicillin and tilmicosin. There is no significant ($p>0.05$) association between colistin susceptibility pattern and year or resistance pattern to other antibiotics.

Keywords: colistin, poultry, multidrug-resistant, *E. coli*, susceptibility pattern

INTRODUCTION

In 2015, the plasmid-mediated colistin resistant bacteria in animals and humans was discovered to be widespread in China (Liu *et al*., 2016). Colistin is an important drug in human medicine, thus, the consequence of its veterinary use that could affect human health must be ascertained (WHO, 2014). Poultry industry is one the most important livestock in Malaysia. The use of antibiotics in control of diseases is rampant. Thus, it is important to understand the antibiotic susceptibility pattern to these antibiotics to ensure that there is no adverse effect on human health (EMA-ESVAC, 2016). In this retrospective study, the susceptibility pattern *E. coli* from poultry to colistin was determined.

MATERIAL AND METHOD

A retrospective study on 69 antimicrobial susceptibility profiles of multidrug-resistant (MDR) *E. coli* from several poultry farming in Malaysia was determined. Data for the period of 1stJanuary 2014 to 1st December 2015 on the antimicrobial susceptibility profiles were obtained from a private laboratory from. The association between colistin susceptibility pattern, year, and resistance to others tested antibiotics were determined at 95% confidence and significant at $p<0.05$ using Chi Square Test. All the statistical analyses were performed using SPSS software version 20 (SPSS, IBM Inc, USA).

RESULT

The MDR *E. coli* showed highest susceptibility to colistin at 90%, followed by fosfomycin at 57%. The bacteris was totally non-susceptibility to amoxicillin/clavulanic acid, amoxicillin, and tilmicosin. There is no significant association between colistin susceptibility pattern and year or resistance pattern to other tested antibiotics.

DISCUSSION

Colibacilosis is a common disease of economic importance in poultry. Colistin is one of the choice antibiotic to treat colibacilosis. The study showed that colistin is more effective against MDR *E. coli* than other antibiotics. There susceptibility pattern of *E. coli* to colistin did change over the period of the study. This finding suggests that the use colistin in Malaysia is prudent and well under control. However, this study was conduct on a small sample and there is need to conduct a large scale survey to determine the true picture on the use antibiotic in the region (EMA-ESVAC, 2016). Since information on use of antimicrobials in poultry farms in Malaysia is limited, the authorities must institute close monitoring on their use.

CONCLUSION

The study showed that *E. coli* is most sensitive to colistin than other antibiotics. However, to ensure reliability of results, more sensitive techniques as the polymerase chain reaction technique must be employed by testing laboratories.

REFERENCES

EMA-ESVAC (2016). Update on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. European medicines Agency. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/05/WC500207233.pdf (Accessed on 13 September 2016).

WHO (2014). Antimicrobial resistance: global report on surveillance 2014. Geneva; World Health Organization. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1 (Accessed on 13 September 2016).

Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Ly L, He D, Zho H, Liang Z, Liu J-H, Shen J (2014). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 I animals and human being in China: a microbiological and molecular biological study. *The lancet Infectious Diseases*, 16(2); 161-168.

EFFECT OF EDIBLE-BIRD'S NEST IN TRIS AND BIOXCELL EXTENDERS ON CRYOPRESERVATION OF BULL SEMEN

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ABSTRACT

Semen cryopreservation is a procedure of preserving semen for an indefinite period. The aim of this study was to evaluate the effects of supplementing edible-bird's nest (EBN) into Tris (Tr) and Bioxcell (Bx) extenders for bull sperm cryopreservation. Twelve semen samples was collected from mature bulls by electro-ejaculation. The semen samples were evaluated both freshly and after cryopreservation for quality based on % sperm general and progressive motility, viability, and abnormal morphology. Fresh samples were diluted and extended using the two extenders containing 0 (control), 0.03, 0.06, and 0.12% of EBN, chilled at 4 °C for 3 hours before packaging into 0.25 mL straws and frozen into liquid nitrogen at -196 °C for 48 hours. The results revealed insignificant ($p>0.05$) differences in all parameters between treatment groups. However, extenders containing 0.12% EBN showed the lowest percentage of abnormality that is close to the fresh samples. Moreover, Bx produced lower percentage of abnormality than Tr with the same EBN treatment, implying it has a more protective role on bull semen. In conclusion, EBN in semen extenders did not significantly improve sperm quality after freezing.

Keywords: semen cryopreservation, edible-bird nest, bulls, Tris and Bioxcell extenders

INTRODUCTION

Semen cryopreservation is a biotechnology to preserve and store sperm for a short or long time for many purposes such as in assisted reproduction technologies, species or breed conservation, and clinical medicine. However, the quality and life-span of frozen-thawed semen decrease with storage (Barbas and Mascarenhas, 2009). One of the cause of loss of semen quality is reactive oxygen species (ROS). Antioxidants are present in the seminal plasma of the bull (Karunakaran *et al.* (2012). However, when the production of ROS exceeds the antioxidant capacity of

the seminal plasma during cryopreservation, it leads to oxidative stress, resulting in loss of sperm motility and viability.

Edible-bird's nest (EBN) is a dried glutinous secretion from the salivary glands of several different swiflet species, mainly *Aerodramus fuciphagus*, and contains 35.8% of protein and 46.5% of carbohydrate (Lee *et al.*, 2015). Thus, being rich in essential nutrients and antioxidant activity, EBN is thought to have a positive effect in maintaining sperm quality during cryopreservation. Hence, this study was conducted to determine the effect of adding EBN to Tris and Bioxcell extenders on the post-thaw quality of cryopreserved bull sperm.

MATERIALS AND METHODS

Twelve semen samples were collected from seven sexually matured bulls in Ladang 16, Taman Pertanian Universiti (TPU), Universiti Putra Malaysia using the electroejaculator. The volume, colour and concentration of fresh samples were recorded and semen quality evaluated before diluting in Tris and Bioxcell extenders that contain 0 (control), 0.03, 0.0%, or 0.12% edible-bird nest (EBN). The diluted semen was then chilled at 4 °C for 3 hours before packaging into 0.25 mL straws and frozen into liquid nitrogen at -196°C for 48 hours. The straws were randomly selected for each treatment group and thawed in the 37 °C water bath for 30 seconds, and semen parameters determined. Semen quality parameters that were assessed were general and progressive motility, viability and abnormal morphology such as bent tail, coil tail or dag defect, taillessness, and decapitated and head abnormalities. All the data were analyzed using a one-way analysis of variance (ANOVA) and the differences among means were tested for significance by *post hoc* Tukey test at α=0.05.

RESULTS AND DISCUSSION

The mean semen volume was 7.88 mL with mean semen concentration of 1.19 × 10^9 sperm/mL. The results show that the general motility, progressive motility and viability of the fresh semen were significantly ($p< 0.05$) higher compared to the post-thawed semen sample of treatment groups. The abnormalities of the post-thawed treatment were higher than fresh semen, although not statistically significant ($p>0.05$). Besides, Bioxcell and Tris extenders with 0.12% of EBN show the lowest semen, but not significant ($p>0.05$) different abnormalities among treatment groups.

According to Bansal and Bilaspuri (2011), the reactive oxygen species (ROS) increases and antioxidant level decreases during cryopreservation. Thus, during exposure of sperm to cold shock and atmospheric oxygen during cryopreservation, the occurrence of lipid peroxidation may result in damage of sperm plasma membrane with consequential loss of sperm motility and membrane and morphological integrity, impairment of cell functions, and apoptosis of sperm.

CONCLUSION

The study showed that the concentrations of the EBN used were inadequate to provide significant positive effect to the bull semen quality after cryopreservation. However, there was observable reduction in percentage semen abnormality with the addition of 0.12% EBN in the extenders used for crypreservation. The results warrant further study on the use EBN as a component of semen extenders.

REFERENCES

Bansal AK and Bilaspuri GS (2011). Impacts of oxidative stress and antioxidants on semen functions. http://www.hindawi.com/journals/vmi/2011/686137/
(Accessed on 8 October 2015).

Barbas JP and Mascarenhas RD (2009). Cryopreservation of domestic animal sperm cells. *Cell Tissue Bank,* 10 (1): 49-62.

Karunakaran M, Devanathani TG, Kulasekar K, Sridevi P, Jawahar TP, Loganatahsamy K, Dhali A, Selvaraju S (2012). Effect of fertility-associated protein on oxidative stress of bovine sperm cells. *Indian Journal of Animal Reproduction,* 30 (1): 43-46.

Lee TH, Tan ETT, Wani WA, Aziz R (2015). Investigations into the physicochemical, biochemical and antibacterial properties of edible bird's nest. *Journal of Chemical and Pharmaceutical Research,* 7(7): 228-247.

COMPARISON OF CYTOLOGIC STAINING TECHNIQUES ON THE MORPHOLOGY AND MORPHOMETRY OF BOER GOAT SPERMATOZOA

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ABSTRACT

Visual observation of sperm has led to widely varying results due to numerous factors such as the use of different staining procedures and lack of standardisation of staining techniques. This study was conducted to compare cytology staining techniques to be used for the identification of the best staining methods for semen evaluation. Seven semen samples were collected using an electro-ejaculator on Boer bucks at Universiti Agricuture Park, Universiti Putra Malaysia (n = 4) and a Labu, Negeri Sembilan, Malaysia (n = 3) farm. Each sample was processed and assessed using routine semen evaluation protocols. Semen smears were stained by Eosin-Nigrosin, Giemsa, Diff-Quik, and Hematoxylin-Eosin (HE) stains. The stained semen was examined and morphometric measurements of 50 randomly selected spermatozoa in each slide made. There were significant ($p<0.05$) differences in all morphometric parameters between the stained spermatozoa. Morphometric analysis revealed for spermatozoa head measurement the lowest values were with the HE stain. Higher means of head width and length were recorded for all stains except HE. It is concluded that Boer goat sperm morphology vary with the stain used.

Keywords: spermatozoa, Boer goat, staining, morphology, morphometry,

INTRODUCTION

Reproduction in males is closely related to semen quality and sperm structure. Semen analysis to determine quality routinely includes assessment of volume, appearance, sperm concentration and motility, as well as sperm morphology, and presence of foreign cells. The morphological evaluation, now expanded to include detailed spermatozoon morphometry is considered to be a useful tool in the clinical

diagnosis of sub-fertile animals.

There are several staining methods that can be used to evaluate the morphology of spermatozoa (Uven *et al*., 1998) and to predict male fertility. The goal of sperm staining technique is to assist in the visualisation of the cells and to provide a better identification of the abnormalities through light microscopy (Villaverde *et al*., 2008). However, a common encountered problem during evaluation of the morphology and morphometry of sperm is the lack of standardisation in the staining techniques. Therefore, this study was conducted to compare cytology staining techniques and to identify the best staining method for evaluation of goat semen.

MATERIALS AND METHODS

Semen collection in animals

Semen was collected from 7 adult Boer-cross goats from University Agriculture Park (TPU), Universiti Putra Malaysia (n=4) and the Tok Seri Buak Agrofarm, Labu, Negeri Sembilan, Malaysia (n=3). Semen was collected from the goats using the electro-ejaculation method.

Semen evaluation

Sperm concentration, gross motility, progression and sperm motion were analyzed by light microscopy.

Preparation of smears

A minimum of five semen smears were prepared for each sample and air-dried.

Staining procedure

The semen smears were stained with either Eosin-Nigrosin (EN), Giemsa (G), Diff-Quik (DQ), or Hematoxylin-Eosin (HE) stain. Morphology and morphometric evaluation were done via a light microscopy connected to an image analyser. Fifty sperms from random fields were selected form each smear, assessed and measured. Staining efficiency was scored based on the modified staining technique described by Lingappa *et al*., (2015) with score 0=pale/poorly stained, +=not clear, ++=clear and +++=very clear.

Statistical analysis

Data were analyzed using Kruskal–Wallis test and further elaborated using the Mann–Whitney test for pair-wise mean comparison.

RESULTS AND DISCUSSION

The staining efficiency mean scores are summarised in Table 1. Under EN staining, dead spermatozoa stained pink. The morphology of the head could be seen clearly

whereas, the boundary of the mid-piece and the remainder of the tail was not easily distinguishable. The spermatozoa stained dark-blue purple with Giemsa. The acrosome part stained rose-pink while the post-acrosome area to the mid-piece and tail stained dark-blue purple. Head morphology and condensation could be clearly but the middle piece and the tail could not be clearly differentiated.

The head was stained homogeneously dark red under DQ. The acrosome stained red and the post-acrosome area stained dark red. The mid-piece part and tail clearly distinguishable, therefore, all morphological defects of spermatozoa could be easily evaluated. Under HE staining, the buck sperm head was basophilic in colour. The acrosome was lighter in colour, gradually becoming darker towards post-acrosome area. The mid-piece and tail stained eosinophilic, and it was difficult to distinguish between the end of the tail and the boundary between mid-piece and tail. The background of the smear was light and unstained, which did not hinder evaluation. From the results, DQ stain produced the clearest spermatozoa morphology among the stains used.

Table 1: Staining efficiency of different staining techniques

Staining technique	Morphology			
	Head	Acrosome	Midpiece	Tail
Eosin-Nigrosin	++	++	+	+
Giemsa	++	+++	+	+
Diff-Quik	+++	++	+++	+++
Hematoxylin-Eosin	+++	+++	+	++

+ least efficient to +++ most efficient.

Table 2: Means and SE for of head length and width, mid-piece and tail length of spermatozoa stained with different staining techniques

Stains	Measurement (μm)			
	Head length	Head width	Mid-piece length	Tail length
Eosin-Nigrosin	8.18±0.03	4.11±0.02	11.70±0.07	39.11±0.23
Giemsa	8.24±0.03	4.12±0.02	11.97±0.05	38.36±0.20
Diff-Quik	8.22±0.03	4.11±0.02	12.02±0.05	38.69±0.20
Hematoxylin-Eosin	7.55±0.02	3.77±0.02	12.13±0.04	38.33±0.18

Comparative assessment of the sperm morphometry is shown in Table 2. The results showed that HE had the lowest mean head length and head width (7.55±0.02 μm) and the highest mean belonged to G (8.24±0.03 μm). The lowest mean of mid-piece length was with EN whereas the highest with HE stain. The

lowest mean of tail length was with HE, while the highest mean was with EN stain. There were significant ($p<0.05$) lower mean head length and head width under HE stain compared to the other stains.

CONCLUSION

In conclusion, the study shows that staining techniques affect sperm morphology. The choice of stain has the potential to cause small alterations in sperm dimensions. The results showed that the best staining method for sperm is Diff-Quik. Haematoxylin-Eosin stain is not recommended to be used in sperm morphometric assessment.

REFERENCES

Lingappa HA, Govindashetty AM, Krishnamurthy A, Puttaveerachary AK, Manchaiah S, Shimoga I C, Mallaradhya SH, Gowda SBM (2015). Quest for an ideal, simple and cost-effective stain for morphological assessment of sperms. *Journal of Clinical and Diagnostic Research*, 9(10): EC01.

Uven M, Can B, Saran Y (1998). Ultrastructural changes in the spermatozoa of infertile men. *Turkiye Klinikleri Journal Medical Research*, 16:103-5.

Villaverde AISB, Melo CM, Corrente JE, Papa FO, Lopes MD (2008). Comparison between two staining methods for morphological analysis and acrosome of sperm domestic cat (*Felis catus*). *Ciênce Animmal Brasil,* 9(3): 686-692.

MYCOBACTERIUM AVIUM SUBSPECIES *PARATUBERCULOSIS* INFECTION IN BEEF CATTLE AT UNIVERSITY AGRICULTURE PARK, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the aetiological agent for Johne's disease that is a chronic, contagious bacterial intestinal tract infection affecting ruminants. The infection is characterised by chronic or intermittent diarrhoea, progressive weight loss, decreased production and causing substantial economy losses to the farmer. This study was conducted to determine the presence of MAP antigen and antibodies in the faeces and serum of the cattle, respectively. Two hundred and thirteen faecal samples and 71 serum samples were collected from 71 cattle at University Agriculture Park (TPU), Universiti Putra Malaysia. These faecal samples were subjected to Ziehl-Neelsen acid fast staining for antigen detection and serum samples to complement fixation test (CFT) for antibody detection. The results showed that 60 (28.2%) faecal samples were positive at Ziehl-Neelsen staining while 3 (4.2%) were positive for *M. avium* antibodies. In conclusion, MAP infection was present in the herd at TPU.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), intermittent diarrhoea, CFT, Ziehl-Neelsen acid fast stain, complement fixation test.

INTRODUCTION

Mycobacterium avium subsp. paratuberculosis (MAP) is the aetiological agent for chronic bacterial enteritis in ruminants known as Johne's disease or paratuberculosis (Mercier, 2014). This organism is characterised as small, acid-fast bacilli on Ziehl-Neelsen stain. The disease caused by MAP is characterised by chronic enteritis with diarrhoea, unthriftiness and progressive weight loss despite good appetite. This disease causes significant economic loss to the farmer if left untreated. Infected animals can produce large amount of the bacteria and shed it in the faeces, which the primary source of infection. In approximately 33% cows infected with this disease, whether with or without clinical signs, contain the

bacteria in their colostrum and milk (Haskell, 2008).

Mycobacterium avium subsp. paratuberculosis upon ingestion, survives and replicates within the macrophages of the intestinal wall and regional draining lymph nodes, since is resistant to intracellular degradation (Hayton, 2007). In macrophages, the bacteria replicate slowly and stimulate inflammatory and cellular response.

The economic impact of the disease in beef production is devastating particularly from loss of production. There is no known treatment for this disease. Control measures for the infection involve good hygiene and husbandry management (Mercier, 2014).

The method to diagnose Johne's disease is either by the detection of antigens in faecal or tissue samples or serological techniques. The most common serological method used for Johne's disease diagnosis are complement fixation test (CFT), absorbed enzyme-linked immunosorbent assay (ELISA), and agar gel immunodiffusion (AGID). However, the sensitivity and specificity of serologically and specificity relies on faecal culture results.

This study was undertaken to determine the presence of MAP in the cattle herd in University Agriculture Park (TPU), Universiti Putra Malaysia.

MATERIALS AND METHODS

Seventy-one beef cattle or Kedah-Kelantan (n=34) and Brangus breed (n=37) were used in this study. Faecal samples (n=213) and blood samples (n=71) were processed using Ziehl-Neelsen acid fast staining and complement fixation test (CFT), respectively.

RESULTS AND DISCUSSION

The result of faecal Ziehl-Neelsen staining and serum CFT are presented in Table 1. The Ziehl-Neelsen staining showed 28.2% (60/213) were positive for acid-fast organisms while only 4.2% (3/71) serum samples were positive for the antibacterial antibodies.

Table 1: Faecal acid-fast bacteria and serum anti-bacteria antibody

Cattle Breed	Faecal organism (Ziehl-Neelsen staining)			Serum antibody (Complement fixation test)		
	Positive	Negative	Total	Positive	Negative	Total
KK	46	56	102	1	33	34
Brangus	14	97	111	2	35	37
Total	60	153	213	3	68	71

KK=Kedah-Kelantan

However, since MAP not being the only acid-fast organisms, *Nocardia sp* and *Corynebacteria sp* can be positive results that is a diagnosis for Johne's disease. The test method used in this study is of low sensitivity and specificity, therefore, other test particularly faecal culture and the isolation and identification of the organism are required to confirm diagnosis. Faecal culture can a definitive diagnosis because it is 100% specific. However, the method is time-consuming and very difficult to perform because of contamination (Mercier, 2014). Although CFT produced low positive MAP samples, it does not mean that the herd at TPU had low MAP. Based on stages of MAP infection, positive samples by acid-fast staining indicate that the animals were in subclinical infection stage.

CONCLUSION

This study indicates there are presence of MAP infection among beef cattle in TPU based on the CFT and acid fast staining method. However, for acid fast staining method result, it should be supported with other diagnostic method to identify it as true MAP organism.

REFERENCES

Haskell SRR (2008). Johne's disease: Blackwell's five-minute veterinary consult: ruminant, 1st Edition. Ames, Iowa, Wiley-Blackwell. Pp444 – 445.

Hayton AJ (2007). Johne's disease. *Johne's management*. http://www.holstein-uk.org/media/legacyhw/Hot%20Topic/Johnes%20Management%20Paper (Accessed on 5 January, 2016).

Mercier P (2014). Paratuberculosis (Johne's disease). *Manual of diagnostic tests and vaccines for terrestrial animals 2015 (Chapter 2.1.11)* http://www.oie.int/international-standard-setting/terrestrial/manual/ (Accessed on 2 September 2016).

A SURVEY ON FOOT AND MOUTH DISEASE IN CATTLE AND BUFFALOES AT SELECTED LOCATIONS IN MALAYSIA FOR YEAR 2010 TO 2015

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. Malaysia has been importing live ruminant for domestic demand and this had poses risks to the local ruminant industry. Movement of FMD-infected animals is one of the main factors for outbreaks. The objectives of this study were to determine the seroprevalence of FMD in cattle and buffaloes in Rantau Panjang and Padang Besar Animal Quarantine Stations, Malaysia from 2010 to 2015, and to describe the outbreaks of FMD in six selected states. The existing data between 2010 and 2015 were reviewed to include results from the enzyme-linked immunosorbent assay (ELISA) and the 3ABC non-structural protein (NSP) test for FMD retrieved from the Regional Veterinary Laborator, Kota Bharu, Kelantan. The outbreaks data were extracted from the World Organization for Animal Health-Regional Coordination Unit (OIE-RCU) website. Based on the NSP tests, the overall seroprevalence of FMD in quarantine stations averaged at 36.4% with 40.7 and 34.9%, for Rantau Panjang and Padang Besar, respectively. There was significant ($p<0.05$) differences between stations. The highest FMD seroprevalence was recorded in 2011 and the lowest in 2012. There was significant ($p<0.05$) difference among in seroprevalence among years. Cattle showed significantly ($p<0.05$) higher seroprevalence than buffaloes at 36.6 and 30.7%, respectively, and in males than females at 39.1 and 30.4%, respectively. There 69 FMD outbreaks of FMD the northern states of Peninsular Malaysia from 2010 to 2015. Terengganu recorded the highest number of outbreaks at 26 while Perlis the lowest with one outbreak. The FMD outbreaks was the highest in September of each year. Virus serotypes O and A were the main cause of outbreaks in these states. The findings showed that FMD is endemic in Malaysia and control strategies need to be improved.

Keywords: foot and mouth disease, cattle, buffalo, seroprevalence, outbreaks, NSP, ELISA, quarantine station, Peninsular Malaysia

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals including cattle, pigs, sheep and many wildlife species. The FMD virus is of the *Aphthovirus* genus and family *Picornaviridae*. Common clinical signs of FMD infections are acute febrile reaction with vesicular formation consistently in and around the mouth and feet (Alexandersen *et al*., 2003). This disease is considered as an important disease in Peninsular Malaysia and is one of the major transboundary animal diseases (TAD) that had affect trade of livestock and their products to FMD free-zone country (Abila and Foreman, 2006; Kibore *et al*., 2013). The FMD test for cattle and buffaloes to differentiate infected from vaccinated animals is the determination of presence of FMD virus antibodies using the nonstructural protein (NSP) enzyme linked immunosorbent assay (ELISA).

This study was conducted with the objective of determining the seroprevalence of FMD in cattle and buffaloes at two animal quarantine stations of Peninsular Malaysia and to describe the outbreak of FMD in the northern border states of Peninsular Malaysia between 2010 and 2015.

MATERIALS AND METHODS

Seroprevalence study

Serology results from non-structural protein enzyme-linked immunosorbent assay (NSP-ELISA) test for cattle and buffaloes in the Rantau Panjang Animal Quarantine Station, Kelantan and Padang Besar Animal Quarantine Station, Perlis, Malaysia from 1st January 2010 until 31st December 2015, were obtained from the Veterinary Diagnostic Laboratory, Kota Bharu, Kelantan. The test was done using the PRIONICS PrioCHECK® FMDV antibody test kit to differentiate infected and vaccinated animals regardless of vaccination status and managed using Microsoft Excel 2010® with the following information: submitter address, date of sample submission, herd age, herd sex, species, no. of serum sample sent, no. of serum sample tested, number of serum sample tested positive. The data was imported to Statistical Package for Social Science (SPSS) version 22 for statistical analysis. Descriptive statistical analysis was done after obtaining the seroprevalence of FMD Chi square and odds ratio was calculated to determine association of risk factors with FMD (Nawaz *et. al*. 2014) and strength of the association.

Outbreak description study

The FMD outbreak data for Kedah, Kelantan, Perak, Perlis, Pulau Pinang and Terengganu from 1st January 2010 until 31st December 2015 were obtained from OIE FMD outbreak report and Wahis Interface. The data were described according to month, year, and number of outbreaks and the FMD virus serotype.

RESULTS AND DISCUSSION

Seroprevalence

Based on the NSP tests, the overall seroprevalence of FMD in quarantine stations was 36.4% with Rantau Panjang and Padang Besar stations showing 40.7 and 34.9%, respectively. There was significant ($p<0.05$) differences between stations ($\chi^2=42.3$, df=1, $p<0.05$). The highest FMD seroprevalence was recorded in 2011 and the lowest in 2012. There was significant ($p<0.05$) differences in FMD seroprevalence among years. In 2010, the outbreaks were reported to be high in the South East Asia region. Significantly higher seroprevalence ($p<0.05$) were also found in cattle than buffaloes at 36.6 and 30.7, respectively) the result of extensive introduction of exotic and cross-breed blood cattle that are highly susceptible to FMD (Zulfiqar, 2003). It was also found that males had higher seroprevalence than females at 39.1 and 30.4%, respectively, which were contrary to findings published by Nawaz *et al.* (2014).

Outbreak description study

The total number of outbreaks of FMD in the northern states of Peninsular Malaysia from 2010 to 2015 was 69. As reported by the World Animal Health Organization (OIE), FMD outbreaks were high in 2010 in the South East Asia region. In Malaysia, Terengganu recorded the highest number of outbreaks at 26 while Perlis the lowest with one outbreak. The high number of outbreaks due increase in cattle and buffalo importation from Thailand and Myanmar. These cattle and buffaloes may have brought the disease into the country and causing the outbreaks. Illegal inter-state movements of animal could have also led to the spread of disease (Abdul-Hamid *et al.,* 2011; Abila, 2011).

The FMD outbreaks were highest in September with 13, followed by November with 11, and October with 10 incidences. The foot and mouth disease seemed to be clustered in northern part of Malaysia during the end and early calendar year (Abila and Foreman, 2006) and this coincide with festive season of the country when the demand for livestock is high (Ramanoon *et al*., 2013).

The *Aphthovirus* virus serotypes O and A were the main cause of outbreaks in in Peninsular Malaysia. The serotype O virus is the more aggressive of the two (Kitching, 2005). The findings in this study show that FMD is endemic in Malaysia.

REFERENCES

Abdul-Hamid NF, Hussein NM, Wadsworth J, Radford AD, Knowles NJ, King DP (2011). Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. *Infection, Genetics and Evolution*, 11(2): 320-328.

Alexandersen S, Zhang Z, Donaldson A, Garland AJM (2003). The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology*, 129(1): 1-36.

Abila RC (2011). The SEACFMD Progress Report. In: OIE SEACFMD (South-East Asia and China Foot and Mouth Disease Campaign), 17th Meeting of the OIE Sub-Commission for Foot and Mouth Disease Control in South-East Asia and China. Bali, Indonesia 7-11 March 2011. Paris: OIE.

Abila RC and Foreman S (2006). Control of foot and mouth disease in Southeast Asia. In: Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics. 11: pp1103-1105.

Kibore B, Gitao CG, Sangula A, Kitala P (2013). Foot and mouth disease sero-prevalence in cattle in Kenya. *Journal of Veterinary Medicine and Animal Health*, 5(9): 262-8.

Kitching RP (2005). Global epidemiology and prospects for control of foot-and-mouth disease. *Current Topics in Microbiology and Immunology*, 288: 133-148.

Nawaz Z, Arshad M, Iqbal Z (2014). Epidemiological of foot and mouth disease in buffaloes and cattle of Punjab using non-structural proteins ELISA. *Pakistan Journal of Agricultural Sciences*, 51(2): 497-501.

OIE FMD outbreak report. http://www.arahis.oie.int/reports.php (Accessed on 14 September 2016).

Ramanoon SZ, Robertson ID, Edwards J, Hassan L, Isa KM (2013). Outbreaks of foot-and-mouth disease in Peninsular Malaysia from 2001 to 2007. *Tropical Animal Health and Production*, 45(2): 373-377.

Walis Interface. http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail (Accessed on 14 September 2016).

World Organization for Animal Health (OIE) (2011). Report of 17th Meeting of the OIE Sub-Commission for Foot and Mouth Disease Control in South-East Asia and China, 7-11 March 2011, Bali, Indonesia. Office International des Epizooties, Paris.

Zulfiqar M (2003). Draft Report for Development of National Disease Control Policy for Foot and Mouth Disease in Pakistan under the FAO Project "Support for Emergency Prevention and control of main transboundary animal diseases in Pakistan Rinderpest, FMD, PPR".

COMPARISON OF CHROMOSOMAL KARYOTYPE AND REPRODUCTIVE PERFORMANCE BETWEEN THE BRAFORD COW AND ITS GAUR-CROSS OFFSPRING

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ABSTRACT

Interspecific hybridisation is a significant evolutionary process resulting in significant shifts of allele frequencies among interbreeding species. Selembu is a cross-breed between Malayan Gaur (*Seladang*) and domestic cattle (*Lembu*). The objective of this study was to compare the chromosomal karyotype and individual reproductive performance between the Braford cow and the Gaur-cross Braford cow (*Selembu*) offspring, which was produced by artificial insemination using the semen from the Malayan Gaur. Peripheral blood lymphocyte cultures from both cows were prepared and chromosomes at metaphase stage of the cell cycle were harvested, giemsa-stained and their morphology and number determined. Well-spread chromosomes were used to establish karyotypes under photomicroscope using Video Test Kayo 2.1 and 3.1 software. The results showed obvious differences in chromosomal morphology and diploid number between the Bradford and *Selembu* cow. The Braford cow had a diploid chromosome number, 2n = 60, while the *Selembu* presented an intermediate number of its parents, 2n = 58. However, the fundamental number remained the same at 62, implying the impact of the hybridisation and possible evolutionary process that took place from the same ancestor. The reproductive performance of Braford cow and *Selembu* was similar.

Keywords: *Selembu*, Braford cow, chromosome, karyotype, reproductive performance

INTRODUCTION

The theory of evolution by natural selection is the process by which organisms change over time as an outcome of changes in heritable physical or behavioural trait (Nonaka, 2014). Interspecific hybrids reveal novel genomes that are exposed to natural selection, hence providing a key to exhibit the ultimate causes of adaptation and speciation (Storz and Hoekstra 2007). The Braford breed is a cross

between Hereford and Braford, produced for beef production and durability, along with the ability to thrive in difficult climates. *Selembu* is a cross-breed between the Malayan Gaur (*Seladang*) and domestic cattle (*Lembu*).

The objective of the present study is to compare the chromosomal karyotype between the Braford cow and *Selembu*, which was the result of artificial insemination using the semen from the Malayan gaur, and their reproductive performance on records.

MATERIALS AND METHODS

Blood was collected into heparinised venoject tube for lymphocyte culture. Although whole blood is cultured, T lymphocytes are usually used for blood cytogenetic investigations (Smith, 2006). The procedure for short-term lymphocyte culture was based on the method described by Hayes and Dutrillaux (2000) with minor modifications.

Chromosomes at metaphase stage of the cell cycle were harvested, giemsa-stained and their morphology and number detemined. Well-spread chromosomes were used to establish karyotypes under photomicroscope using Video Test Kayo 2.1 and 3.1 software. The reproductive performance assessment of the animals was based farm records.

RESULTS AND DISCUSSION

The Braford cow have a diploid chromosome number, 2n = 60, while the *Selembu* presented to have an intermediate number of its parents, 2n = 58 (Figure 1).

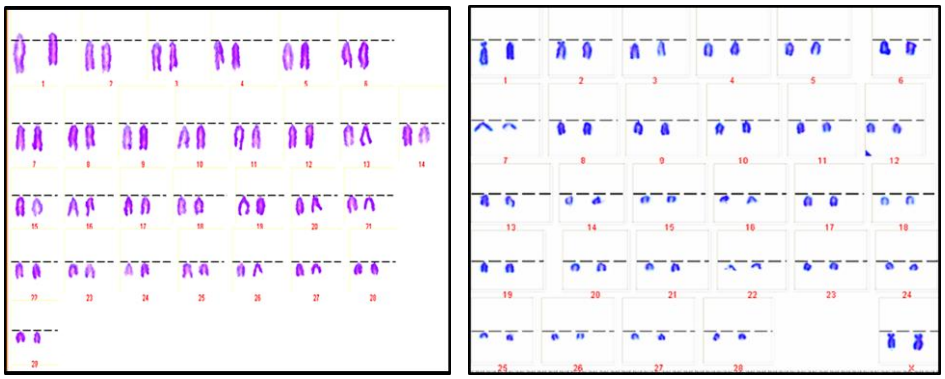

Figure 1: Complete chromosome karyotype. Braford cow (left) and *Selembu* (right).

However, the fundamental number remained the same at 62 (Table 1), implying that the impact of the hybridisation and possible evolutionary process that took place from the same ancestor. The reproductive performance of Braford cow and *Selembu* was similar (Table 2).

Table 1: Numerical and morphological description of chromosomes in Braford cow and selembu hybrid

Breed	Fundamental number	Autosomes	Sex chromosomes (xx)
Braford	62	2 = Submetacentric 54 = Acrocentric	Submetacentric
Selembu	62	58 = Acrocentric	Submetacentric

Table 2: Comparative reproductive performance of Bradford cow and Selembu.

Animal	Date of arrival/date of birth	Age at first calving (years)	No. of calving	Calving interval (months)	Post-partum period (months)
Bradford Cow	05/01/2004	3	6	19-25 (Avg. 22)	10-16 (Avg. 13)
Selembu	15/03/2011	3.5	1	Est. 23	14

CONCLUSION

There are obvious differences in chromosome number and morphology between the Braford cow and its *Selembu* offspring, which is the result of hybridisation. The fundamental number of the Bradford cow and *Selembu* was the same. This is the result of the evolutionary process from the same ancestor that allowed for the production of natural fertile interspecific offspring. In spite of factors that could influence reproduction performance of animals in the farm, the Braford cow and *Selembu* offspring had almost the same calving interval and post-partum period.

REFERENCES

Storz JF and Hoekstra HE (2007). The study of adaptation and speciation in the genomic era. *Journal of Mammalogy,* 88(1): 1-4.

Hayes H and Dutrillaux, B (2000). *In situ* Hybridization techniques. In: Techniques in Animal Cytogenetics, Popescu P, Hayes H, Dutrillaux B (Editors), Springer Verlag, Berlin. Pp69-84.

Nonaka E (2014). Evolutionary consequences of ecological interactions. *Umeå universitet*.
https://www.diva-portal.org/smash/get/diva2:710674/FULLTEXT01.pdf (Accessed on 14 September 2016).

Smith, K (2006). Basic Cytogenetic Techniques: Culturing, Slide Making, And G-Banding. Cell biology: A laboratory handbook, Volume 2, Celis JE (Editor). Elsevier Academic Press. Pp381-385.

EFFECT OF SELENIUM SUPPLEMENT ON ANTI-OXIDANT STATUS AND SERUM ASPARTATE AMINOTRANSFERASE CONCENTRATION IN BEEF CATTLE

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ABSTRACT

Selenium (Se) is an essential micronutrient required for normal growth development and antioxidant defense. The objective of this study is to determine the effect of Se supplementation on the serum oxidative stress parameters, malondialdehyde (MDA) and glutathione peroxidase (GSH-Px), and serum muscle enzyme, aspartate aminotransferase (AST) in beef cattle. Ten 1-year-old cows were selected and divided into equal control and treatment groups. The control group was treated with 10 mL normal saline intramuscularly and the treatment group with 0.1 mg/kg body weight Se, twice at the start of the experiment and two weeks later. Blood was collected weekly for 4 weeks and plasma MDA, erythrocyte GSH-Px, and serum AST concentrations determined. respectively. There was no significant ($P>0.05$) difference MDA and AST concentrations between treatment and control groups. However, the GSH-Px concentration increased significantly ($p<0.05$) on weeks 1 and 2 post-treatment only. It is concluded that Se supplementation is not effective in alleviating oxidative stress and improving muscle integrity in beef cattle.

Keywords: selenium, oxidative stress, malondialdehyde, glutathione peroxidase, aspartate aminotransferase, beef cattle

INTRODUCTION

Selenium (Se) was first recognised as an essential micronutrient for the maintenance of animal in 1957 (Schwarz and Foltz, 1957). Selenium and vitamin E both have complementary but independent roles in the protection of cells against the damaging effects of free radicals produced during normal metabolism (Villar *et al*., 2002). On the other hand, Se and vitamin E deficiency can result in nutritional muscular dystrophy, infertility, stillbirths or retained placenta (Davis and Myburg, 2016). The acidic soil of Malaysia with a range of pH of 3 to 5 may cause

formation of Se-iron hydroxide complexes leading to low uptake by pastures and thus become less bioavailable to animals (Lyons *et al*., 2007; Shamshuddin e*t* al., 2011). Selenium fertilization of pasture and supplementation programmes in animals via feed concentrate, mixture or boluses would be an effective way to increase Se content in the animal diets (Lyons *et al.,* 2007). Thus, the investigation into the role of Se in grazing ruminants in Malaysia is warranted. Therefore, the objectives of this study are to determine the oxidative stress status and muscle integrity via serum aspartate aminotransferase (AST) concentration in beef cattle given Se supplementation.

MATERIALS AND METHODS

Ten healthy, Kedah-Kelantan cows aged approximately 1 year were selected from Ladang 16, Taman Pertanian Universiti, Universiti Putra Malaysia. The animals were divided equally into two groups; control and treatment. Approximately 3 mL blood each were collected via jugular venipuncture in heparinised plain tube from all cows. The cows in the treatment group were treated intramuscularly with with 0.1 mg/kg body weight Se while the control group was given 10 mL of normal saline. The Se treatment was done twice at the beginning of the experiment and 2 weeks later. Blood was collected via jugular venipuncture every week for 4 weeks for plasma malondialdehyde (MDA), erythrocyte glutathione peroxidase (GSH-Px), and serum aspartate aminotransferase (AST) analyses.

RESULTS AND DISCUSSION

The MDA concentration in treatment and control groups decreased with time (Table 1). Although there was no significant difference in MDA concentration between groups, cows treated with Se showed lower concentration after 4 weeks.

The GSH-Px concentration did not differ significantly ($p>0.05$) between Se-treated and control groups (Table 2). However, at Weeks 1 and 2, those of the treated cows has significantly higher ($p<0.05$) concentrations than that of control group.

Although the cows in the control showed an increasing trend in serum AST concentration with time, the concentration was lower from 1 week after Se compared to beginning of experiment (Table 3). However, this change was not significant ($p>0.05$).

The plausible explanation for the lack of efficacy of the Se in the treatment of the Kedah-Kelantan cows could be attributed to presence of vitamin B12, adenosine triphosphate tetrasodium, potassium aspartate semihydrate, and magnesium aspartate tetrahydrate in the Se concoction. These compounds may have negated or antagonised the effect Se. It is also possible that the concentration of Se used in this study is too low to produce effect (Young and Lowe, 2001).

Table 1: Plasma malondialdehyde of Kedah-Kelantan cows treated with selenium.

Group	Plasma malondialdehyde (nmol)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control	0.3±0.04	0.2±0.12	0.2±0.04	0.2±0.02	0.3±0.05
Treatment	0.4±0.25	0.2±0.05	0.2±0.03	0.2±0.05	0.2±0.04

Values are mean±standard error.
Control: Given 1m mL normal saline; Treatment: Intramuscular 0.1 mg/kg body weight Se.

Table 2: Erythrocyte GSH-Px concentration of Kedah-Kelantan cows treated with selenium.

Group	Erythrocyte GSH-Px concentration (U/gHb)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control	3.2*±1.39	2.5±0.23	5.2*±0.41	3.2±0.83	3.2±0.52
Treatment	3.5*±0.52	4.1±0.42	6.7*±0.70	4.1±0.67	3.9±0.59

Values are mean±standard deviation.
*Means within row are significant different at $p<0.05$.
Control: Given 1m mL normal saline; Treatment: Intramuscular 0.1 mg/kg body weight Se.

Table 3: Serum aspartate aminotransferase concentration of Kedah-Kelantan cows treated with selenium.

Group	Serum aspartate aminotransferase (U/L)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control	83.8±24.92	88.1±13.89	86.6±14.14	107.6±32.02	105.8±30.22
Treatment	103.9±23.04	78.0±11.03	73.2±10.52	81.5±13.18	88.9±27.15

Values are mean±standard deviation.
Control: Given 1m mL normal saline; Treatment: Intramuscular 0.1 mg/kg body weight Se.

CONCLUSION

In conclusion, Se does play a role in reducing oxidative stress and aids in maintaining muscle integrity. However, supplementation of selenium alone is insufficient to effectively reduce oxidative stress.

REFERENCES

Davis A and Myburg JG (2016). Investigation of stillbirths, perinatal mortality and weakness in beef calves with low selenium whole blood concentration. *Journal of South African Veterinary Association*, 87(1): a1336.

Lyons MP, Papazyan TT, Surai PF (2007). Selenium in food chain and animal nutrition; Lessons from nature. *Asian-Australian Journal of Animal Science*, 20(7): 1135-1155.

Schwartz K and Foltz CM (1957). Selenium as an integral part of factor 2 against dietary necrotic liver degeneration. *Journal of the American Chemical Society,* 79; 3292-3293.

Shamshuddin J, Fauziah, CI, Anda M, Kapok J, Shazana MARS (2011). Using ground basalt and/or fertilizer to enhance productivity of acid soils in Malaysia for crop production. *Malaysian Journal of Soil Science*, 5: 127-146.

Villar-Patiño G, Díaz-Cruz A, Ávila-González E, Guinzberg R, Pablos JL, Piña E (2002). Effects of dietary supplementation with vitamin C or vitamin E on cardiac lipid peroxidation and growth performance in broilers at risk of developing ascites syndrome. *American Journal of Veterinary Research*, 63(5): 673-676.

Young AJ and Lowe GM (2001). Mini review: Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics,* 385(1) 20-27.

ENDOPARASITE INFESTATION IN SEMI-COMMERCIAL AND FREE-RANGING VILLAGE CHICKEN

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ABSTRACT

Endoparasitic infestation in semi-commercial village chicken and scavenging village chicken in Simpang Renggam, Kluang, Johor, Malaysia was compared. Six faecal samples were collected each from semi-commercial village chicken and chicken found scavenging freely in the village were obtained. McMaster technique performed to quantify parasites in all faecal samples did not produce any helminths ovum. In semi-commercial chickens, 3 faecal samples had 50 oocysts/g and one had 150 oocysts/g, and anther had 1000 oocysts/g. In free-ranging village chicken 4 samples did not show any oocyst while one had 150 oocysts/g and another 250 oocysts/g. The study showed that no significant ($p>0.05$) difference in faecal oocyts count between semi-commercial and free-ranging village chicken.

Keywords: endoparasitic infestation, village chicken, helminths ova, coccidial oocysts

INTRODUCTION

Village chickens (*Gallus domesticus*) are medium-sized fowl with small heads, large bodies, and bare scally legs. These chickens often have fleshy wattles and combs. Their beaks are small and wings relatively short wings for their body weight. The colour of the village chicken vary with breed. The first village chickens were domesticated Red Jungle fowl (*Gallus gallus*). The Red Jungle fowls are found naturally in most of Southeast Asia and is believed to have been hybridized with the Grey Jungle fowl (*Gallus sonneratii*). Some researchers suggest that the origin of the Red Jungle fowl is some areas of South and Southeast Asia, including North and South China, Thailand, Burma and India (Kris, 2014).

In most rural villages of Malaysia, poultry are often left to scavenge around house compounds during the day for food. Among their diet are insects, seeds and kitchen leftovers. The free-ranging and scavenging habits of these chickens caused them to be in great contact with insects and soil. Malaysian soils, especially when humid and warm, serves as reservoir and transmission site for the larval stages of helminths (Abdul *et. al.*, 2009).

This study was conducted to determine the level of endoparasite infestation in Malaysian village chicken in Simpang Renggam, Johor.

MATERIALS AND METHODS

The study was conducted at a poultry farm and surrounding area in Kampung Melayu Bukit Nyamuk, Simpang Renggam, Kluang, Johor, Malaysia. Six faecal samples each from semi-commercial and free-ranging village chickens. were collected and assigned as commercial village chicken. Each sample was subject to McMaster flotation technique to identify and quantify helminths eggs and coccidia oocysts. Chi-square test was performed to determine difference in parasite infestation between semi-commercial and free-ranging village chickens.

RESULT AND DISCUSSION

The faecal samples did not produce any helminthes ovum. However, coccidial oocysts were detected in some of the samples. In semi-commercial chickens, 3 faecal samples had 50 oocysts/g and one had 150 oocysts/g, and anther had 1000 oocysts/g. In free-ranging village chicken 4 samples did not show any oocyst while one had 150 oocysts/g and another 250 oocysts/g. Coccidial oocyst was found absence in only 1 fecal sample of semi commercial chicken. In free-ranging village chicken 4 samples did not show any oocyst while one had 150 oocysts/g and another 250 oocysts/g. The study showed that no significant ($p>0.05$) difference in faecal oocyts count between semi-commercial and free-ranging village chicken.

According to Irungu *et. al,* (2004), high helminths load can be expected in avian that are not well-managed and treated. There was also an evidence that nutritional status of the chicken can have profound effects on the course of helminth infestations because dietary deficiency leads to stress that predisposes them to the infestation (Chubb *et. al,* 1963). It has been shown that chickens reared under the cage system are not usually burdened with helminthes while free-ranging chickens tend to have high infestation rates (Irungu, *et. al,* 2004).

Wet and humid climate of Malaysia is favourable environment for the sporulation of oocysts and coccidiosis while poor hygiene and multiage flock practices can cause contamination that also lead to coccidiosis (Pitersky, 2014).

REFERENCES

Abdul RW, Salim H, Ghause MS (2009). Helminthic parasites of scaven-ging chickens (*Gallus domesticus)* from villages in Penang island, Malaysia. *Tropical Life Science Research*, 20(1): 1-6.

Chubb LG and Wakelin D (1963). Nutrition and helminthiasis in chickens. *Proceedings of the Nutrition Society*, 22(01): 20-25.

Irungu LW, Kimani RN, Kisia SM (2004). Helminth parasites in the intestinal tract of indigenous poultry in parts of Kenya: short communication. *Journal of the South African Veterinary Association*, 75(1): 58-59.

Kris K (2014). The History of Chickens (*Gallus domesticus*). http://archaeology.about.com/od/domestications/qt/chicken.htm.

(Accessed on 10 February 2016)

Pitersky M (2014). Coccidiosis in Chicken: Transmission, Diagnosis and Treatment, *Chicken Whisperer Magazine.*

http://www.chickenwhisperermagazine.com/articles/coccidiosis-chickens-transmission-diagnosis-and-treatment. (Accessed on 10 February 2016).

KARYOTYPES OF FALLOW AND SPOTTED DEER

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ABSTRACT

Various deer species had introduced new germplasm and risk of developing germplasm combinations. In this study, the chromosomes of two deer species raised in Malaysia, namely *Dama dama* (fallow deer) and *Axis axis* (spotted deer) were karyotyped. Lymphocytes were cultured in medium consisting RPMI-1640, supplemented with pokeweed mitogen, foetal calf serum, penicillin-streptomycin, amphotericin B, and pokeweed mitogen for the production of good quantity and quality chromosome metaphase spreads. The fallow deer and spotted deer had diploid number (2n) of 68 and 66, respectively. The conventional karyotype showed that fallow deer has 33 pairs of autosomes, 32 pairs acrocentric and 1 pair metacentric, while spotted deer has 32 pairs of autosomes, 31 pairs acrocentric and 1 pair metacentric chromosomes. The X chromosome is long acrocentric while the Y chromosome is small sub-metacentric for both species. The fundamental number for fallow deer is 71 for males and 70 for females and for spotted deer, 69 for males and 68 for females. The study showed that fallow deer and spotted deer have different diploid and fundamental numbers but similar chromosome morphology.

Keywords: *Dama dama*, *Axis axis*, lymphocyte culture, karyotype, fundamental number

INTRODUCTION

Various deer species had brought new germplasm and risk developing germplasm combinations into Malaysia. There is a need to evaluate the genetic background of these deer species before they are indiscriminately diluted or altered. In an earlier study on genetic characterisation of deer species raised in Malaysia, only *Cervus timorensis* (Rusa), *Cervus unicolor* (Sambar), and *Cervus nippon* (Sika) have been karyotyped (Habiba *et al*., 2005). The present study was conducted to determine the diploid number (2n), fundamental number (NF), and to construct the karyotypes of males and females, and to describe the chromosome morphology of *Dama dama* (fallow deer) and *Axis axis* (spotted deer).

MATERIALS AND METHODS

Animals and sample collection

The animals used in this study comprised of 2 males and 2 females each of fallow deer and spotted deer. The fallow deer belonged to University Agriculture Park, University Putra Malaysia while the spotted deer were the property of the Livestock Animal Centre, Lenggong, Perak, Malaysia. The deer were kept, physically restrained, in the dark room in the morning of blood collection. The deer were sedated with approximately 4 mg/kg body weight xylazine. Blood samples were collected from the jugular veins into heparinised tubes by using a 18G venoject needle and transported immediately in a cold box to the Cytogenetic Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia to be processed.

Cytogenetic analyses

The basic short-term lymphocyte culture technique described by Moorhead ***et. al.,*** 1960 was adopted with some modifications. Blood samples were centrifuged at 1800 rpm for 10 min to obtain the buffy coat containing leucocytes. The buffy coat was aspirated and placed in culture flasks containing culture medium comprising of 8.0 mL RPMI-1640 medium, 2.0 mL foetal bovine serum, 1000 µg penicillin G-streptomycin, 1000 µg amphotericin B, and 1µg pokeweed mitogen. The mixture was incubated at 37°C for 72 hours. One hour before harvesting, 0.1 mL colcemid solution was added into the culture flask. The suspension was transferred into a 15 mL conical centrifuge tube and centrifuged for 8 min at 1800 rpm. The supernatant was discarded and 6.0 mL of pre-warmed 0.075 M potassium chloride solution was added to each tube. The tubes were then incubated at 37°C for 20 minutes, centrifuged at 1800 rpm for 8 min and the supernatant discarded. 6.0 mL Carnoy's fixative (1 part glacial acetic acid to 3 parts methanol) was added. The fixation procedure was repeated 3 times and the culture was kept at 4°C overnight. Two drops of culture suspension were placed on a pre-cleaned glass slide. The slide was dried in warm air and stained with 10% giemsa solution for 2.5 minutes and examined under light microscopy and photographed using image analyzer. The diploid number of chromosomes were calculated manually. The karyotypes were constructed from chromosomes cut and arranged according to International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDA, 1989).

RESULTS AND DISCUSSION

The diploid numbers were determined from the percentage distribution chromosomes of ≥50%. From this study, the diploid number for fallow deer was 68 with 65.1 and 53.2% chromosome distribution in male and female, respectively (Figure 1). The spotted deer had a diploid number of 66 with 69.7 and 67.7% chromosome distribution in male and female, respectively. The conventional karyotype showed that fallow deer has 33 pairs of autosomes, 32 pairs acrocentric and 1 pair metacentric, and one pair of sex chromosome. The X chromosome is a

long acrocentric chromosome while the Y chromosome is a small sub-metacentric chromosome (Figure 2). The results are in agreement with that of Kozubska-Sobocińska *et. al.,* (2013).

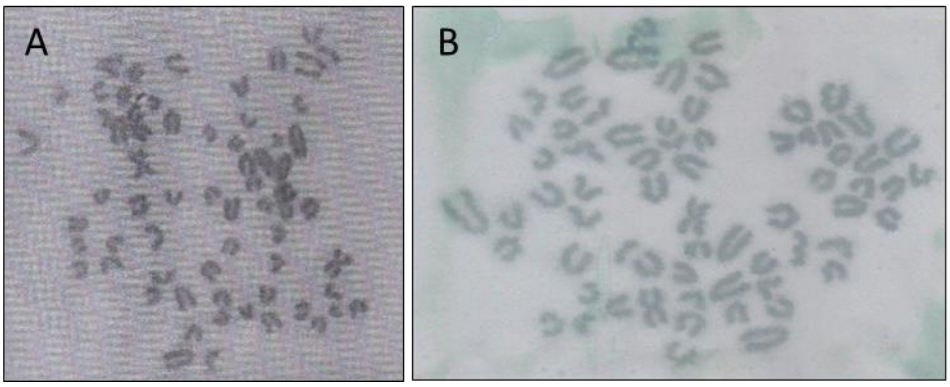

Figure 1: Conventional karyotype of fallow deer. (A) Male; (B) Female.

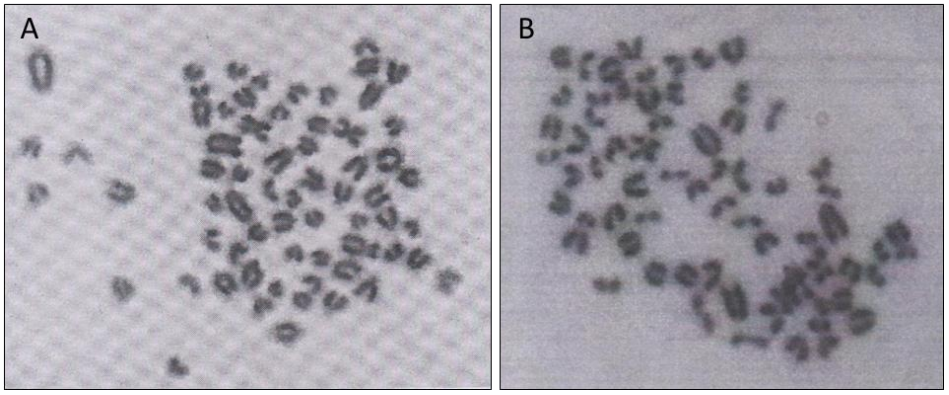

Figure 2: Conventional karyotype of spotted deer. (A) Male; (B) Female

Spotted deer had 32 pairs of autosomes, 31 pairs acrocentric and 1 pair metacentric chromosome, and one pair of sex chromosome. The X chromosome is a long acrocentric chromosome while the Y chromosome is a small sub-metacentric chromosome. The findings are in agreement with those of earlier studies (Shanthi *et. al.,* 2008; Robinson and Elder, 1993). The fundamental number of fallow deer is 71 for male and 70 for female, while for the spotted deer they were 69 and 68 doe males and females, respectively.

A previous study that the diploid number of rusa, sambar, and sika are 60, 62 and 66 chromosomes (Habiba Ali, 2005). These deer also also differ in autosomes number and chromosome morphology. There is similarity in morphology of sex chromosome between rusa, sambar and sika were similar. However, the fundamental number of rusa, sambar and sika were similar for males and females at 70.

The differences in chromosome constitution between fallow deer, spotted deer, rusa, sambar, and sika is the result of evolution and geographical isolation. The genus *Cervus* had given rise to different species with different features.

It is recommended that further studies be conducted to determine the risk development of germplasm combinations in these deer.

REFERENCES

Habiba Ali, AE (2005). Genetic characterization of three deer species in Malaysia. PhD thesis, Universiti Putra Malaysia. http://psasir.upm.edu.my/6178/1/FP_2005_12%281-24%29.pdf (Accessed on 15 September 2016).

ISCNDA (1989). International System for Cytogenetic Nomenclature of Domestic Animals (1989): 2nd International Conference on Standardization of Domestic Animal Karyotypes, Jouy-en-Josas, May 1989 1st Edition, DiBerardino, Hayes H, Fries R, Long S (Editors), S. Karger, Basel, Germany.

Kozubska-Sobocińska A, Danielak-Czech B, Babicz M, Bąk A, Rejduch B (2013). Interspecies hybridizations *in situ* with bovine heterosome painting probes for identification of sex chromosomes in fallow deer (*Dama dama*). *Annales Universitatis Mariae Curie-Skłodowska Sectio EE Zootechnica* 31(4): 95-99.

Moorhead PS, Nowell PC, Mellman WJ, Batipps DM and Hungerford DA (1960). Chromosome preparations of leucocytes cultured human peripheral blood. *Experimental Cell Research,* 20: 613-616.

Robinson TJ and Elder FFB (1993). Cytogenetics: its role in wildlife management and the genetic conservation of mammals. *Biological Conservation,* 63: 47-51.

Shanthi G, Balasubramanyam D, Thangaraju P, Srinivasan R (2008). Karyological studies in spotted deer (*Axis axis*). *Tamilnadu Journal of Veterinary and Animal Sciences,* 4(6): 244-246.

ISOLATION AND IDENTIFICATION OF NORMAL FLORA IN THE CLOACA OF MALAYAN BOX TURTLES

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ABSTRACT

Malayan Box turtle, *Cuora amboinensis kamaroma,* is a reptile of the order Testudines, under the family of *Geoemydidae* and subfamily *Geoemydinae*. This species is listed by the International Union for Conservation of Nature and Natural Resources Red List of Threatened Species as a vulnerable species in Malaysia. The aim of this study was to determine the presence of bacteria in the cloaca of Malayan Box turtles. Eight cloacal sterile swab samples were collected from healthy adult Malayan Box turtles for bacteria isolation and identification. Nine species of bacteria were isolated and identified from the samples. One species of gram-positive, *Staphylococcus aureus,* and 8 species of gram-negative bacteria, *Acinetobacter lwoffi, Escherichia coli, Pasteurella testudinis, Pantoea agglomerans, Acinetobacter calcoaticus, Salmonella spp., Klebsiella oxytoca,* and *Serratia spp.* Most of the isolated bacteria were normal flora except for *S. aureus, E. coli, Salmonella spp., K. oxytoca* and *Serratia spp.* were isolated and identified. These microorganisms can cause severe infection especially in sick or immunocompromised people. In conclusion, appropriate hygienic precautions need to be taken during handling and transporting of the animals to prevent transmission of zoonotic bacteria from the animal to humans.

Keywords: Malayan Box turtle, cloaca, zoonosis, normal flora

INTRODUCTION

Malayan Box turtle, *Cuora amboinensis kamaroma*, is a semi-aquatic turtles species that is listed as a vulnerable species in Malaysia by IUCN Red List of Threatened Species (IUCN Red list, 2000). Many bacteria have been identified as causing illness in turtles kept in captivity (Glazebrook *et. al.,* 1993). A variety of bacteria can be present in healthy turtles, but can become pathogenic in susceptible animals living under stressful conditions (Santoro *et al*., 2006). The objective of this study was to isolate the normal flora from the cloaca of the Malayan Box turtles and identify and differentiate the types of normal flora and to deterimine whether they opportunistic or zoonotic bacterial organisms in Malayan Box turtles.

MATERIALS AND METHODS

Cloaca sterile swab samples were collected from 8 healthy adult Malayan Box turtles. The samples were cultured onto blood agar and MacConkey agar. After incubation for 24 hours, colonies present were then inoculated and incubated onto blood agar to obtain pure culture colonies. Standard bacteria isolation and identification procedure was done to determine the species of bacteria. Pure colonies were subjected to gram-staining. Gram-positive bacteria were then subjected to catalase, coagulase, Voges-Proskauer, mannitol, and gelatin liquefaction tests while gram-negative bacteria subjected to oxidase, triple sugar iron, sulphide motility indole, urease, and citrate tests. The species of the bacteria were then determined.

RESULTS AND DISCUSSION

Nine species of bacteria, one gram-positive, *Staphylococcus aureus,* and 8 gram-negative bacteria, *Escherichia coli, Acinetobacter lwoffi, Serratia sp., Pantoea agglomerans, Pasteurella testudinis, Klebsiella oxytoca, Acinetobacter calcoaticus* and *Salmonella sp*. were isolated and identified. The most prevalent bacteria species was *P. agglomerans* (20%), followed by *S. aureus* (15%), *A. lwoffi* (15%), *P. testudinis* (10%), *K. oxytoca* (10%), *Serratia sp.* (10%), *Salmonella spp.* (10%), *E. coli* and *A. calcoaticus* (5.0%).

From the results, the normal flora of the cloaca of healthy Malayan Box turtles include *Salmonella sp., Acinetobacter sp., Serratia sp.* and *E. Coli*, which are opportunistic and can cause disease in stressed animals. Some isolates are opportunistic pathogens of humans and are especially dangerous to the immunosuppressed. Zoonotic transmission can occur between animals and humans. Thus, handlers of the turtles run the risk of being infected by the opportunistic pathogens.

CONCLUSION

In conclusion, some of the normal flora from the cloaca of Malayan Box turtles. have zoonotic potentials and can cause detrimental effects to humans when in contact. Appropriate hygienic precautions need to be taken during handling and transporting of these animal.

REFERENCES

Glazebrook JS, Cambell RSF, Thomas AT (1993). Studies on an ulcerative stomatitis – obstructive rhinitis – pneumonia disease complex in hatchling and juvenile sea turtles *Chelonia mydas* and *Caretta caretta*. *Diseases of Aquatic Organisms*, 16: 133-147.

IUCN Red List (2000). Asian Turtle Trade Working Group (2000). *Cuora amboinensis*. The IUCN Red List of Threatened Species 2000:e.T5958A11953035. http://dx.doi.org/10.2305/IUCN.UK.2000.RLTS.T5958A11953035.en. (Accessed on 21 February 2016).

Santoro M, Hernandéz G, Caballero M, Gárcia F (2006). Aerobic bacterial flora of nesting green turtle (*Chelonia mydas*) from Tortuguero National Park, Costa Rica. *Journal of Zoo and Wildlife Medicine*, 27: 549-552.

EFFECT OF *FERULA ASAFOETIDA* POWDER ON RAT CONCEPTION RATE

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ABSTRACT

Rats are reservoirs for leptospirosis and several vector-borne diseases. According to the Malaysian Pest Control Association, the rat population in Kuala Lumpur in 2013 was approximately 6.8 million. Despite massive trapping, the rat population did not decrease significantly. This study aimed to determine the effect of *Ferula asafetida* (asafetida) powder on the conception rate of rats. One male and 6 female Sprague-Dawley rats were used for this study. Three females in the treatment group were given 400 mg asafoetida powder in water daily while three from the control group were given water only. The male rat was introduced to the females for one week to allow mating. The results showed that animals in the control group increased in body weight, had distended abdomen and hair loss around the teats. The treatment group did not get pregnant suggesting either masking of female pheromone or failure of implantation. The study showed that asafoetida powder could be a natural, safe herb to be used in the control of rat population.

Keywords: rat population, *Ferula asafoetida*, biological control

INTRODUCTION

Rats are thin long tailed, medium-sized rodents of the family Muridae. They originated from Asia and Australia and are now found all over the world. The best known rat species are the black, *Rattus rattus*, and brown *Rattus norvegicus* rats, (Musser, 2014). According to the Malaysian Pest Control Association, the estimated rat population in metropolitan Kuala Lumpur in 2013 was 6.8 million rats (Kuala Lumpur City Hall report, 2013).

Rats have been identified as the main cause of leptospirosis in livestocks and humans (Izurieta et al., 2008). Malaysia in 2010, recorded 1,976 cases of leptospirosis recorded with 69 deaths, in 2011, 2,268 cases with 55 deaths, and in 2012, 3,665 cases with 48 deaths. In 2014, the leptospirosis cases peaked at 7,808 cases with 92 deaths but decreased again to 5,370 cases with 30 deaths in 2015 (Zainudin Abdul Wahab, 2015). These leptospirosis cases were attributed to rat

population in the country.

Asafoetida, the oleo gum resin extracted from the root and rhizomes is said to have many beneficial properties such as anti-flatulent, antidiabetic, and contraceptive properties. The asafetida powder from the plant *Ferula asafetida* is consumed in small amount in foods by Indians in Malaysia. It is postulated that asafetida powder is an effective compound to control the rat population. Thus, this study was undertaken to determine the effect asafetida power on the conception rate of rats.

MATERIALS AND METHODS

Seven Sprague-Dawley rats comprising of 1 male and 6 females, all proven fertile, were kept in plastic cages under a constant ambient of approximately 24 °C. The male rat was kept separate in one cage while the 6 females divided into 2 groups of 3 rats each; the control group and the treatment group in separate group cages. The male cage was located in between the female cages to induce oestrus (Whitten, 1968). All rats were fed pellets.

After 4 days of acclimatisation, the treatment group was given 400 mg/kg body weight asafoetida powder in 125 mL of water while the control group received water. This treatment was given daily for 21 days. The rats were observed daily for signs and feed intake and body weight were recorded.

The male was then introduced first to control group, followed by the treatment group, each time for 4 hours. The male-female reception and the mating behaviour were recorded.

RESULTS AND DISCUSSION

There was a gradual increase in body weight in the control female rats with abrupt large increase from day 6 peaking on day 29 of the experiment. The control rats showed prominent teats and distended abdomen and all delivered pups on day 29. The treatment group showed a gradual increase in body weight, but there was no sign of pregnancy. It is suggested that failure of the treated rats to become pregnant is possible due to pheromone-masking effect or implantation impairment. The pheromone-masking is suggested to be due to the foetid odour of asafoetida in breath, secretions, flatus, and gastric eructations of the rats (Wickes, 1998) that discouraged mating of the rats. The *F. asafoetida* plant also lacks phytoestrogens, which may have interrupted pregnancy by disrupting uterus metabolism during implantation (Keshri *et al*., 2004).

CONCLUSION

The *F. asafoetida* powder is a safe and non-toxic product for people and to the environment. In this study, the powder was shown to prevent conception rats and thus it has bpotential to be used as an agent in the biological control of rat populations. Controlling rat population is one of the means to control spread of diseases such as leptospirosis, salmonellosis and vector-borne diseases.

REFERENCES

Izurieta R, Galwankar S, Clem A (2008). Leptospirosis: The "mysterious" mimic. *Journal of Emergencies, Trauma and Shock*, 1(1): 21-33.

Keshri GM, Bajpai V, Lakshmi BS and Setty GG (2004). Role of energy metabolism in the pregnancy interceptive action of *Ferula assafoetida* and *Melia azedarach* extracts in rat. *Contraception*, 70(5): 429-432

Kuala Lumpur City Hall report (2013). City Hall: 6.8 million rats in KL. http://www.thestar.com.my/news/nation/2013/09/12/city-hall-68-million-rats-in-kl-this-resulted-in-2925-leptospirosis-cases/. (Accessed on 14 October 2015).

Musser G (2014). Rat: Rodent Genus. Encyclopaedia Britanica. http://global.britannica.com/animal/rat. (Accessed on 10 September 2015).

Whitten WK, Bronson FH and Greenstein JA (1968). Estrus-inducing pheromone of male mice: transport of movement of air. *Science,* 161(3841): 584-585.

Wickes H and Lloyd J (1998). *Ferula asafoetida*, Henriette's Herbal Homepage. King's American Dispensatory. http://www.henriettes-herb.com/eclectic/kings/ferula-asaf.html. (Accessed on 16 November 2015).

Zainudin Abdul Wahab (2015). Epidemiology and current situation of leptospirosis in Malaysia. http://jkt.kpkt.gov.my/resources/index/pdf/Persidangan 2015/persidangan%20kesihatan/Leptospirosis_in_Malaysia.pdf (Accessed on 15 September 2016).

SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS IN BIRDS IN MALAYSIA

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ABSTRACT

Japanese Encephalitis is one of the most important zoonotic diseases and it is caused by Japanese Encephalitis virus (JEV) from family *Flaviviridae* and genus *Flavivirus*. The virus is transmitted through *Culex* mosquito primarily by *Culex tritaeniorhynchus* with ardeid birds as reservoir. Pigs and birds play an important role as the main vertebrate amplifier and humans and horses as the dead-end host. Forty-five birds consisted of village chicken, Jungle Fowl cross, and water birds were sampled in Tanjung Piandang, Perak, Jenderam Hulu, Selangor, and Putrajaya Wetland, Malaysia, respectively. Serum samples were subjected to double-antigen sandwich ELISA for detection of antibody against Japanese Encephalitis virus (MyBiosource®). Out of forty-five sample, 28.89% (13/45) were positive for JEV antibodies. Jenderam Hulu samples showed the highest seroconversion (50%) followed by Tanjung Piandang (21.74%) and Putrajaya Wetland (20%). Young birds showed higher seroconversion (35.71%) than adult (25.81%). The seroconversion in the Jungle Fowl cross and American flamingo was 50% and in the village chicken 21.74%. Male showed higher seroconversion (50%) than females (28.13%). There was no association between the risk factors and JEV seroprevalence. In conclusion, the seroconversion towards JEV in birds in this study showed that all birds have similar risk to JEV infection irrespective of age, sex, species, and location.

Keywords: Birds, Japanese Encephalitis, Malaysia, seroconversion, antibody, risk factors, double-antigen sandwich ELISA

INTRODUCTION

Japanese Encephalitis virus (JEV) is a mosquito-borne *Flavivirus* of the *Flaviviridae* family. This virus infection is the most important cause of viral

encephalitis in humans and animals (Chen *et al*., 1990). Transmission of JEV involves the *Culex tritaeniorhynchus* mosquito and similar species that lay eggs in paddy fields and water bodies. Pigs and aquatic birds are the main vertebrate amplification host (Halsted and Jacobson, 2008). Japanese encephalitis an important endemic zoonotic disease, particular to the Southeast Asian and other developing countries. Currently there is no report on the prevalence of JE in birds in Malaysia or on species involvement in the maintenance of the virus in the environment (Tsuchie *et al.,* 1994; Halsted and Jacobson, 2008; Cleton *et al.,* 2014). Therefore, the objective of this study is to determine the serological prevalence of JE in birds in Malaysia and also to determine the association between seropositivity against JEV and potential risk factors.

MATERIALS AND METHODS

Forty-five birds were sampled from three t locations in Malaysia; Tanjung Piandang, Perak, Jenderam Hulu, Selangor, and Putrajaya Wetlands in January 2016. Twenty-three village chicken (*Gallus gallus domesticus*) samples were from Tanjung Piandang, 12 Jungle Fowl cross (*Gallus gallus*) samples are from Jenderam Hulu, and 10 water bird samples from the Putrajaya Wetlands. All the birds were healthy and the areas were not known to had history of JE outbreak.

The risk factors investigated in this study were species, age, sex, and location. One to 3 millilitre blood were collected via wing or medial metatarsal venipuncture from the birds only using either 25G or 23G needles. Blood was processed and serum was stored at -20°C. Serum was subjected to ELISA (MyBiosource®) to determine the anti-JEV IgG antibodies. The ELISA provides qualitative determination of IgG antibody using the double-antigen sandwich ELISA concept. The sample is considered positive when the mean sample optical density of sample ($O.D_{Sample}$) is more or equal to the cut-off value calculated using the following formula:

$$\text{Cut-Off} = O.D_{NC} + (0.15 \times O.D_{PC})$$

Test sample is considered negative when the mean sample $O.D_{Sample}$ is less than the cut-off value. Chi-square test was used to determine association between risk factors and presence of anti-JEV antibodies at α=0.05. Odds ratio was calculated to determine potential risk factor.

RESULTS AND DISCUSSION

Antibodies against JEV was detected in 28.89% (13/45) of bird samples. The prevalence of JEV infection is considered low in comparison with earlier reports of 86.7% (Yang *et al*., 2011) and 85.9% (Saito *et al*., 2009). The reason for the discrepancy could be the differences in location of sampling. Japanese encephalitis

viral transmission is stronger in areas with overlapping rice production and pig-rearing activities compared to those that are not (Erlanger *et al*., 2009). Our study did not show any association between risk factors and JEV seroprevalence. By location, the highest seroprevalence was in Jenderam Hulu at 50% followed by Tanjung Piandang at 21.74%, and Putrajaya Wetland at 20%. The high JEV seroprevalance was in fact reported in an earlier study (Vythilingam *et al.,* 1997). In that report, the *Culex tritaeniorhynchus* and *Culex gelidus* mosquitoes was found to be in abundance in the Jenderam Hulu area.

Young birds have higher JEV seroprevalence than adult birds, suggestin that age is a risk factor in JEV seroconversion. Young birds tend to develop more significant viraemia than old birds (Cleton *et al.,* 2014).

This study also showed that male tend to have showed higher seroprevalence than female birds.

There no association between bird species and JEV seroprevalence, although the Jungle Fowl crosses and flamingoes showed higher seroprevalence than village chickens.

Japanese encephalitis cases occur sporadically and in tropical areas such as Malaysia, Indonesia, southern Vietnam, southern Thailand, southern India and Philippines, the disease usually peaks during the rainy season from May to October of each year. Development of water bodies conducive for mosquito breeding during this season contribute to the transmission of JEV.

In conclusion, the seroconversion towards JEV in birds in this study showed that all birds have similar risk to JEV infection irrespective of age, sex, species, and location.

REFERENCES

Chen W, Tesh R, Rico-Hesse R (1990). Genetic variation of Japanese encephalitis virus in nature. *Journal of General Virology*, 71(12): 2915-2922.

Cleton N, Bosco-Lauth A, Page M, Bowen R (2014). Age-related susceptibility to Japanese encephalitis virus in domestic ducklings and chicks. *American Journal of Tropical Medicine and Hygiene*, 90(2): 242-246.

Erlanger T, Weiss S, Keiser J, Utzinger J, Wiedenmayer K (2009). Past, present, and future of Japanese encephalitis. *Emerging Infectious Diseases,* 15(1): 1-7.

Halsted SB and Jacobson J (2012). Japanese encephalitis vaccines. In: Vaccines, Plotkin SA, Orenstein WA, Offit PA (Editors), 6th Edition, Elsevier Saunders, Philadelphia. Pp312-351.

Saito M, Osa Y, Asakawa M (2009). Antibodies to flaviviruses in wild ducks captured in Hokkaido, Japan: risk assessment of invasive flaviviruses. *Vector Borne Zoonotic Diseases*, 9:253–258.

Tsuchie H OdaK, Vythilingam I, Thayan R, Vijayamalar B, Sinniah M, Hossain MM, Kurimura T, Igarashi A. (1994). Genetic study of Japanese encephalitis viruses isolated in Malaysia. *Japanese Journal of Medical Science and Biology*, 47(2): 101-107.

Vythilingam I, Oda K, Mahadevan S, Abdullah G, Thim C, Hong C, Vijayamalar B, Sinniah M, Igarashi A (1997). Abundance, parity, and Japanese encephalitis virus infection of mosquitoes (Diptera: *Culicidae*) in Sepang District, Malaysia. *Journal of Medical Entomology*, 34(3): 257-262.

Yang D-K, Oh Y-I, Kim H-R, Lee Y-J, Moon O-K, Yoon H, Kim B, Lee K-W, Song J-Y (2011). Serosurveillance for Japanese encephalitis virus in wild birds captured in Korea. *Journal of Veterinary Science*, 12(4): 373-377.

BLOOD FATTY ACIDS ANALYSIS IN CAPTIVE FALSE GHARIAL (*TOMISTOMA SCHLEGELII*)

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ABSTRACT

Fatty acid is an important component of body metabolisms. Fatty acid profile is an indicator of the physiological status of animals. Therefore, a study was undertaken with the objective of establishing the plasma fatty acids profile of false gharial fed raw chicken and catfish. The fatty acid analysis was done on the plasma and feed on five captive false gharials using gas chromatography following the procedure of total lipid extraction and fatty acids methyl esters (FAME) preparation. The results showed all false gharials showed highest polyunsaturated (PUFA), followed by saturated and monounsaturated fatty acids in their plasmas. The plasma eicosapentaenoic acid (EPA, C20:5 n-3) concentration in false gharials was found to be low but the docosahexaenoic acid (DHA, C22:6 n-3) concentration was significantly ($p<0.05$) high. The omega-3 concentration in chicken feed was low and the fish did not contain any omega-3 PUFA.

Keywords: fatty acid composition, false gharial, essential fatty acid, diet

INTRODUCTION

False gharial, *Tomistoma schlegelii,* categorised as freshwater crocodile, is one of the species listed as 'vulnerable' by the IUCN Red List (2000). Usually, captive *T. schlegelii* are fed chicken, beef, pigs, rats, and fish meats with feeding frequency varying with management practice. The crocodile diet is believed to be directly related to their body fatty acid composition. Currently, there is no data of fatty acid profile for *T. schlegelii*.

Unlike unsaturated, saturated fatty acids (SFA) are considered to be bad cholesterol. Unsaturated fatty acids consist of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The PUFA namely omega-3 and omega-6 are essential fatty acids as they can only be acquired from the diet. A balanced ratio of omega-6 and omega-3 PUFA is needed for normal growth and development. This balance also helps to reduce cardiovascular and other chronic diseases while

improving mental health in humans.

This study was undertaken with the objective of establishing the plasma fatty acids profile of captive false gharial.

MATERIALS AND METHODS

Animals

This study was carried out at the Wildlife Conservation Centre, Sungai Dusun, Kuala Kubu Baru, Malaysia. Five false gharials comprising of 2 males and 3 females were selected. The false gharials were kept in an earthen freshwater pond and fed raw commercial chickens once a week and catfish once a month.

Sample collection

Blood samples were collected from the lateral tail vein using 5 mm 18G spinal needle fitted to a 10 mL plastic syringe. Approximately 10 mL blood samples were collected into 6 mL ethylenediaminetetraacetic tubes. Plasma by centrifugation at 1000 × g for 10 min and feed samples were obtained for fatty acid analyses.

Fatty acid analysis

Total fatty acids were extracted from plasma and feed using chloroform:methanol 2:1 (v/v) based on the method of Folch *et al*. (1957) modified by Rajion (1985). Approximately 1 mL plasma and 1 g of feed samples were transferred to glass tubes and 6 mL fresh chloroform-methanol (2:1, v/v) added. Non-lipid contaminants were retained in the aqueous upper phase. Transmethylation of fatty acids to their fatty acid methyl esters (FAME) were done with 20% methanolic boron trifluoride. The methyl esters were quantified by gas-liquid chromatography by injecting 1 µL auto-sampler. Identification of fatty acids was carried out by comparing relative FAME peak retention times of sample to standard (Sigma). The gravimetric calculations and normalised total fatty acid % were used to determine the fatty acids composition.

Data analysis

Data were analysed using descriptive analysis by establishing mean, standard deviation, minimum and maximum values and expressed as %.

RESULTS AND DISCUSSION

Plasma fatty acids

Based on total mean value, the dominant fatty acids were oleic, palmitic, docosahexanoic, linoleic, and stearic acids. The highest fatty acid concentrtion was docosahexanoic acid (DHA) and the lowest eicosopentaenoic acid (EPA). In the false gharial total PUFA as highest in concentration followed by SFA and MUFA. The plasma omega-3 and omega-6 fatty acid was 28.33% and 18.97%, respectively.

Feed fatty acids

Chicken, a dietary source of omega-6 fatty acids, contained linoleic acid (18:2 n-6) as one of the dominant fatty acids at 17.16%. However, chicken contained only 0.73% arachidonic acid.

The catfish in the false gharial diet when analysed did not seem to contain any omega-3 fatty acid. This feature may be associated with the metabolism of this freshwater fish that requires much less dietary omega-3 fatty acids than the marine species. This observation supports findings from earlier studies that showed muscles of freshwater fishes, patin (*Pangasius pangasius*), tilapia *(Oreochromis niloticus),* and catfish *(Clarius batrachus)* contained 0, 0.05 and 0.16% DHA, respectively (Zzaman, 2014).

We are not certain as to why false gharial has high plasma DHA concentration while this fatty acid concentration is low. Since, the false gharial was reared in a semi-wild environment, there is possibility the they may have gained access to preys that contributed to its high plasma DHA.

CONCLUSION

In conclusion, the fatty acids profile of captive false gharials in this study showed a predominant increase in unsaturated fatty acids specifically polyunsaturated fatty acids due to an increase in omega-3 and omega-6 polyunsaturated fatty acids. In relation to diet provided to the captive false gharials, the chicken and catfish are known to be the dietary source of omega-6. Nonetheless, this shows that the diet provided is suitable for the consumption by the false gharials as the diet contains essential fatty acids needed for their well-being.

REFERENCES

Zzaman W, Suseno SH, Nadiah, WA, Tajul AY (2014). Fatty acid profile and antioxidant capacity of muscle and byproduct oil from selected fresh water fish. *Food Science and Technology*, 2(3): 41-46.

IUCN Red List (2000). Asian Turtle Trade Working Group (2000). Cuora amboinensis. The IUCN Red List of Threatened Species 2000:e.T5958A11953035.

EFFECT OF CINNAMON (*CINNAMOMUM VERUM*) ON BACTERIA ISOLATED FROM CATS WITH OTITIS EXTERNA

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ABSTRACT

Otitis externa is a chronic inflammation of external ear canal has a variety of causes including bacteria infection, parasite infestation, food allergy, drug reaction, foreign body, keratinisation and autoimmune disease. This study was undertaken to determine the effect of cinnamon, *Cinnamomun verum*, on bacteria isolated from cats with otitis externa. Thirty ear swab samples taken from cats were cultured on blood agar for isolation and identification of bacteria. *C. verum* paste was prepared by mixing cinnamon powder with sterile distilled water. Antibacterial properties of *C. verum* were determined by antibiotic sensitivity test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) towards *Staphylococcus pseudintermedius, Staphylococcus intermedius, Staphylococcus hyicus, Enterococcus faecalis, Corynebacterium ulcerans, Bacillus* sp. and *Aggregatibacter actinomycetemcomitans* isolated from the samples. Some bacteria were resistant to marbofloxacin, doxycycline, and enrofloxacin while All were susceptible towards cinnamon paste. The MIC test showed that the maximum activity cinnamon paste ranged from 0.03 to 1.00 g/mL. The MBC test showed that the concentration of cinnamon paste that could inhibit growth or kill the bacteria ranged from 0.03 to 0.67 g/mL. Based on the test results, it suggested that *C. verum* paste had antibacterial properties toward bacteria isolated from cats with otitis externa. Thus, the C. verum paste has potential to be developed for the treatment of otitis externa.

Keywords: *Cinnamomum verum*, antibacterial effect, otitis externa

INTRODUCTION

Otitis is inflammation of ear canal or pinna regardless of the cause or clinical presentation (Jackson and Marsella, 2012). Cats subjected toear-cleaning by removal of ear canal excretion with cotton swabs, raised in humid environment, frequent bathed are high risk of developing otitis (Kennis, 2013). Under normal circumstances, opportunistic bacteria are present in low numbers and are considered as normal flora of the ear. However, in affected ear canal, these resident bacteria will proliferate and cause secondary infection. In cases of otitis in cat,

Staphylococcus spp., Escherichia coli, Corynebacterium spp., and *Pseudomonas spp.* are commonly isolated (Dowling, 1996). In acute otitis due to infections with or without purulent infiltrate, *Malassezia* or gram-positive cocci bacteria, typically *Staphylococcus pseudintermedius,* can be isolated (Jackson and Marsella, 2012). Aminoglycoside and fluoroquinolone antibiotics are efficacious towards staphylococci and gram-negative bacteria have been used to treat otitis externa. However, there are adverse effects of in the use antibiotics. Some bacteria may develop resistant to these antibiotics. *Cinnamomum verum* as essential oil and components compounds such as cinnamaldehyde and eugenol has been shown to have antibacterial properties (Gill and Holley, 2004). One of its mechanism of antibacterial action of these compounds is alteration of bacterial membrane permeability. Currently, there is no information on the antibacterial properties of *C. verum* against bacteria isolated from cats with otitis externa. Thus, the objectives of this study were to isolate and identify the bacteria that are present in the ears of cats with otitis externa and to determine the effect of *C. verum* on these bacteria.

MATERIALS AND METHODS

Ear swab collection
Thirty samples were taken from 30 cats that showed signs of ear scratching and had waxy ears.

Cinnamon paste preparation
C. verum sticks were grounded and filtered to obtain fine cinnamon powder. The cinnamon powder was mixed with sterile distilled water or nutrient broth at dilution of 1.0 g/mL.

Bacteria isolation and identification
The ears of all cats were swabbed and cultured on blood agar and incubated at 37°C for 24 hours. The isolates were then sub-cultured on blood agar before gram-staining to determine the reaction and cell morphology. Biochemical tests were conducted on the isolate according to the standard bacterial identification procedures.

Antibiotic sensitivity test
The concentration of bacterial inoculums was determined by comparing turbidity with 0.5 McFarland Standard. The inoculums were overlaid by using sterile cotton swab on Mueller Hilton or blood agar. A well was cut off from the agar and filled in with *C. verum* paste. Blank disc was included in each plate as negative control. Marbofloxacin (5µg), amoxicillin-clavulanic acid (30µg), doxycycline (30µg) and enrofloxacin (5µg) antibiotic discs were used as positive controls. Six replicates were made for each bacterium. The diameter of inhibition zone (DIZ) was determined in millimetres.

Minimum inhibitory concentration

Broth dilution method using 10mL test tube was performed to determine the minimum inhibitory concentration (MIC) for *Staphylococcus hyicus, Enterococcus faecalis* and *Bacillus* sp. One gram *C. verum* powder was diluted in. The diluted *C. verum* was then serially diluted two-fold with 1.0 mL nutrient broth (NB) to concentrations ranging from 1.0 to 0.03 g/mL. Positive control was prepared by adding antibiotic discs (Amoxicillin-Clavulanic Acid and Doxycycline) into test tubes containing NB while negative control tube only contains NB. One millilitre bacterial inoculum was then added to each test tube containing diluted cinnamon and negative and positive controls. Each treatment was done in triplicates. All test tubes were then incubated at 37°C for 24 hours. Bacteria growth inhibitions was determined by visual assessment of turbidity to obtain MIC values.

Minimum bactericidal concentration

Ten microlitres mixture was withdrawn from each MIC test tubes and inoculated onto blood agar. The blood agar plates were then incubated at 37°C for 24 hours. The minimum bacterial concentration (MBC) values were determined through visual assessment of areas with no bacteria growth.

RESULTS AND DISCUSSION

Eleven isolates were isolated from the 30 samples were gram-positive *Staphylococcus*. Among the samples, 30% were positive for non-pathogenic staphylococci and this finding is similar to that by Hariharan *et al.* (2006). In cases of acute otitis associated with infections, with or without purulent infiltrate, *Malassezia* or gram-positive cocci bacteria particularly *S. pseudintermidius* were isolate from the ear of cats (Jackson and Marsella, 2012).

Staphylococcus hyicus are usually present in skin infections in pigs and cattle. The organism has also been isolated from milk of cows (Songer and Post, 2004). In our study, *S. hyicus* was present in the ears of cats with otitis externa at a rate of 20% of isolates, suggesting that the bacteria is quite ubiquitous in animals. Approximately 6% of isolates comprised of *S. intermedius, E. durans,* and *Bacillus* sp. Other bacteria isolated at 3% each were *E. faecalis, Chromobacterium, C. ulcerans, Dermatophilus* sp. and *Aggregatibacter actinomycetemcomitans*.

C. verum paste was found to be sensitive towards *S. pseudintermedius, S. intermedius, S. hyicus, E. faecalis, C. ulcerans, Bacillus* sp. and *A. actinomycetemcomitans*. There was significant ($p<0.05$) differences in DIZ of each bacterium towards *C. verum* paste and the antibiotic treatments. *S. hyicus* producing a DIZ of 19.17mm was most susceptible to the *C. verum* paste while *C. ulcerans* with a value of 8.41mm was least susceptible. *A. actinomycetemcomitans* was also susceptibility to the *C. verum* paste with a DIZ value of 15.40mm. The results from this study are consistent with the findings of a previous study (Prabuseenivasan *et al.,* 2006) that showed V. verum was equally effective against both gram-negative and gram-positive bacteria.

The MIC and MBC values of *C. verum* against *E. faecalis* was lowest among bacteria tested at 1.00 and MBC=0.66 g/mL, compared to *Bacillus* sp. at 0.29 and 0.14 g/mL, and to *S. hyicus* at 0.03 and 0.03 g/mL, respectively. This may suggest emergence of resistance by the bacterium towards antibiotics (Fraser *et al.*, 2015). The presence of endospore in *Bacillus* sp. may be reason for the MIC and MBC values of *C. verum* towards this bacterium to be lower than towards to be than *S. hyicus*. Endospore is a resistant differentiated cell produced under stressful conditions that make the bacteria relatively resistant to the effect of antibiotics. Thus, higher concentrations of *C. verum* paste may be to ensure effect towards the bacteria.

Based on the findings of this study, *C. verum* paste has potential to be used as alternative treatment for otitis externa in cats. However, further researches are need to ascertain whether or not *C. verum* paste is toxic to animals.

REFERENCES

Dowling PM (1996). Veterinary Pharmacology: Antimicrobial therapy of skin and ear infections. *Canadian Veterinary Journal*, 37(11): 695-699.

Fraser SL, Salata RA, Donskey CJ (2015). Enterococcal infections. http://emedicine.medscape.com/article/216993-overview (Accessed on 25 February 25 2016).

Hariharan H, Coles M, Poole D, Lund L, Page R (2006). Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Canadian Veterinary Journal*, 47(3): 253-255.

Jackson H and Marsella R (Editors) (2012). BSAVA manual of canine and feline dermatology, 3rd Edition). Gloucester, British Small Animal Veterinar Association. Pp110-120.

Kennis RA (2013). Feline otitis: Diagnosis and treatment. *Veterinary Clinics of North America Small Animal Practice*, 43(1): 51-56.

Cornell University. Department of Microbiology. Bacterial Endospores https://micro.cornell.edu/research/epulopiscium/bacterial-endospores (Accessed on 24 February 2016).

Prabuseenivasan S, Jayakumar M, Ignacimuthu S (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC complementary and alternative medicine*, 6:39.

Songer J and Post K (Editors) (2004). Veterinary microbiology. Elsevier Saunders, St. Louis. Pp35-42.

Gill AO and Holley RA (2004). Mechanism of bactericidal action of cinnamaldehyde against Listeria monocytogenes and Eugenol against L. monocytogenes and Lactobacillus sakei. *Applied and Environmental Microbiology*, 70(10): 5750-5755.

MICROBIOLOGICAL QUALITY OF *CERITHIDEA OBTUSA* AND ANTIBIOTIC SENSITIVITY OF ITS BACTERIA ISOLATES

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ABSTRACT

Cerithidea obtusa is a delicacy known as *Siput sedut* in Malaysia. *C. obtusa* is a filter-feeder and is therefore suspected to have poor microbiological quality, especially when the hygiene and cleanliness of water environment of their origin are poor. Currently, the microbiological quality of the *C. obtusa* available in Malaysian markets have not been evaluated. Fourteen pooled *C. obtusa* samples purchased from 10 wet markets in Kuala Lumpur and Selangor, Malaysia were examined for occurrence of *Escherichia coli*, and *Salmonella* and *Vibrio spp*. and the antibiotic susceptibility of isolates determined. The *C. obtusa* sold in wet markets were from Malaysia (14.3%) and Indonesia (85.7%). Microbiological analysis showed that 28.6% of *C. obtusa* samples tested for standard plate count (SPC) had counts beyond the accepted limit of 5,000,000 CFU/g set by Microbiological Reference Criteria for shellfish. All samples tested for coliform plate count (CPC) had count of >1,000 CFU/g. There was no significant ($p>0.05$) difference in microbiological quality between *C. obtusa* from Malaysia and Indonesia. *E. coli* was detected in 21.4% of samples while 14.2 and 7.21% of samples were positive for *Salmonella* and *Vibrio spp*, respectively. The *E. coli* and *Salmonella* spp. isolates were all resistant to oxytetracycline. Among *E. coli* isolates, two were resistant to trimethoprim/sulfamethoxazole, florfenicol, azithromycin, ciprofloxacin, and ceftriaxone. Among *Salmonella* spp. isolates, one was susceptible to trimethoprim/sulfamethoxazole, florfenicol, amoxycillin, ciprofloxacin and ceftriaxone. The *Vibrio* spp. isolate was resistant to erythromycin only. In conclusion, the study showed that locally sold *C. obtusa* was of poor microbiological quality and harbour multidrug resistant pathogens.

Keywords: *Cerithideaobtusa, E. coli, Salmonella, Vibrio*, antibiotic sensitivity

INTRODUCTION

Cerithidea obtusa is a marine gastropod from the class *Gastropoda orthogastropoda*, order of *Sorbeoconcha*, super family *Cerithidea* and family *Potamididae*. This shellfish, known in Malaysia as *Siput sedut* can be found in

abundance in mangroves and estuarine mudflats in tropical and subtropical areas (Sealifebase.org, 2012). The *C. obtuse* is a delicacy in Malaysia.

Sewage is the main source of river pollution (Azni and Abdullah, 2006). Foodborne bacteria such as *E. coli* and *Salmonella* spp. are present in these water ways from faeces contamination (Feldhusen, 2000; Lyhs, 2009). In addition, *Vibrio* spp. are commonly in aquatic animals exposed to the contaminated rivers.

Judicious use of antibiotic in aquatic farming is good veterinary practice (FDA, 2014). However, the presence of antibiotic residues in aquaculture farms providing food to humans is of great concern, due to potential exposure to antibiotic resistant bacteria in the fishes, shrimps, and shellfishes.

C. obtusa is a filter-feeder therefore may have poor microbiological quality especially when the hygiene and cleanliness of the environment of their origin is poor. Currently, there is little information on the microbiological quality of shellfish produced in Malaysia. Thus, this study was conducted to determine the microbiological quality of *C. obtusa* sold in Malaysian wet markets.

MATERIALS AND METHODS

Sample

Fourteen pooled *C. obtusa* samples were purchased from 10 markets in Selangor and Kuala Lumpur, Malaysia. The samples were placed in an ice boxes and transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for bacteria isolation and identification.

The shells of the *C. obtusa* were cleaned with 70% alcohol and the meat of removed and the entire organism pooled and divided into portions of 3 and 10 g each. The 3 and 10g portions were homogenised with 27 and 90 mL peptone water, respectively, for 2 minutes in a stomacher.

Microbiology Quality

The 10g homogenised product was diluted 10-fold according to the method described by Maturin and Peeler (2001). Aliquots of 0.1 mL from the 10^{-3} to 10^{-8} diluted samples were pipetted onto the surface of standard plate count agar. The inoculum was spread over the entire surface of the agar plate using a L-shaped spreader before incubation at 37 °C for 48 hours under aerobic condition. One millilitre aliquot from the 10^{-3} to 10^{-8} diluted samples was pipetted onto the petrifilm (3M™Petrifilm™Coliform Plate Count) and the plate incubated at 37 °C for 24 hours under aerobic condition. The plates and films are selected and enumerated according to the method described by Maturin and Peeler, 2001).

Isolation and identification of E. coli

Three loopfuls each of 3g homogenised sample was streaked onto EMB agar and the plate incubated at 37 °C for 24 hours under aerobic condition. Typical *E. coli* colonies on EMB agar appeared black with green metalic sheen. Single colonies from EMB agar was picked and gram-stained. The colonies were also subcultured

onto nutrient agar for identification, followed by the sulfide indole motility test for confirmation of identity. After 24 hours, 1 drop of Kovacs reagent was drop into the tube to determine ability of the bacteria to produce indole from the tryptophan.

Isolation and identification of Salmonella spp.
One mL of 3g homogenised sample were transferred to 9 mL buffered peptone water. Pre-enriched samples were incubated at 37° C for 24 hours under aerobic condition. 0.1 mL of each pre-enriched samples was transferred into Rappaport-Vassilidiasis broth and incubated at 42 °C for 24 hours under aerobic condition. Three loopfuls of each enriched sample was streaked onto xylose lysine deoxycholate (XLD) agar and brilliant green agar (BGA) and the plates were incubated at 37° C for 24 hours under aerobic condition. Typical *Salmonella* spp. colonies appear glossy with black centres or completely black on XLD agar and pink colonies on BGA agar. Single colonies from XLD and BGA agar were picked for gram-staining. Typical colonies from XLD and BGA agar were subcultured onto nutrient agar for identification followed by triple sugar iron, lysine iron agar, sulfide indole motility, Citrate, urease, and serological tests. The serological test used *Salmonella* polyvalent agglutination serum. *Salmonella* isolates were sent to Veterinary Research Institute, Ipoh, Malaysia for serotyping.

Isolation and identification of Vibrio spp.
3 g homogenised samples were incubated at 30 °C for 24 hours under aerobic condition. Three loopfuls of each enriched sample was streaked onto thiosulfate citrate bile salts sucrose (TCBS) with 2% NaCl. The plates were then incubated at 30 °C for 24 hours under aerobic condition. Typical colonies are green and yellow on TCBS agar. Single colonies were picked and gram-stained. Typical colonies were subcultured onto tryptic soy agar (TSA) for identification and confirmation by oxidase, triple sugar iron, and oxidative fermentation tests. Species identification was done using the API 20E system test.

Antibiotic sensitivity of E. coli, and Salmonella and Vibrio spp.
The antibiotic sensitivity of *E. coli*, *Salmonella* and *Vibrio* spp. were tested against oxytetracycline (30µg), trimethoprim/Sulfamethoxazole (25µg), florfenicol (30µg), azithromycin (30µg), ciprofloxacin (5µg), ceftriaxone (30µg), amoxycillin (10µg), doxycycline (30µg) and erythromycin (15µg). Colonies from non-selective agar were transferred into test tubes containing TSA and vortexed to obtain bacterial suspension. The suspension was incubated at 37 ° C for 2 to 6 hours under aerobic condition. *E. coli* and *Salmonella* were isolated on nutrient agar and *Vibrio* on TSA. The turbidity of bacterial suspension was adjusted using the 0.5 McFarland standard. Sterile swabs were dipped into the bacterial suspension and then streaked over the entire surface of Mueller-Hinton agars. The plates were inverted and incubated at 37° C for 24 hours under aerobic condition. The zone of complete inhibition was measured using calipers. The growth inhibition zone was compared with zone-size interpretative table (Rao, 2011).

RESULTS AND DISCUSSION

Based on the information provide by retailers, 14.3% of the *C. obtusa* was from Malaysia and 85.7% from Indonesia. Microbiological analysis revealed that 28.6% of *C. obtusa* samples tested for SPC had a mean count of 30,809,000 CFU/g which was beyond the allowable 5,000,000 CFU/g set by the Microbiological Reference Criteria for Food, October 1995 (Food Administration Manual, 1995) while samples tested for CPC had a mean count of 1,026,428 CFU/g, which more than the allowable limit of 1,000 CFU/g. There was no significant ($p>0.05$) difference in SPC and CPC values between *C. obtusa* from Malaysia and Indonesia.

Three pooled *C. obtusa* samples (21.4%) tested positive for *E. coli*. Two *E. coli* isolates from the Indonesian and one Malaysia *C. obtusa* showed towards oxytetracycline (30µg). Only one isolate was susceptible to trimethoprim/sulfamethoxazole (25µg), florfenicol (30µg), azithromycin (30µg), ciprofloxacin (5µg) and ceftriaxone (30µg). Two pooled samples from Indonesia tested positive for *Salmonella* spp. These isolates were resistant to oxytetracycline (30µg). One *C. obtusa* sample from Indonesia were presumptively tested positive for *Vibrio* spp. API 20 E system test was done for further identification and the result showed that the isolate was *Vibrio parahaemolyticus*. The antibiotic sensitivity test showed that the *V. parahaemolyticus* was resistant to erythromycin (15 µg).

Filter-feeders seafoods, such as mussels and oysters, are prone to bacterial contamination (Hazzah *et al.*, 2011). *E. coli*, which was at a rather high prevalence, can be used as an indicator of faecal contamination (Feldhusen, 2000). The *E. coli* and *Salmonella* isolates were 100% resistant towards oxytretracycline. This could be due to the use of oxtetracycline in packing water to preserve the seafood for exportation (Shariff *et al*., 2000). In fact, the *V. parahaemolyticus* in freshwater fish sold at wet markets in Selangor, Malaysia was resistant to erythromycin (Noorlis *et al*., 2011).

In Indonesia oxytetracycline and erythromycin are the antibiotics normally used in aquaculture. Oxytetracycline is used widely to treat bacterial diseases in fish and shrimp and erythromycin to control bacterial disease of shrimp (Supriyadi and Rukyani, 2000). This practice had contributed to the antibiotic resistance of their acquaculture products.

REFERENCES

Azni I and Abdullah A-M (2006). Untreated Sullage: A challenge to the rehabilitation of rivers in Malaysia. *IMPAK* (3): 6-7.

Feldhusen F (2000). The role of seafood in bacterial foodborne diseases. *Microbes and Infection* , 2(13): 1651-1660

Food Administration Manual (1995). *Microbiology Reference Criteria for Food.* http://www.foodsafety.govt.nz/elibrary/industry/Microbiological_Reference-Guide_Assess.pdf (Accessed on 15 January 2016).

Hazzah WA, Abaza AF and Bakr WM (2011). Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. *Journal of American Science*, 7(9): 663-668.

Maturin L and Peeler JT (2001). Bacteriological Analytical Manual. Chapter 3: Aerobic Plate Count. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm06334.htm (Accessed on 29 January 2016).

Noorlis A, Ghazali FM, Cheah YK, Tuan Zainazor TC, Wong WC, Tunung R, Pui CF, Nishibuchi M, Nakaguchi Y, Son R (2011). Antibiotic resistance and biosafety of *Vibrio cholerae* and *Vibrio parahaemolyticus* from freshwater fish at retail level . *International Food Research Journal,* 18(4): 1523-1530.

Rao PN (2011). Zone diameters of antimicrobial agents according to CLSI guidelines 2011. http://www.microrao.com/micronotes/pg/kirby_bauer.pdf (Accessed on 15 September 2011).

Sealifebase.org. *Cerithidea obtusa* (Lamarck, 1822) obtuse horn shell. http://www.sealifebase.org/summary/Cerithidea-obtusa.html (Accesses on 29 January 2016).

Supriyadi H and Rukyani A (2000). The use of chemicals in aquaculture in Indonesia. http://hdl.handle.net/10862/603 (Accessed on 17 March 2016).

SURVEY ON PET-OWNER AWARENESS OF PARASITIC DISEASES IN CATS AND DOGS AND THE PREVENTIVE MEASURES IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Many parasitic diseases of cats and dogs are zoonotic and can affect humans. There is information of the awareness of the Malaysian public on the dangers of these diseases. Thus, a cross-sectional study to determine the level of awareness of pet-owners on cat and dog parasitic diseases and preventive measures was conducted in Klang Valley, Malaysia. The subjects were a random sample of 150 respondents who consulted 10 private veterinary clinics in Klang Valley area. A set of closed-ended-structured questionnaire was designed for the study that included questions on client and pet profiles and knowledge on parasitic diseases and preventive medicine in cats and dogs. From the study, 74% of respondents were aware of parasitic diseases while only 67% were aware of parasitic zoonosis. However, only 33% of respondents were aware of preventive medicine such as deworming, ectoparasite control, and heartworm prevention to be instituted on their pets. The association between educational status and the level of awareness of the respondents was determined. The study significantly ($p>0.05$) showed that pet owners with higher education level are aware of parasitic diseases but not clearly aware of parasitic zoonosis. In conclusion, the majority of the pet owners have limited knowledge and understanding on parasitic zoonosis.

Keywords: parasitic zoonosis, preventive medicine, pet-owners, awareness.

INTRODUCTION

In Malaysia, dogs and cats are the most common animals kept as pets. Malaysia has a hot and humid climate all year that provides a conducive environment for parasites to survive. In cats and dogs in Malaysia, the most common ectoparasites are fleas, mites, lice, and ticks, whereas hookworm, roundworm, tapeworm and heartworm are the most common endoparasites. This study was conducted to determine the level of awareness among pet-owners of parasitic diseases and preventive measures, and zoonosis in cats and dogs.

MATERIALS AND METHODS

Study Design and Population
A cross-sectional study on level of pet owner awareness of cat and dog parasitic diseases and their preventive measures was conducted from 11th to 31st January, 2016. This target of the study was 150 respondents who were cat or dog owners and had consulted small animal veterinary clinics in the Klang valley, Malaysia. The areas of study were in Seri Kembangan, Bangi, Kajang, Petaling Jaya, Subang Jaya, Shah Alam, Damansara, and Kuala Lumpur.

Study conduct
A random sample of consenting respondents who consulted 10 private veterinary clinics in Klang valley area were recruited in the study. The questionnnaires were either given to the participants or left with the clinic to be filled by the participants and collected after one week.

Data collection and analysis
Data were compiled in Microsoft Excel and analysed by the SPSS software Version 12.0 for descriptive statistics. The Chi-Square test was used to determine association between educational status and awareness of the pet-owner of parasitic diseases, preventive medicine, and zoonosis in cats and dogs.

RESULTS AND DISCUSSION

General pet health care
Most (41%) of the respondents kept their pets as semi-roamers. The majority (68%) of the respondents practiced ectoparasite control for their pets. Only 59% of respondents dewormed their pets regularly and 31% dewormed their pets only occasionally when referring their pets to veterinary clinics. Although 60% of respondents vaccinated their pets only 58% followed proper vaccination regime. These evidences highly suggestive that the general pet health care of cats and dogs by pet-owners in the Klang Valley is at moderate level.

Parasitic disease, preventive medicine, and zoonosis
Regarding parasitic diseases awareness, the majority (73.3%) of the respondents are aware of parasitic diseases (Figure 1). As shown by the analysis, awareness of parasitic disease is closely associated with level of education. In fact, the majority (82%) of the respondents in this study had high education. This finding is consistent with that of previous reports (Chee *et al*., 2008; Matos *et al*., 2013).

The majority (86.7%) of the pet-owners are aware of parasitic preventive medicine. This is clear because the majority of the pet owners subjected their pets to deworming (80%) and ectoparasite control (68%). However only 33% of pet-owners refer their pets to veterinary clinics for heartworm prevention. This

shows that there is general lack of awareness of heartworm prevention among pet-owners (Duarte *et al.,* 2010).

The level of awareness on parasitic zoonosis among respondents was only 32.7% and the remaining 67.3% have no idea of zoonosis. The situation is different in developed countries where in the Netherlands, for example, 53% knew of parasitic zoonosis (Massar, 2014) and in Portugal the awareness on parasitic zoonosis is even higher at 85% (Stull *et al*., 2016). The study shows pet-owners in Klang valley, Malaysia have a limited knowledge and awareness on parasitic zoonosis and consequently they would not understand the dangers of these diseases.

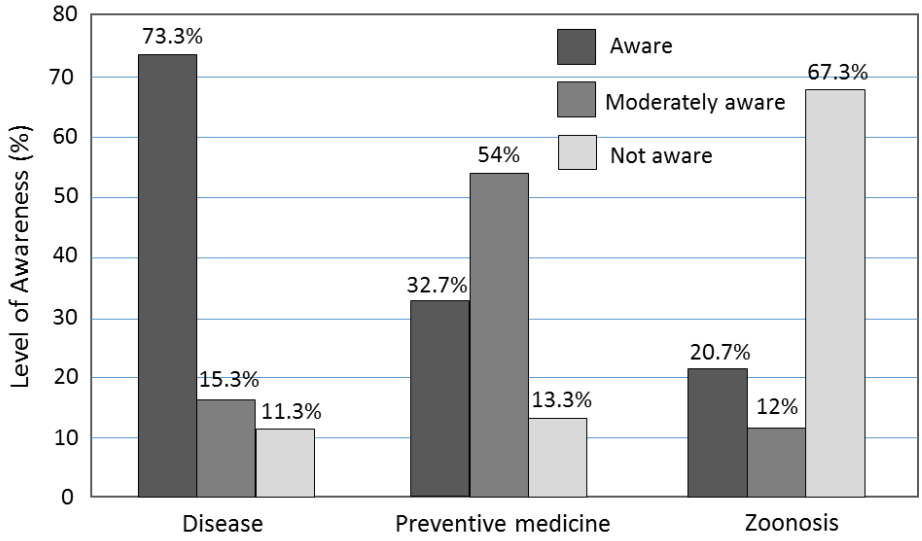

Figure 1: The level of awareness among pet owner-about parasitic diseases, parasitic preventive medicine and parasitic zoonosis in Klang Valley.

This study revealed that veterinarians are the most common sources of information for pet-owners of parasitic preventive medicine (75%) and parasitic zoonosis (62%). There is need for public education on importance of zoonotic diseases.

In conclusion, the majority of pet owner in Klang Valley, Malaysia are aware of parasitic diseases and c preventive medicine in cats and dogs but not aware of parasitic zoonosis.

REFERENCES

Chee J-H, Kwon J-K, Cho H-S, Cho K-O, Lee Y, Abdel-Aty AM, Shin S-S (2008). A survey of ectoparasite infestations in stray dogs of Gwang-ju City, Republic of Korea. *Korean Journal of Parasitology*, 46(1): 23-27.

Duarte A, Castro I, Pereira da Fonseca IM, Almeida V, Madeira de Carvalho L, Meireles J, Fazendeiro MI, Tavares L, Vaz Y (2010). Survey of infectious and parasitic diseases in stray cats at the Lisbon Metropolitan Area, Portugal. *Journal of Feline Medicine and Surgery*, 12(6): 441-446.

Stull JW, Carr AP, Chomel BB, Berghaus RD, Hird DW (2016). Small animal deworming protocols, client education, and veterinarian perception of zoonotic parasites in western Canada. *The Canadian Veterinary Journal*, 48(3): 269-276.

Matos M, Alh, AM, Owen SP, Nunes T, deCarvalho LM (2015). Parasite control practices and public perception of parasitic diseases: A survey of dog and cat owners. *Preventive Veterinary Medicine*, 122(1-2): 174-180.

Massar R (2014) Prevalence and awareness of zoonotic parasites of dogs on Curaçao. Thesis; Faculty of Veterinary Medicine, Utrecht University. http://dspace.library.uu.nl/handle/1874/273632 (Accessed on 16 September 2016)

IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROCOCCAL SPECIES ISOLATED FROM ANTIBIOTIC-EXPOSED CATS

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ABSTRACT

Enterococci are part of normal microbial flora in the gastrointestinal tract of humans and animals. The bacteria have emerged as a significant public health concern because o of its opportunistic characteristic in causing nosocomial infections and gaining resistance to many antimicrobial agents. The appearance of vancomycin-resistant enterococci (VRE) are causing serious problems in human and veterinary medicine. Thus, the objectives of this study were to isolate and identify enterococci in cats and determine the antimicrobial susceptibility pattern bacterial isolates. Thirty rectal swabs from cats known to have been treated with antibiotics at a veterinary clinic in Klang Valley, Malaysia, were collected processed for the isolation and identification of enterococci. The samples were inoculated onto blood agar and subjected to antimicrobial susceptibility test to amoxicillin, amoxicillin and clavulanic acid, enrofloxacin, marbofloxacin, doxycycline, and vancomycin. Six rectal swab samples were found positive for enterococci. The enterococci isolates were *E. faecalis* (3 isolates), *E. durans* (3 isolates) and *Enterococcus* sp. (1 isolate). None of the isolates were resistant to the 6 antibiotics tested. *Enterococcus* sp. isolate was resistant to amoxicillin, amoxicillin and clavulanic acid, enrofloxacin, marbofloxacin, and doxycycline (6 antibiotics). One isolate of *E. faecalis* was resistant to amoxicillin and clavulanic acid, enrofloxacin, marbofloxacin, and doxycycline (5 antibiotics). One *E. faecalis* and one *E. durans* isolate was resistant to amoxicillin and clavulanic acid, enrofloxacin, and marbofloxacin (4 antibiotics). Two *E. durans* and one *E. faecalis* isolates were resistant to enrofloxacin and marbofloxacin (2 antibiotics). All enterococci isolates were resistant to enrofloxacin and marbofloxacin and resistant to vancomycin. Thus, the study revealed 2 multidrug-resistant (MDR) enterococci and 2 extensively drug resistant (XDR) enterococci in cats.

Keywords*:* cat, rectal swab, enterococcus

INTRODUCTION

The enterococci are the normal microbial flora in the gastrointestinal tract of humans, animals and are commonly found in the environment, contaminated faecal materials, and food products derived from animals. *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus durans* are the major enterococcal species (Akhter *et al.*, 2011). These bacteria have emerged as a significant public health threat due their opportunistic characteristics causing nosocomial infections and the ability to acquire resistance to many antimicrobial agents (Kataoka *et al.*, 2013). The bacteria are known to cause urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infection, bacteraemia, and neonatal sepsis in humans (Poh *et al.*, 2006).

In cats, enterococci can be isolated in cases of bacterial urinary tract infections feline lower urinary tract and liver diseases, and inflamed ileum mucosa of terminally ill kittens with clinical signs of diarrhea. The infection can easily spread to humans through physical contact. Therefore, the objectives of this study were to isolate and identify enterococci in cats and determine antimicrobial susceptibility pattern of the isolates.

MATERIALS AND METHODS

Animal and samples

Thirty cats from a veterinary clinic history of being treated with antibiotics, amoxicillin 18/30, amoxicillin and clavulanic acid 5/30, marbofloxacin 5/30, enrofloxacin 4/30, and metronidazole 1/30 were selected for the study. Rectal swab was taken by using sterile cotton swab.

Isolation and identification of enterococcus

The faecal swab samples were streaked onto blood agar and the plate incubated at 37^{o}C for 24 hours. The colonies were examined for morphology and gram-stained. The presumptive enterococci were then subcultured onto blood agar and subjected to biochemical tests according to the standard bacterial identification procedures.

Antimicrobial Susceptibility Tests

Several colonies were diluted with sterile saline to obtain turbidity equivalent to 0.5 McFarland Standard. The inoculum was streaked evenly onto blood agar using a sterile cotton swab. Six (6) antibiotic discs, amoxicillin (10 µg), amoxicillin and clavulanic acid (30 µg), enrofloxacin (5 µg), marbofloxacin (5 µg), doxycycline (30 µg) and vancomycin (30 µg) were placed on the blood agar. The plates were then incubated at 37^{o}C for 24 hours and zone of inhibition was measured in millimeter usuing calipers.

RESULTS AND DICUSSION

Six (20%) samples were positive for enterococci, which was lower than that reported by other studies (43.8%) (Kataoka *et al.*, 2014; Jackson *et al.*, 2009). This difference in result may be attributed to differences in culture method. In their studies, the enterococcosel agar was used as selective medium for enterococci isolation.

The enterococci isolates were *E. faecalis* (3 isolates), *E. durans* (3 isolates) and *Enterococcus* sp. (1 isolate). None of the isolates were resistant to the 6 antibiotics tested. *Enterococcus* sp. isolate was resistant to amoxicillin, amoxicillin and clavulanic acid, enrofloxacin, marbofloxacin, and doxycycline (6 antibiotics). One isolate of *E. faecalis* was resistant to amoxicillin and clavulanic acid, enrofloxacin, marbofloxacin, and doxycycline (5 antibiotics). One *E. faecalis* and one *E. durans* isolate was resistant to amoxicillin and clavulanic acid, enrofloxacin, and marbofloxacin (4 antibiotics). Two *E. durans* and one *E. faecalis* isolates were resistant to enrofloxacin and marbofloxacin (2 antibiotics). All enterococci isolates were resistant to enrofloxacin and marbofloxacin and resistant to vancomycin. The study revealed 2 multidrug-resistant (MDR) enterococci and 2 extensively drug resistant (XDR) enterococci in cats.

REFERENCES

Akhter S, Asna ZH, Rahman MM (2011). Prevalence and antimicrobial susceptibility of enterococcus species isolated from clinical specimens. *Mymensingh Medical Journal,* 20(4): 694–699.

Jackson CR, Fedorka-Cray PJ, Davis JA, Barrett JB, Frye JG (2009). Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. *Journal of Applied Microbiology* 107(4): 1269-1278.

Kataoka Y, Ito C, Kawashima A, Ishii M, Yamashiro S, Harada K, Ochi H, Sawada T (2013). Identification and antimicrobial susceptibility of enterococci isolated from dogs and cats subjected to differing antibiotic pressures. *Journal of Veterinary Medicine and Science,* 75(6): 749-753.

Kataoka Y, Umino Y, Ochi H, Harada K, Sawada T (2014). Antimicrobial susceptibility of enterococcal species isolated from antibiotic-treated dogs and cats. *Journal of Veterinary Medical Science,* 76(10): 1399-1402.

Poh CH, Oh HML, Tan AL (2006). Epidemiology and clinical outcome of enterococcal bacteraemia in an acute care hospital. *The Journal of Infection,* 52(5): 383-386.

SALMONELLA, ESCHERICHIA COLI AND COLIFORM CONTAMINATION OF LAYER CHICKEN EGGS FROM FARM TO MARKET

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ABSTRACT

Contamination of eggs can occur at laying process, from farm and market environment, during handling, transportation and storage. This study was done to determine possible *Salmonella, Escherichia coli* and other coliform contaminations of layer chicken eggs and to determine the points of contamination along the egg production chain. Sixty layer chicken eggs were collected from three farms at spots; soon after laying, egg conveyor belt, grading, and market spots. Five eggs were collected at each spot. Egg surface swab was done for isolation of *Salmonella*, *E. coli* and coliform count. The egg content was used for isolation of *Salmonella* only. Xylose lysine sesoxycholate agar and Brilliant Green agar was used for isolation of *Salmonella* and eosin methylene blue agar for *E. coli*. Coliform count was done using Coliform 3M ® Petrifilm. From this study, 6.67% of surface of eggs collected at grading spot was positive for *Salmonella*. No *Salmonella* was isolated from egg contents. The low occurrence of *Salmonella* contamination on egg shell and content can most probably be attributed to the strict poultry farmer medication and care practices in their chickens. The *E. coli* isolation rate at market place, egg conveyor belt, and grading spot was 46.67, 20, and 13.33%, respectively. Thus, precautions need to be taken at farm and market level to prevent bacteria contamination along the production chain.

Keywords: *E. coli*, coliform, *Salmonella*, layer chicken eggs, farm, market

INTRODUCTION

Chicken eggs are popular and considered cheap, affordable food and can be consumed by people of all ethnic groups and religions. Egg is a nutritionally complete food and an excellent source of protein (Ruxton *et al*., 2010). In 2011, approximately 10.3 million eggs were produced in Malaysia and the consumption of eggs among Malaysian were 314 eggs per capita (DVS, 2014). Contaminated egg

and its products can increase risks of illnesses in humans because of salmonellosis and *E. coli* infection (Chousalkar *et al*., 2010). These diseases in humans can vary from mild symptoms to life threatening conditions. Among possible sources of infections is consumption of bacteria-contaminated foods. For example, salmonellosis can be acquired through consumption of infected raw or undercooked egg causing gastroenteritis. Chicken eggs are important source of *E. coli* infection that frequently cause sudden onset of cramps and abdominal pain followed by diarrhoea within 24 hours of exposure. Thus, coliform count can be used as an indicator of sanitary quality of foods. In this study, layer chicken eggs were analysed from production to market to determine bacteria contamination.

MATERIALS AND METHODS

Sixty layer chicken eggs were collected from three farms. Four spots of along the egg production identified for collection were soon after laying, at egg conveyor belt, grading, and market spots. Five eggs were collected in each spot. Egg surface swabs were used for isolation of *Salmonella* and *E. coli* and coliform count. Egg content was used for isolation of *Salmonella* only. Xylose lysine desoxycholate and Brilliant Green agars were for isolation of *Salmonella* and eosin methylene blue agar for isolation of *E. coli*. Coliform count was done using Coliform 3M® Petrifilm.

RESULTS AND DISCUSSION

From this study, 6.67% of surface of eggs collected at grading spot was positive for *Salmonella.* No *Salmonella* was isolated in egg content. The low occurrence of *Salmonella* contamination in egg shell and content are probably due the strict medication and care practices by the farmer on of their chickens. Prophylactic antibiotics given in feed by the farmer during the growing and laying stages may have reduced occurrence of *Salmonella* in eggs.

The grading and market spot eggs had higher coliform count compared to laying and conveyor spots. The rate *E. coli* isolation at market, egg conveyor belt, and grading spot was 46.67, 20, and 13.33%, respectively. The higher occurrence of *E. coli* in market place eggs could be due during poor handling, processing, transportation, and/or storage under unsuitable environments.

Although coliforms do not cause serious illness to people, they can still cause mild cases of foodborne infections and remain a health risk for consumers.

CONCLUSION

The study shows that eggs at the market were contaminated with *E. coli* and had high coliform count. Since bacteria was isolated in eggs soon after laying, the contamination of the eggs had occurred during transportation, handling, storage,

and/or during marketing. Greater precautions must be taken to ensure eggs reaching consumers are free from bacteria contaminations.

REFERENCES

Chousalkar KK, Flynn P, Sutherland M, Roberts JR, Cheetham BF (2010). Recovery of Salmonella and Escherichia coli from commercial egg shells and effect of translucency on bacteria penetration in eggs. *International Journal of Food Microbiology*, 142(1-2): 207-213.

Bahrouz MA and Al-Jaff (2005). The risk of bacterial contamination in hen eggs of Sulaimani poultries. *Journal of Zankoy Sulaimani*, 8A (1): 63-71.

DVS (Department of Veterinary Services, Malaysia) (2014). Malaysia: Per capita consumption of livestock products 2004-2013.

Ruxton CHS, Derbyshire E, Gibson S (2010). The nutritional properties and health benefits of eggs. *Nutrition and Food Science*, 40(3): 263-279.

EFFECT OF ROUTE OF VACCINATION ON IgM ANTIBODY RESPONSE TO *STREPTOCOCCUS AGALACTIAE* INFECTION IN THE RED HYBRID TILAPIA (*OREOCHROMIS* SP.) FINGERLINGS

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ABSTRACT

Streptococcosis is a disease caused by *Streptococcus sp*. This disease is a major problem for fish production industry because it is associated with great economic losses. This study was aimed at investigating the effect of vaccination routes against *Streptococcus agalatiae* infection in the Red hybrid tilapia fingerlings. Ninety fingerlings were randomly divided into three groups of 30 each. Formalin-killed feed-based and spray vaccine formulations were used in the study. Group 1 was vaccinated with spray vaccine for 3 consecutive days followed by booster doses on 3 consecutive days in the third week of the study. Group 2 was vaccinated once with spray vaccine followed by one booster dose of feed-based vaccine in the third week of the study. Group 3 served as a control group without treatment. All fingerlings were challenged with 100 µL 1 × 10^9 CFU/mL *S. agalactiae* intraperitoneally. Following challenge, the fingerlings were observed for clinical sign and mortality. Mucous swab samples from skin surfaces and gut lavage fluid were collected and the samples subjected to indirect enzyme-linked immunosorbent assay to determine the concentration of anti-*S. agalactiae* IgM antibody. PCR analysis positively identified the *S. agalactiae*. The results showed that the IgM antibody responses in mucus and gut lavage fluids in the Red Hybrid Tilapia fingerlings did not differ significantly ($p>0.05$) among methods of vaccine applications.

Keywords: *Streptococcus agalactiae*, Red hybrid tilapia, spray vaccine, feed-based vaccine, enzyme-linked immunosorbent assay

INTRODUCTION

Streptococcosis can be acute or chronic. Acute streptococcosis usually occurs during seasons when the water temperature is high causing peaks of fish mortality (Intervet, 2006). Streptococcosis is due infection by *Streptococcus* sp., a bacteria spherical or ovoid in shape and of 0.5 to 2.0 µm in diameter in size. The organisms occur in pairs or chains when grown in liquid media, are non-motile,

non-spore-forming and appear purple or blue when gram-stained (Amal and Zamri-Saad, 2011). Streptococcal bacteria are part of the normal flora on animal bodies, infections and diseases can occur when the bacteria enter the body through cuts, abrasions, wounds or when the immune system weakens, especially in stress. In fishes, streptococcosis can be caused by *S. agalactiae* (Suanyuk *et al*., 2005) *S. iniae* (Shoemaker *et al*., 2000) or *S. difficile* (Berridge *et al*., 2001). Group B *S. agalactiae* is an emerging pathogen of freshwater and saltwater fish species throughout the world (Evans *et al*., 2002). Fishes are highly susceptible to streptococcosis when their immunity is compromised. In Malaysia, high fish mortalities frequently between April and July, which are the dry months of the year (Ismail *et al*. 2016). Streptococcosis in fishes is commonly presented with anorexia, exophthalmia, ascites, and erratic swimming (Evans *et al*., 2002; Salvador *et al*., 2005) and meningoencephalitis (Eldar *et al*., 1995).

Vaccines are an antigenic material that stimulates the immune systems. The main aim of vaccinations is to stimulate host adaptive immune responses towards pathogens. Vaccination is the most effective method to combat and prevent diseases (Plant and LaPatra, 2011). The use of vaccine in aquaculture had significantly reduced antibiotic use in fish production (Shoemaker *et al*., 2009).

This study was conducted to determine the effect of different mode of vaccine applications on the antibody response the Red tilapia.

MATERIALS AND METHODS

Study design and data collection

Ninety Red tilapia fingerlings were equally divided into three groups. Group 1, was vaccinated with spray formalin-killed vaccine trice on 3 consecutive days and booster doses give trice, once per day on 3 consecutive days in week 3 of the experiment. Group 2 was vaccinated with spray formalin-killed once and followed one booster dose using the formalin-killed feed-based vaccine in week 3 of the experiment. Group 3 was not treated and served as a control group. Each fingerling was challenged with 100 µL 1×10^9 CFU/mL *S. agalactiae* live pathogenic via the intraperitoneal route. Following challenge, the fish were observed for 14 days for clinical signs and mortality.

Five fingerlings from each group were sampled for collection of mucus and gut lavage fluids. Mucus samples were collected using sterile cotton from the fish body surfaces. Gut lavage fluid was collected by infusing the 10 cm length of the hindgut with 1 mL of sterile phosphate-buffered saline and 0.02% (w/v) sodium azide using sterile pipette and tips.

Analysis

Mucous and gut lavage fluids were subjected to indirect enzyme-linked immunosorbent assay (ELISA) to determine the IgM antibody against *S. agalactiae*. Samples from the brain, eyes, and kidneys were taken in week 2 post-challenge for bacterial isolation, which was done by subculturing onto blood agar. The bacteria

were subjected to PCR for identification and confirmation of *S. agalactiae*.

Statistics

The data were analysed using MedCalc for Windows, version 12.7.0.0 (MedCalc Software, Mariakerke, Belgium) and tested at 5% level of significance. The differences of between time-points was determined by one-way analysis of variance (ANOVA) and the Student-Newman-Keuls pair-wise comparison tests.

RESULTS AND DISCUSSION

The mean antibody concentrations in the gut lavage fluid and mucous of vaccinated and challenged fish were significantly ($p<0.05$) higher than in the untreated control (Figure 1). The antibody responses peaked at week 4 post-vaccination.

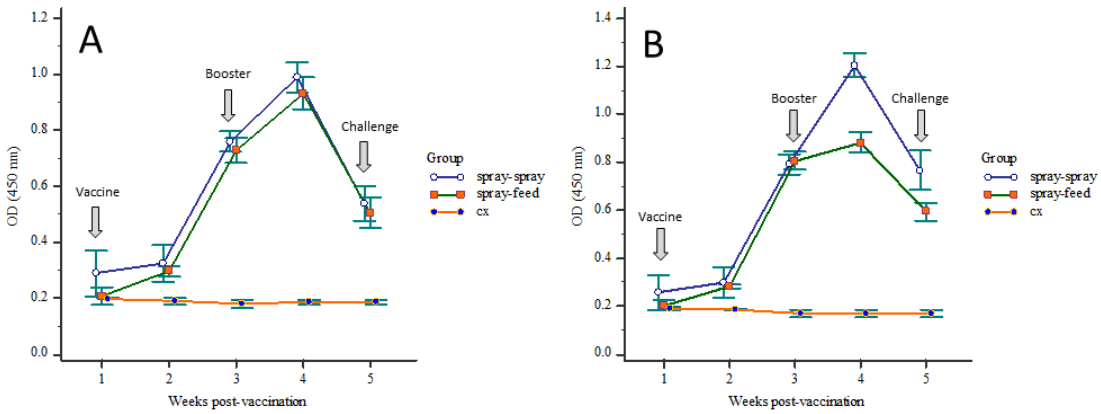

Figure 1: IgM antibody response in vaccinated Red tilapia challenged with *Streptococcus agalactiae*. (A) Gut lavage fluid, (B) skin mucous. Spray-spray=group treated with 3 consecutives dose of spray vaccine in weeks 1 and 3, spray-feed=group treated with one spray vaccine dose each in week 1 and 3, ex=untreated control.

There was no significant ($p>0.05$) difference in gut antibody response between applications spray and feed-based vaccine on antibody response of the Red Tilapia. PCR analysis was positive for *S. agalactiae.*

Spray vaccine administration may provide high level of protection towards infections. In fact, it was shown that one spray of formalin-killed *S. agalactiae* vaccine was able to induce antibody response in the Red tilapia (Noraini *et al.*, 2013).

In conclusion, this study shows that irrespective of mode of application, S. agalactiae vaccine can provide protection to infections in the Red tilapia.

REFERENCES

Amal MNA and Zamri-Saad M (2011). Streptococcosis in Tilapia (Oreochromisniloticus): A Review. *Pertanika Journal Tropical Agriculture Science*, 34(2): 195-206.

Berridge BR, Bercovier H, Frelier PF (2001). Streptococcus agalactiae and Streptococcocus difficile 16S-23S intergenic rDNA: genetic homogeneity and species-specific PCR. *Veterinary Microbiology*, 78(2): 165-173.

Eldar A, Bejerano Y, Livoff A, Horovitcz A, Bercovier H (1995). Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary Microbiology*, 43(1): 33-40.

Evans JJ, Klesius PH, Gilbert PM, Shoemaker CA, Al Sarawi MA, Landsberg J, Duremdez R, Al Marzouk A, Al Zenk S (2002). *Journal of Fish Diseases*, 25(9): 505-513.

Ismail MS, Siti-Zahrah A, Syafiq MRM, Amal MNA, Firdaus-Nawi NM, Zamri-Saad M (2016). Feed-based vaccination regime against streptococcosis in red tilapia, *Oreochromis niloticus* × *Oreochromis mossambicus. BMC Veterinary Research,* 12(1): 194.

Noraini O, Sabri MY, Siti-Zahrah A (2013). Efficacy of spray administration of formalin-killed *Streptococcus agalactiae* in Hybrid Red Tilapia. *Journal of Aquatic Animal Health*, 25(2): 142-148.

Plant KP and LaPatra SE (2011). Advances in fish vaccine delivery. *Developmental & Comparative Immunology*, 35(12): 1256–1262.

Salvador R, Muller EE, Freitas JC, Leonhadt JH, Giordano LGP, Dias JA (2005). Isolation and characterization of *Streptococcus* spp. group B in Nile Tilapia (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northen region of Parana State, Brazil. *Ciencia Rural*, 35(6): 1374-1378.

Shoemaker CA, Evans JJ, Klesius PH (2000). Density and dose: factors affecting mortality to *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). *Aquaculture*, 188(3-4): 229-235.

Shoemaker CA, Klesium PH, Evans JJ, Arias CR (2009). Use of modified live vaccines in aquaculture. *Journal of the World Aquaculture Society*, 40(5): 573-585.

Suanyuk N., Kanghear H., Khongpradit R, Supamattaya K. (2005). *Streptococcus agalactiae* infection in Tilapia (*Oreochromis niloticus*). *Songklanakarin Journal Science Technology,* 27(Suppl. 1): 307-319.

IMMUNE RESPONSE IN *STREPTOCOCCUS INIAE*-CHALLENGED RED HYBRID TILAPIA (*OREOCHROMIS* SP.) FINGERLINGS IMMUNISED WITH FEED-BASED *STREPTOCOCCUS INIAE* VACCINE

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ABSTRACT

Streptococcosis, a *Streptococcus iniae* infection causes morbidity and mortality in fishes. This study was done to determine the antibody responses in mucous and gut lavage fluids of Red Hybrid Tilapia fingerlings treated with feed-based formalin-killed *S. iniae* vaccination and their survivability upon challenge with the pathogen. The study was conducted over 5 weeks. Ninety Red Hybrid Tilapia fingerlings were divided into 3 equal groups. Group 1 was vaccinated once with feed-based vaccine followed by booster dose in week 3 of the experiment. Group 2 was vaccinated trice on 3 consecutive days in week 1 and 3 of the experiment. Group 3 was untreated and served as the control. Skin suface mucous and gut lavage fluid were collected weekly for six weeks to determine mucosal and systemic antibody responses. All fingerlings were challenged in week 5 with intraperitoneal 100 µL 1.5×10^9 CFU/mL *S. İniae* and observed for clinical signs and mortality for 7 days. There was no mortality recorded in the fish at day 7 post-challenge.

Keywords: streptococcosis, *S. iniae,* feed-based formalin-killed *S. iniae* vaccine, antibody response.

INTRODUCTION

Streptococcus iniae is a β-haemolytic, gram-positive, sphere-shaped bacterium that is associated with invasive disease outbreaks in cultured fish (Agnew and Barnes, 2007). Presently, most vaccines used in fishes are inactivated vaccines administered either by injections or as immersions (Gudding *et al*., 1999). This study was done to determine the effectiveness of the feed-based vaccine against *S. iniae* through the determination of antibody responses in the gut and skin of the Red Hybrid Tilapia fingerlings (*Oreochromis sp*.) and to isolate the bacterium from the eye, brain and kidneys of fish challenged with live pathogenic *S. iniae*.

MATERIALS AND METHODS

The study was conducted over 5 weeks. Ninety Red hybrid tilapia fingerlings were divided into 3 equal groups. Group 1 was vaccinated once with feed-based vaccine followed by a booster dose in week 3 of the experiment. Group 2 was vaccinated trice one a day on 3 consecutive days in week 1 and 3 of the experiment. Group 3 was untreated and served as the control. All groups were challenged in week 5 with 100 µL 1.5×10^9 CFU/mL *S. iniae* intraperitoneally. The fish were observed for clinical signs and mortality over 7 days before fishes were sacrifices. Brain, eye and kidney swabs were collected for bacterial isolation and subjected to polymerase chain reaction to confirm the bacteria species.

Mucous from body skin surfaces and gut lavage fluid were collected weekly. The mucous samples were collected using clean, sterile cotton to swab, immersed into 1 mL of sterile phosphate-buffered saline (PBS) with 0.02% (w/v) sodium azide, and kept overnight at 4°C. The solution was then centrifuged at 250 × *g* for 5 minutes, and the supernatant was collected and kept at -20°C until used. Gut lavage fluid was collected by infusing the hindgut with 1 mL of sterile PBS with 0.02% (w/v) sodium azide using a sterile needle and syringe. The fluid was kept at 4°C for 4 hours before being centrifuged 250 × *g* for 5 minutes. The supernatant was collected and subjected to ELISA antibody titre estimation.

RESULTS AND DISCUSSION

The mucous and gut lavage fluid antibody titre in vaccinated fish with *S. iniae* vaccine showed an increasing pattern begining on week post-vaccination peaking at week 4. The antibody titre of the control group remained low throughout the experimental period. There was no significant ($p>0.05$) difference in antibody titre of mucous and gut lavage fluid groups treated with the two modes of vaccination. The study showed that *S. iniae* vaccine protects the Red Hybrid Tilapia against the bacteria infection.

The bacterial culture showed no growth in samples from the vaccinated group. There were growth in samples from the eye, kidney, and brain of the untreated control fish. The *S. iniae* vaccine produced is safe as suggested by all fish surviving the immunisation. However, vaccine has short-term stability once mixed with feed for oral application.

CONCLUSION

Vaccination is one of preventive steps in controlling diseases. Feed-based *S. iniae* vaccination proves to be effective in increasing antibody titres in immunised Red hybrid tilapia. The study shows that vaccination with 1 day or 3 consecutive day regimes did not differ in antibody response in Red hybrid tilapia.

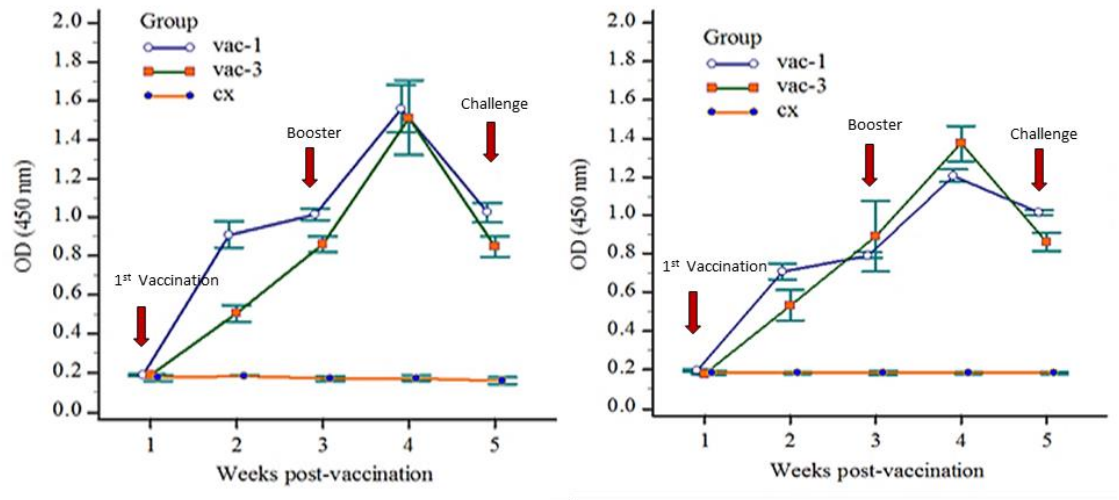

Figure 1: IgM antibody response in vaccinated Red tilapia challenged with *Streptococcus agalactiae*. (A) skin mucous, (B) Gut lavage fluid. Vac 1=group treated with one feed-based vaccine with booster dose, vac 3=group treated with 3 dose vaccine in week 1 and 3. Ex=untreated control.

REFERENCES

Agnew W and Barnes AC (2007). *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology*, 122(1-2): 1-15.

Gudding R, Lillehaug A, Evenson Ø (1999). Recent developments in fish vaccinology. *Veterinary Immnology and Immunopathology*, 72(1-2): 203-212.

ASSOCIATION BETWEEN BUFFALO PLASMA GROWTH HORMONE CONCENTRATION AND PHENOTYPE

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ABSTRACT

Growth performance is associated with the level of growth hormone in the body. The level of this hormone may also determine phenotype of animals. In this study, 40 buffaloes of various phenotypes were used. The phenotypic parameters chosen were breed, sex, body weight, age, birth weight, and age at puberty that were accessed from farm records for the period of 2006 to 2015. Blood samples were collected from the coccygeal vein into ethylenediamine tetraacetic acid (EDTA) tubes. Plasma was harvested to determine concentration of bovine growth hormone (bGH) using a commercial sandwich ELISA. There was no significant ($p>0.05$) difference in plasma bGH concentration between male and female, age of, or between swamp and Murrah-cross buffaloes. However, there was a negative correlation between plasma bGH concentration and body weight ($r = -0.069$), body weight, age ($r = -0.111$), birth weight ($r = -0.141$), and age at puberty ($r = -0.062$). The study shows that plasma bGH concentration is not associated with the buffalo phenotypes.

Keywords: buffaloes, growth hormone, phenotypes, ELISA

INTRODUCTION

Plasma growth hormone (GH) concentration is positively associated with growth performance. Thus, plasma GH level is a good parameter to be used in the selection of animals with superior growth traits (Wickramaratne *et al*., 2010). Plasma GH concentration was also found to be associated with breed (Shingu *et al*., 2001), sex (Irvin and Trenkle, 1971), body weight (Mears and Schaalje, 1993), age (Sarkar *et al*., 2008) and birth weight (Purchas *et al*., 1970). It is presumed that plasma bovine growth hormone (bGH) profile can an aid in selection of buffaloes with enhanced growth performance. Thus, the objective of this study was to determine the concentration of bGH in buffaloes and to investigate the association between plasma bGH concentration and selected phenotypes in buffaloes.

MATERIALS AND METHODS

Ten male and 30 female buffaloes were selected from the Buffalo Breeding and Research Center, Telupid, Sabah, Malaysia for the study. Approximately 10 mL blood samples were collected by coccygeal venipuncture using 18G needles. into EDTA tube. The blood samples were centrifuged at 250 × *g* for 15 minutes and plasma harvested and stored at -20°C pending analysis. Retrospective data for the period of 2006 and 2015 were collected from farm records. The parameters recorded were breed, sex, current body weight, age, birth weight and age at puberty of the buffaloes. Bovine growth hormone analysis was conducted at the Faculty of Veterinary Medicine, Universiti Putra Malaysia using a commercial ELISA kit (Novateinbio) based on quantitative sandwich immunoassay principle.

Statistical analysis was performed using IBM SPSS version 22. The Non-parametric tests, Mann-Whitney U test and Kruskal-Wallis H test, were used to determine the significant difference among groups of parameter. The association of the parameters was analysed using Spearman's Rank Correlation Coefficient. Correlation coefficient were correlated at $p<0.05$. The values were expressed as mean and standard error of mean.

RESULTS AND DISCUSSION

The plasma bGH concentratios of buffaloes were between 8.81 to 31.79 ng/mL and they varied among the buffaloes sampled according phenotypes. In an earlier study by others, the Bgh concentrations was reported to be between 7.0 and 17.0 ng/mL (Mishra *et al*. (2007). These differences in the results obtained by that study and ours can be due to difference in buffalo types. There was no association between plasma bGH concentration and selected buffalo phenotypes. This outcome could due to factor including hormonal behavior, buffalo breed, and sampling time. Growth hormone is secreted in bursts or pulses rather than at constant rate. This pulsative pattern causes wide diurnal fluctuations in plasma GH concentrations (Grigsby and Trenkle, 1986).

Differences in plasma bGH concentrations among breeds and sexes were determined by the Mann-Whitney Test. There was no significant ($p>0.05$) difference in plasma bGH concentration between the swamp and Murrah-cross buffaloes. Similar findings were obtained in the study on Angus-sired steers and Charolais-sired steers (Trenkle and Topel, 1978).

There was no significant ($p>0.05$) difference in plasma bGH concentrations between female and male buffaloes. In contrast, male dairy cattle seemed to have high plasma bGH concentrations than female (Irvin and Trenkle (1971).

Our study showed no significant ($p>0.05$) difference in plasma bGH concentrations among buffaloes of different age groups. This finding is not consistent with that of other studies in cattle. Young Holstein steer calves had higher plasma GH concentrations than old steers (Mears and Schaalje, 1993).

In this study the plasma bGH concentrations of the buffaloes were negatively correlated with body weight ($r = -0.069$), age ($r = -0.111$), birth weight ($r = -0.141$)

and age at puberty (r = -0.062). Based on other reports, the plasma bGH concentrations are expected to be positively correlated with body and birth weights (Purchas *et al*., 1970; Mears and Schaalje, 1993). However, our study was conducted on a small sample size without repetition; thus, results that are not consistent with thoses of other studies can be expected.

In conclusion, this study shows that plasma bGH concentration is not associated with buffalo phenotypes.

REFERENCES

Grigsby ME and Trenkle A (1986). Plasma growth hormone, insulin, glucocorticoids and thyroid hormone in large, medium and small breeds of steers with and without an estradiol implant. *Domestic Animal Endocrinology*, 3(4): 261-267.

Irvin R and Trenkle A (1971). Influences of age, breed and sex on plasma hormones in cattle. *Journal of Animal Science*, 32: 292-295.

Mears GJ and Schaalje GB (1993). Growth and growth hormone kinetics in Holstein steer calves. *Canadian Journal of Animal Science*, 73(2): 277-285.

Mishra A, Goswami T K, Shukla DC (2007). An enzyme-linked immunosorbent assay (ELISA) to measure growth hormone level in serum and milk of buffaloes (*Bubalus bubalis*). *Indian Journal of Experimental Biology,* 45(7): 594-598.

Purchas RW, Macmillan KL, Hafs HD (1970). Pituitary and plasma growth hormone levels in bulls from birth to one year of age. *Journal of Animal Science,* 31(2): 358-363.

Sarkar M, Nandankar UA, Duttaborah BK, Das S, Bhattacharya M, Prakash BS (2008). Plasma growth hormone concentrations in female yak (*Poephagus grunniens* L.) of different ages: Relations with age and body weight. *Livestock Science,* 115(2-3): 313-318.

Shingu H, Hodate K, Kushibiki S, Ueda Y, Watanabe A, Shinoda M, Matsumoto M (2001). Profiles of growth hormone and insulin secretion, and glucose response to insulin in growing Japanese Black heifers (beef type): comparison with Holstein heifers (dairy type). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 130(2): 259-270.

Trenkle A (1971). Growth hormone secretion rates in cattle. *Journal of Animal Science,* 32(1): 115-118.

Trenkle A and Topel DG (1978). Relationships o some endocrine measurements to growth and carcass composition of cattle. *Journal of Animal Science*, 46(6): 1604-1609.

Wickramaratne SHG, Ulmek BR, Dixit S, Kumar SP, Vyas MK (2010). Use of growth hormone gene polymorphism in selecting *Osmanabadi* and *Sangamneri* goats. *Tropical Agricultural Research*, 21(4): 398–411.

SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN LONG-TAILED MACAQUE (*MACACA FASCICULARIS*)

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ABSTRACT

Japanese Encephalitis Virus (JEV) is among the most important viral encephalitis in Asia caused by an envelope single-stranded positive-sense RNA virus of genus *Flaviviridae* and family *Flavivirus*. Japanese encephalitis virus is maintained in a zoonotic cycle with mosquitoes, principally *Culex tritaeniorhychus,* as the vector. Pigs and aquatic birds are the reservoir and amplifying hosts whereas humans, horses, and wild and domestic mammals are the dead-end hosts. According to World Health Organisation, JEV is widely endemic in Southeast Asia and Western Pacific regions. This study was conducted to determine the seroprevalence of JEV in macaques in Peninsular Malaysia. Forty-four of long-tailed macaque (*Macaca fascicularis*) serum samples collected from Pahang, Kelantan, and Perlis, Malaysia in 2015 and 2016 were used in the study. The macaques were of both sexes, various ages, and from different habitats were analysed. The antibody titres were determined by quantitative competitive ELISA assay. The result revealed that 20.45% (9/44) macaques were seropositive for JEV with the highest prevalence in Kelantan (33.33%). This study shows that long-tailed macaques in Peninsular Malaysia have been exposed to the JEV infection.

Keywords: Japanese Encephalitis Virus, long-tailed macaque, ELISA, seropositive, prevalence

INTRODUCTION

Japanese Encephalitis Virus (JEV) is a single-stranded RNA virus of genus *Flavivirus* and family *Flaviviridae***.** The virus is transmitted among vertebrate hosts by the mosquitoes, primarily *Culex tritaeniorhynchus*. The vertebrate hosts of JEV are humans, horse, domestic and wild animals, with pigs and aquatic birds playing a major amplification role (Zuckerman *et al*., 2009). The virus was first

documented as a viral encephalitis in the 1870s in Japan (Solomon *et al.*, 2003) and its geographic range extends from Eastern to Southeast Asia including Malaysia (Van den Hurk *et al*., 2009). It is estimated that 67,900 Japanese encephalitis (JE) cases occur annually in 24 endemic countries putting more than 3 billion people at risk to the infection (WHO, 2015). In Malaysia, the first JE case was reported in 1952 and the first outbreak occurred in Langkawi in 1974 with 10 cases and two deaths. Recently, in 2014, 16 JE cases were reported nationwide that resulted in four human deaths (Ismail, 2014).

One study conducted in the Philippines showed that *Macaca fascicularis* can be naturally infected with JEV with a consistent antibody prevalence rate of 35.2% (Inoue *et al*., 2003). In Japanese macaques *(Macaca fuscata)* JEV seropositivity was reported to be 44% (Shimoda *et al*., 2014). In Thailand, according to Nakgoi *et al*. (2013), 23.7% (9 out of 38) of captive monkey (*Macaca nemestrina*) colonies in the Northern region were seropositive for JEV.

This study was conducted to determine the seroprevalence of JEV in macaques in Peninsular Malaysia.

MATERIALS AND METHODS

Sample and data collection

Forty-four long-tailed macaque serum samples of 21 male and 23 female juvenile, sub-adult, and adult long-tailed macaque (*Macaca fascicularis*) collected from Pahang, Kelantan, and Perlis, Malaysia in 2015 and 2016 were used in the study. These samples were subdivided according to habitat of the macaque; suburban, urban, plantation, and secondary forest.

Enzyme-linked Immunosorbent assay

The presence of JEV-specific IgG antibodies in the monkey serum samples were determined by a commercial quantitative competitive Monkey Japanese Encephalitis Antibody IgG (JEAb-IgG) ELISA test kit.

Statistical Analysis

Statistical Package for the Social Science (SPSS) 22 was used to analyse data. The categorical data were assessed in cross-tabulation analyses using the Chi-square and odds ratio tests to determine association between risk factors and JEV antibody titre.

RESULTS AND DISCUSSION

Among macaque serum samples 20.45% (9/44) were seropositive for JEV. Samples from Kelantan showed the highest JEV antibodies prevalence (33.33%) followed by Perlis (31.58%), and Pahang at 5.26% (Table 1). The study also revealed significant ($p<0.05$) association between JEV antibody prevalence and state of

sample origin. The JEV antibody titre of samples from male macaques were significantly ($p< 0.05$) than in males than females. There is no difference ($p>0.05$) in JEV antibody titre among habitat and between ages. The prevalence of JEV antibodies is highest in macaques from plantation (40%) followed by urban (36.36%), secondary forests (16.67%), suburban (9.09%) samples. The prevalence of JEV antibodies is highest in the juvenile (29.31%) macaque than sub-adult (21.43%) or adult (7.69%) samples (Table 2).

Table 1: Japanese encephalitis virus serovalence in long-tail macaque (*Macaca fascicularis*) according to age and state of origin.

Parameter	Macaque information					
	Age			State of origin		
	Juvenile	Subadult	Adult	Pahang	Kelantan	Perlis
Number examined	17	14	13	19	6	19
Number positive	5	3	1	1	2	6
% positive	29.41%	21.43%	7.69%	5.26%	33.33%	31.58%

Table 2: Japanese encephalitis virus serovalence in long-tail macaque (*Macaca fascicularis*) according to habitat and sex.

Parameter	Macaque information					
	Habitat				Sex	
	Suburban	Urban	Plantation	Secondary forest	Female	Male
Number examined	22	11	5	6	21	23
Number positive	2	4	2	1	1	8
% positive	9.09%	36.36%	40.00%	16.67%	4.76%	34.78%

CONCLUSION

This study revealed that long-tail macaques in Peninsular Malaysia have been exposed to JEV infection with the overall prevalence of 20.45%. Therefore, there may be a risk of JEV transmission from macaques to human handlers.

REFERENCES

Inoue S, Morita K, Matias RR, Tuplano JV, Resuello RR, Candelario JR, Cruz DJ, Mapua CA, Hasebe F, Igarashi A, Natividad FF (2003). Distribution of three arbovirus antibodies among monkeys (*Macaca fascicularis*) in the Philippines.

Journal of Medical Primatology, 32(2), 89-94.
Ismail Z (2014). Another mosquito virus: How the Japanese encephalitis affects our brain. http://www.thestar.com.my/lifestyle/health/2014/07/20/its-no-jest/ (Accessed on 15 September 2016).
Nakgoi K, Nitatpattana N, Wajjwalku W, Pongsopawijit P, Kaewchot S, Yoksan S, Siripolwat V, Souris M, Gonzalez JP (2013). Dengue, Japanese encephalitis and Chikungunya virus antibody prevalence among captive monkey (*Macaca nemestrina*) colonies of Northern Thailand. *American Journal of Primatology*, 76(1): 97-102.
Shimoda H, Saito A, Noguchi K, Terada Y, Kuwata R, Akari H, Takasaki T, Maeda K (2014). Seroprevalence of Japanese encephalitis virus infection in captive Japanese macaques (*Macaca fuscata*). *Primates*, 55(3): 441-445.
Solomon T, Ni H, Beasley DWC, Ekkelenkamp M. Cardosa MJ, Barrett ADT (2003). Origin and evolution of Japanese encephalitis virus in Southeast Asia. *Journal of Virology*, 7(5): 3091-3098.
Van den Hurk AF, Ritchie S, Mackenzie J (2009). Ecology and geographical expansion of japanese encephalitis virus. *Annual Review of Entomology*, 54: 17-35.
WHO (World Health Organization) (2015). Japanese encephalitis. Fact sheet No 386. http://www.who.int/mediacentre/factsheets/fs386/en
(Accessed on 9 March 2016)
Zuckerman A, Banatvala J, Schoub B, Griffiths P, Mortimer P (Editors) (2009). Principles and Practice of Clinical Virology, 6th Edition, Wiley-Blackwell. Pp668-695.

COMPARISON OF DUCK EGG CONTAMINATION BETWEEN EGG-LAYING SPOTS

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ABSTRACT

Duck eggs can easily be contaminated by unclean egg-laying spots. This study compares bacteria contamination on shell of duck eggs obtained various egg-laying spots. Thirty Khaki-Campbell duck eggs were collected from pond-side, floor, and sand in two farms that used the same commercial diet for their ducks. Egg contamination was determined by coliform count, *Salmonella spp* isolation and identification, and egg-laying spot temperature detection methods. Coliform counts involved quantification of the coliforms *Escherichia spp*, *Enterobacter spp*, *Citrobacter spp*, and *Klebsiella spp*. on the duck eggshell and egg content. The results show that eggs from pond-side were more contaminated with coliform than from those from the sand. There was no difference in coliform count among eggs from pond-side, floor, and sand. With the except of one egg content sample, all eggshells and egg contents were negative for *Salmonella* sp. There was no correlation between temperature of egg-laying spot and coliform count. Thus, the study showed that egg-laying spots did not contribute significantly to egg coliform contamination.

Keyword: Khaki-Campbell layer, coliform, contamination, egg-laying spots, *Salmonella*, temperature

INTRODUCTION

In Malaysia Khaki-Campbell is the best known duck laying breed used for egg production. In some tropical areas, commercial duck farms practice fully-integrated farming system with aquaculture. This practice takes advantage of duck droppings to feed fish for high yields (Cullimore 2000). In this system, duck-houses are built beside a pond to allow full access to water for their natural behaviour. Ducks are allowed to freely range and as a result eggs can be found on all over the farm including in pond, at pond-side, in sand, and on the floor of duck houses. These eggs are manually collected, cleaned, packed and marketed.

In this study, it is assumed that eggs laid at pond-side will more contaminated with coliform from faeces than those on duck-house floor and sand. Currently,

there is no knowledge on the rate of coliform contamination of duck eggs laid at various sites on the farm. Thus, this study attempts to determine the rate of contamination of egg collected from the farm.

MATERIALS AND METHODS

Duck Eggs Sampling and Temperature Detection

The study was conducted at 2 commercial duck farms, A and B, in Tanjung Tualang, Perak, Malaysia with duck populations of 15,000 and 40,000 ducks, respectively. Thirty duck egg samples were collected randomly from three egg-laying spots; pond-side, duck-house floor, and sand. Each egg was place in individual small plastic bags to avoid cross-contamination. The bags were sealed, placed into a larger plastic bag, and transported in an ice box to the Microbiology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The temperature of the spot where the egg was taken was determined using a thermometer.

Coliform Plate Count

A sterile cotton swab moistened with peptone water (PW) was used to swab the egg surface randomly. The swab is then placed in 10 mL PW and shaken vigorously which formed the dilution 10^{-1}. Serial dilutions were done from 10^{-1} to 10^{-9}. After dilution was done, 1 mL aliquot of each dilution was transferred onto petri-film. The petri-film was incubated at 37ºC for 24 hours. All petri-films were counted at the same time by using the colony counter. The results were computed into excel and the data was selected according to the rules for selecting plates and counting colonies accredited by American Public Health Association, United States Food and Drug Administration (Bacteriological Analytical Manual).

Salmonella spp isolation and identification

Sterile cotton swab moistened with buffered peptone water (BPW) was used to swab egg surfaces and the swab immediately placed into a bottle with 10 mL BPW. The eggshells were cleaned with alcohol. Each egg was cracked open using a sterile forceps and the content obtained and placed into a sterile plastic bag. The egg content was thoroughly homogenised using a Bag Mixer and 1 mL egg content mixture was transferred to bottles containing 9 mL BPW. The bottles with shell and content were incubated at 37°C for 24 hours. 0.1 mL aliquots of the mixture were pipetted into 10 mL RV broth and incubated at 42°C for 24 hours. Loopfuls of RV broth with mixture were plated by streaking on the surface of xylose lysine desoxycholate agar and Brilliant Green agar and the plates were incubated at 37°C for 24 hours. Three typical colonies were picked and purified by streaking on the Nutrient agar and incubation at 37ºC for 24 hours.

Suspected colonies were subjected to the confirmatory triple sugar iron, lysine iron agar, SIM, citrate, and urease tests. Serological test was done by slide agglutination test with Salmonella O polyvalent antisera. Colonies of confirmed *Salmonella* sp. were inoculated into a slant nutrient agar and sent to the Veterinary

Research Institute, Ipoh, Malaysia for serotyping.

RESULTS AND DISCUSSION

Coliform Plate Count

There was no significant ($p<0.05$) difference in egg coliforms count between the egg laying spots in Farm A. However, in farm B, the pond-side eggs were more contaminated with coliform than the sand eggs. The differences in result can be attributed to the fact that Farm A treated its pond-side area with limestone powder approximately one month the conduct of this study. The last time Farm B treated its pond-side was more than a year ago. Limestone powder neutralises acid soils and lakes and indirectly kill bacteria through the heat of neutralization (Harwood and Lodge, 2014). The other explanation is that the layer density in the ponds of farm A is less because of their much lower duck population and pond contamination is not as high as in Farm B. It was shown that among risk factors for layer duck colisepticaemia include close proximity to other poultry farms or contaminated pond from high stocking density (Swayne, 2013).

Salmonella spp. isolation and identification

From the results, none of the eggshell yielded *Salmonella spp.* and only one egg content sample was positive for the organism. This contaminated egg is suggested to be the result of ovarian (Board and Fuller, 1994).

Egg-laying spot temperature

There was no correlation between egg-laying spot temperature and coliforms plate count. and there was no support relationship between temperature and coliform plate count.

CONCLUSION

The study shows that egg-laying spots did not contribute significantly to coliform contamination in duck eggs.

REFERENCES

Bacteriological Analytical Manual.
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm (Accessed on 17 September 2016).

Board RG and Fuller R (Editors) (1994). Microbiology of avian egg, 1st Edition, Chapman and Hall. Pp94-128.

Cullimore DR (2000). A Simplified Guide to Bacteria in Water Part 1. http://www.dbi.ca/Books/Docs/Bacteria.html (Accessed on 7 February 2016).

Harwood R and Lodge I (Editors) (2014). Limestone. In: Cambridge IGCSE® Chemistry Coursebook, 4th Edition, Cambridge University Press.

Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair VL (Editors) (2013). Colibacillosis. In: Diseases of Poultry. 13th Edition. Wiley-Blackwell, USA.

EFFECT OF SOY WASTE ON GROWTH PERFORMANCE AND CRUDE PROTEIN COMPOSITION OF RED HYBRID TILAPIA

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ABSTRACT

One of the commonest interests in tilapia farming worldwide is to diminish production cost particularly feeding cost and shortened output time. Soy waste is one of the locally available waste products that has high protein content and potential to be used as one of the feedstuffs in animal feed. Hence, this study was aimed to determine the effect of feeding soy waste on growth performance and crude protein composition in Red Hybrid Tilapia. Forty-five juvenile Red Hybrid Tilapia was divided into 3 equall treatment groups: Group 1: 40% soy waste + 60% commercial diets; Group 2: 20% soy waste + 80% commercial diets; and Group 3: control, without soy waste. Proximate analysis on formulated diet was done and that protein content was shown to be higher with the inclusion of soy waste in the diet. The results showed that fish with soy waste, especially at 40%, included in their commercial diet, showed significantly ($p<0.05$) greater body weight, length, girth, and fish protein composition than control fish. Thus, soy waste supplement improves growth performance and protein composition in the Red Hybrid Tilapia.

Keywords: Red Hybrid Tilapia, soy waste, growth performance, protein composition

EXPERIMENTAL INTRAOCULAR INFECTION OF JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*) WITH INFECTIOUS BURSAL DISEASE VIRUS INTERMEDIATE STRAIN

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ABSTRACT

Infectious bursal disease (IBD) was first discovered in 1957 in Gumboro, Delaware, USA and was first described in Malaysia in 1991. Infectious bursal disease becomes an important viral disease in poultry industry because it can cause profound immunosuppression, high mortality, and significant economic losses in birds. This experiment was conducted to determine the effect of intraocular treatment of quails with Japanese quails with IBD-modified live vaccine intermediate strain. The parameters selected for the study were clinical sign, post-mortem lesion, antigen detection using reverse transcriptase polymerase chain reaction (RT-PCR) and histopathological change. Twenty-four one-week old quail chicks were equally divided into three treatment groups; Group A treated with $1 \times 10^{4.8}TCID_{50}$/mL vaccine titre, Group B with $1 \times 10^{5.5}TCID_{50}$/mL of vaccine titre, and Group C with 0.03 mL sterile phosphate buffered saline and served as the negative control. Specific primers were designed to target the viral protein 2 gene. Two chicks were sacrificed on day 5, and two each on day 9 and 15 post-infection and bursas collected. The result reveals minor lymphoid depletion in Group A, prominent clinical signs and mild lymphoid depletion in Group B. None of the chicks showed post-mortem lesion. RT-PCR analyses were negative for all chick. Thus, quails inoculated with IBD virus intermediate strain vaccine showed negative immunological response. However, quails can be potential carrier for IBD.

Keywords: IBD, intraocular, Japanese quails (*Coturnix coturnix japonica*), RT-PCR, histopathology

DISEASE PREVALENCE AND ASSOCIATED PATHOLOGICAL CHANGES IN SMALL ANIMALS PRESENTED TO THE POST-MORTEM LABORATORY, FACULTY OF VETERINARY MEDICINE, UNIVERSITI PUTRA MALAYSIA FROM YEAR 2005 TO 2015

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ABSTRACT

Post-mortem or necropsy is an autopsy on an animal to determine cause of death when possible. The cause of death can be determined by gross lesions, histopathology and laboratory analyses. This retrospective study was conducted to determine the most common disease cats and dogs presented to the Post-mortem Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Thirty-seven cases among dogs were presented during the period of 2005 to 2015. The most common diagnoses among dogs were septicemia (10.2%), mammary gland tumor (8.4%) and canine distemper (7.9%). In cats, 27 diseases were diagnosed with the most common being traumatic injuries (19.5%), followed by feline infectious peritonitis (15.1%), and sporotrichosis (12%).

Keywords: post-mortem, diagnosis, cats, dogs

EXPERIMENTAL INTRAOCULAR INFECTION OF JAPANESE QUAILS *(Coturnix coturnix japonica)* WITH GENOTYPE VII NEWCASTLE DISEASE VIRUS

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ABSTRACT

The prevalence of Newcastle disease (ND) in the quail industry in Malaysia is not well documented. Genotype VII Newcastle disease virus (NDV) is the most predominant virus circulating among poultry farms in Malaysia. Therefore, this study aimed to determine the susceptibility of Japanese quails towards genotype VII NDV. The clinical sign, gross organ pathological change, viral detection in organs and cloacal swabs, and antibody titre were used as parameters to assess the susceptibility of Japanese quails to the infection. In this experimental study, 20 quails were divided into 3 groups: Group A (n= 8) treated intraocularly with 0.03 mL $1 \times 10^{3.5}$ ELD_{50}, Group B (n= 8) treated with 0.03 mL $1 \times 10^{7.0}$ ELD_{50} NDV strain IBS 002, and Control Group (n=4) treated with 0.03 mL 1X phosphate-buffered saline. Quails of infected groups showed depression, ruffled feathers, trachea rales, leg paralysis, and torticollis. However, there was no significant ($p>0.05$) difference in clinical sign between these groups. Necropsy on day 7 post-infection did not reveal gross pathological change in organs of infected quails. Cloacal swabs taken on day 7 post-infection from all quails were subjected to one-step reverse transcription real time polymerase chain reaction (RT-qPCR). The results from this analysis were negative for all groups. Organ samples from infected groups, such from the trachea, proventriculus, and caecal tonsil, were positive for the virus. Haemagglutination inhibition assay showed an increasing mean antibody titres over time in the infected groups. There was no significant ($p>0.05$) difference in antibody titre over time, but significant ($p<0.05$) difference was observed among infected groups. In summary, Japanese quails was shown to be susceptible to genotype VII NDV infection.

Key words*:* Genotype VII Newcastle disease virus, RT-qPCR, haemagglutination inhibition assay, susceptibility

SELF-RECOGNITION IN A YOUNG CHIMPANZEE

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ABSTRACT

Animal cognition refers to the mental capabilities and memory of an animal. The cognitive function also provides evidence of self-awareness, which is defined as the ability to distinguish oneself as an individual separate from the environment and other individuals. The mirror self-recognition (MSR) test or mark test is a behavioural technique used to determine whether or not non-humans possess the ability of self-recognition. The objective of this study was to measure and analyse self-recognition in a young chimpanzee. One chimpanzee (*Pan troglodytes*) was identified at Zoo Negara Malaysia to be used in the study. A 60 × 84 cm acrylic mirror was placed outside its cage. The chimpanzee was marked on the front of the head with odourless non-toxic white paint for the MSR test. Open mirror and mark test behaviours were recorded using a video camera over one week and all data were analysed using the ethogram procedure. Theoretically, animals are considered to be able to recognise themselves in a mirror if they go through four stages of behaviour; social responses, physical inspection, repetitive behaviour, and realisation of seeing themselves. The results showed that the chimpanzee had positive MSR in the open mirror test. However, it did not show self-recognition towards the mark, indicating negative MSR. Several factors such as bars of the cage, insufficient lighting, and fading mark colour may be reasons for the chimpanzee not reacting to the mark. Further researches are needed to understand the real capability of the chimpanzee for self-recognising. The understanding of cognitive ability will assist in the management of animals and the reestablishment of endangered and threatened species.

Keywords: mirror self-recognition, chimpanzee (*Pan troglodytes*), mark test

DETECTION OF AVIAN POLYOMAVIRUS IN PSITTACINE BIRDS FROM KLANG VALLEY, MALAYSIA

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ABSTRACT

Avian polyomavirus (APV) primarily affects young birds and can cause mortality in a wide range of psittacine and non-psittacine birds. Adult birds typically are resistant to infection; they seroconvert and shed the virus for as long as 90 days before the infection is cleared. The typical presentation of APV-infected birds in well-fleshed juveniles just before fledgling age are acute onset of lethargy, crop stasis, and death within 24 to 48 hours. The study was conducted to determine presence of APV among psittacine birds in Klang Valley Malaysia. Isolation of DNA was done on the faecal samples of 85 symptom-free psittacine birds obtained from 4 breeders. The presence of APV was analysed by polymerase chain reaction using primers specific for VP1 and APV-full length genes. The analysis produced positive results in 6 of 30 pooled samples (20%) from yellow-collared macaw, blue-headed parrot, red-crowned macaw, sulphur-crested cockatoo, blue-throated macaw and Pesquet's parrot. In conclusion, APV was shown to be present in psittacine birds in this study. This is a first report on the detection of APV in psittacine birds in Malaysia.

Keywords: avian polyomavirus, psittacine birds, polymerase chain reaction.

COST OF REARING A BUFFALO CALF FROM BIRTH TO WEANING AT THE BUFFALO BREEDING AND RESEARCH CENTRE FARM, TELUPID, SABAH, MALAYSIA

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ABSTRACT

Malaysia has been witnessing a decline in buffalo population because of lack of suitable land and superior breed for extensive farming, reproductive problems, and poor management. Most farmers have poor knowledge on farm economics for farm sustainability. Therefore, the objective of this study determine the cost of rearing a buffalo calf from birth to weaning age. The study was conducted at the Buffalo Breeding and Research Centre Farm, Telupid, Sabah, Malaysia. Information for the year 2015, obtained from the farm through a questionnaire were farm general and health management, healthcare, and labour and treatment costs. In 2015, the farms had 143 buffalo breeder and 133 buffalo calves born. The most common calf diseases were weight loss (15 cases), diarrhoea (17 cases), and respiratory diseases (3 cases). The weaning age of the buffaloes in the farm was 3 months. All buffalo calf was dewormed at birth. The calves were given 0.5 kg pellet/calf for 3 weeks during the preweaning period and remained with their dam until weaning before they were dewormed a second time. The cost of rearing a calf was calculated from feed (RM1.14/kg body weight), ID-tag (RM2/animal), preventive treatment (RM0.50/animal), and labour (RM4.73/person/hour) costs. Milk cost based on current market buffalo milk price was RM3.20/L. The calculation took into account the requirement for 90 consecutive days and average daily gain (0.6 kg) of calves, the cost of rearing a buffalo calf from birth until weaning was RM1842.46. The highest contributing cost was feed (99.85%) of which milk cost contributed 99.57% to overall feed cost. The cost of treatment/herd/year was RM969.44, with diarrhea, weight loss and respiratory diseases requiring RM30.16, RM30.16 and RM1.46 per animal, respectively. Thus, in this farm, the cost of rearing buffalo calves was RM246,016.77/herd/year with treatment costs accounting for 0.39% of total annual rearing cost. Hence, there is need for the farmer to improve the herd health programme to ensure reduction in overall buffalo-rearing cost.

Keywords: distribution cost, rearing buffalo calf

IN VITRO ANTHELMINTIC ACTIVITY OF PAPAYA (*CARICA PAPAYA*) LEAF CHLOROFORM EXTRACT AGAINST THE THIRD-STAGE LARVAE OF STRONGYLES FROM SHEEP

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ABSTRACT

Parasitic gastroenteritis due to helminthiasis is one of the major causes of economic losses in small ruminant farming. In Malaysia, helminthiasis causes severe morbidity and mortality in the small ruminant industry, and chemical anthelmintics are commonly used for treatment and control. However, frequent and indiscriminate use of anthelmintics has resulted in drug resistance. This study aimed to evaluate the effect of papaya leaves (*Carica papaya*) chloroform extract (CPE) as an anthelmintic on the third-stage larvae (L3) of strongyles from sheep. Pooled faecal samples from few sheep were cultured and L3 of strongyles were harvested 7 days later. One hundred L3 per group of six petri dishes each were used in the three CPE groups treated with 7.5, 10, and 12.5 mg/mL CPE, one Levamisole (10 mg/mL) group, and one negative control (10 mg/mL Levamisole plus 0.01% dimethyl sulfoxide) group. Compared with the negative control group, all concentrations of CPE used in this study exerted significant ($p<0.05$) anthelmintic activity towards L3 after 48 hours of treatment, with a mortality rate up to 99%. All L3 died at the second hour of Levamisole treatment. In conclusion, CPE can potentially be developed into an alternative anthelmintic agent for sheep.

Keywords: papaya (*Carica papaya*) leaves, chloroform extract, strongyle, L3 larvae, sheep

OCCURRENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *SALMONELLA* SPP. AND *ESCHERICHIA COLI* ISOLATES FROM PERIDOMESTIC COCKROACHES (*PERIPLANETA AMERICANA*)

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ABSTRACT

Cockroaches are abundant in Malaysia and harbour an array of pathogens. Environmental and sanitary conditions associated with demographic/socio-economic settings of an area contribute to the prevalence of disease pathogens in cockroaches. The aim of this study was to determine the incidence of bacteria of public health interest transmitted by cockroaches. Cockroaches were trapped in Serdang, Dengkil, and Kajang, Malaysia from one week from 11th to 17th January 2016. Forty cockroaches were collected and the species identified was the common peridomestic cockroaches, *Periplaneta americana*. The external body surface (n=40) and gut homogenates (n=40) samples from these cockroaches were analysed for *Salmonella* and *Escherichia coli* and bacteria isolated subjected to antibiotic susceptibility test. The study showed that 6 out 80 cockroaches specimens harboured *Salmonella* spp.; 5% from external body surfaces and 10% from gut contents. Twenty specimens were confirmed to contain *Salmonella* spp. isolates; 42.5% from external body surfaces and 12.5% from gut contents. The *Salmonella* and *E. coli* isolates in this study were multi-drug-resistant (MDR) to some antibiotics. The prevalence and burden of infection in cockroaches is a reflection of the sanitary conditions of these urban areas.

Keywords: cockroach, *Salmonella, E. coli*, antibiotic, multi-drug resistance

OWNER AWARENESS AND RISK FACTORS OF OBESITY IN CATS IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Obesity is a nutritional disease defined by an excess of body fat. Cats that are over nourished, lack the ability to exercise, or that have a tendency to retain weight are at risk for becoming obese. This study was conduct to determine owner awareness of cat obesity. A cross-sectional questionnaire study of cat owners in Klang Valley, Malaysia was done in January 2016. The objectives of this study were to determine the level of awareness of owners on the risks of obesity in their cats and factors contributing to feline obesity. The questionnaires comprised of three sections: demographics of the cat, feed and feeding regime, and owner awareness and knowledge on feline obesity. The body condition score of cats was rated by the owners using a validated five-point scale. One hundred and fifty owner responded to the survey. Data was analysed using descriptive statistics. Overall, the majority of the cat-owners were aware of the risks of feline obesity (88.7%, 133/150). The rate for overweightness and obesity in cats in this study was 47.3% (71/150). The study also showed that there a likely relationship between overweight and obesity with neutering status and age of cat, type of diet, management, and frequency of feeding. The major risks for feline obesity are neutering status, senior age (> 9 years old), commercial diet, keeping indoors, and excessive feeding. This study showed that cat-owner knowledge on the prevention of feline obesity is still low.

Keywords: cat, obesity, body condition score, cat-owner awareness

CHARACTERISTIC, STORAGE TIME, PACKAGING, AND SOURCE OF PRODUCTION OF CATTLE FROZEN-THAWED SPERM

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ABSTRACT

Artificial insemination (AI) with cryopreserved semen is the predominant method used in cattle reproduction around the world. The purpose of using AI, using good quality semen, is to obtain crossbred animals with high milk and beef production and that are resistant to diseases. The aim of this study is to compare the general and progressive motility, viability, and characteristics of frozen-thawed semen of different cattle breeds, and the duration of storage, packaging, and source of production of semen. Sixty-six produced locally semen samples from eleven breeds, packed either in glass ampoules or straws were evaluated. The semen samples were from Brahman, Droughmaster, Jersey, Hereford, Sahiwal, Friesian-cross, Kedah-Kelantan, Brangus, Mafriwal, Nelore, and Holstein cattle. The results showed that the general motility significantly ($p<0.05$) decreased with duration of storage. Package in straws (64.32±1.96%) produced higher general semen general motility than in glass ampules (55.31±2.13%). Holstein (87.22±0.70%) produced semen of the highest general motility while Kedah-Kelantan (37.22±1.02%) cattle the lowest among breeds. Thawed semen produced in 2010 had the lowest viability (31.95±0.80%) and abnormality (1.72±0.34%) and those produced in 1992 the highest viability (76.11±2.28%) and in 2008 the highest abnormality (10.48±1.22%). Based on the results, Holstein frozen-thawed semen was of the best quality among breed evaluated in this study and frozen-thawed semen produced in 1999 was of the best quality.

Keywords: semen, frozen-thawed, motility, viability, morphology,

EFFECT OF FEED FORMULATION ON THE BLOOD PROFILE OF GOATS

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ABSTRACT

Poor animal health and diseases are two causes of low farm productivity level and low profit margin. Therefore, good feeding strategies are imperative for herd health and farm performance. Determination of the nutrition-related blood parameters can be used as an indicator of nutritional status. Thus, this study was conducted to determine the nutrient composition of feed formulations and effect of feed on blood profile in goats. Eighteen female adult Boer cross goats, *Capra aegagrus hircus,* were selected and allocated to three groups (n=6/group) and assigned according to different feed formulation; Diet 1 - current feed provided by farmer, Diet 2 - maintenance diet, and Diet 3 - flushing diet. Blood sampling was conducted before the experiment and after four weeks of feeding trial for the detemination of blood total protein, cholesterol, calcium, glucose and fatty acid concentrations. The diet were subjected to proximate analysis and determination of fatty acid content. The total protein, glucose and calcium concentrations were highest in Diet 3 and lowest in Diet 1 ($p<0.05$). There no significant ($p>0.05$) difference in blood cholesterol and fatty acid concentration among groups. Fatty acid analysis of feed and plasma showed that the content of polyunsaturated fatty acid is higher than saturated fatty acid. Thus, it is important to provide animals with nutrient according to stages of production as sufficent protein and energy will improve breeding efficacy and quality of animal products. In conclusion, this study showed that feed formulation affects nutrition-related blood parameters in goats.

Keywords: proximate analysis, blood profile, fatty acid

PARASITE SPECIES AND BURDEN IN APPARENTLY HEALTHY AND CLINICALLY ILL RED JUNGLEFOWLS (*GALLUS GALLUS*)

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ABSTRACT

The Red Junglefowl, *Gallus gallus,* is one of the four species of Junglefowl found in Asia. It is known as the ancestor of the domestic fowl (*Gallus domesticus*). These birds when domesticated were observed to be very susceptible to diseases that affect domestic chickens. This study aimed to identify the ectoparasites, endoparasites, and blood parasites harbouring Red Jungle fowl and to compare the burden of the parasites between these birds. Twelve clinically ill, with clinical symptoms, and four apparently healthy Red Jungle fowl were sampled from a Red Jungle fowl farm located in Jenderam Hulu, Selango, Malaysia. The birds were examined for ectoparasites, endoparasites, and blood parasites. The species of parasites recovered were *Haemaphysalis wellingtoni*, *Lipeurus caponis*, *Menopon gallinae*, *Menacanthus stramineus*, *Goniodes dissimilis*, *Goniocotes hologaster* (ectoparasites); Strongeloides sp., Capillaria sp., *Heterakis gallinarum*, *Raillietina cesticillus*, *Raillietina tetragona*, *Raillietina echinobothridia*, *Eimeria* sp., (endoparasites); *Plasmodium gallinaceum*, *Leucocytozoon sabrazesi*, *Microfilaria* sp., *Trypanosome* sp. (blood parasites). The results show that there were no significant ($p>0.05$) difference in parasite burden between apparently healthy and clinically ill Red Jungle Fowls.

Keywords: Red Jungle fowl, ectoparasites, endoparasites, blood parasites, apparently healthy, clinically ill

SEROPREVALENCE AND MOLECULAR DETECTION OF LEPTOSPIROSIS AMONG WORKING DOGS IN MALAYSIA

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ABSTRACT

Leptospirosis is an emerging or re-emerging infectious disease and presumed to be the most widespread zoonotic disease in the world. The disease commonly occurs in tropical and subtropical regions. The aim of this study was to determine seroprevalence of leptospirosis among the working dog population in Malaysia. Blood samples were obtained from the Royal Malaysia Police, Federal Land Development Authority Security Police, Royal Malaysian Customs Department, Fire and Rescue Department, and Prison Department of Malaysia. Ninety-six blood samples were collected from clinically healthy working dogs. Serum samples were subjected to microscopic agglutination test (MAT) for 11 *Leptospira* serovars; icterohaemorrhagiae, canicola, pomona, grippotyphosa, australis, bataviae, javanica, tarassovi, hebdomadis, lai and pyrogene, to determine the seroprevalence of canine leptospirosis. Whole blood samples examined by polymerase chain reaction (PCR) assay using primers targeting 531 bp pathogenic-specific and 331 bp genus-specific *Leptospira* genes produced negative results. Three serum samples (3.1%) tested positive for *Leptospira javanica*, *L. australis* and *L. bataviae* on MAT with antibody titre cut-off point of 1:80. Leptospires DNA was not detected in any of the samples. The dogs tested positively with MAT may be carriers of the leptospiral organism. It seems that in spite of annual vaccination, working dogs are still at risk of acquiring leptospirosis.

Keywords: Canine Leptospirosis, Seroprevalence, Working dogs, Microscopic Agglutination Test, Polymerase Chain Reaction

DETERMINATION OF POST-MORTEM INTERVAL VIA IMMUNOHISTOCHEMICAL LOCALISATION AND EXPRESSION OF THE BIOGENIC AMINE, CADAVERINE

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ABSTRACT

Post-mortem interval (PMI) is the time that elapses from death to discovery of carcass or human body. The determination of PMI is of crucial importance in a forensic investigation as it would reduce uncertainties with respect to time and *prima facie*. This study was conducted to determine the parameter to be used for the estimation of PMI, using immunohistochemical localisation and biogenic amine, cadaverine, expression. The brain, liver, and epaxial muscle tissue samples from three dogs were obtained at 0, 12, 18, and 24 hours post-mortem. The samples were then immunohistochemically processed for cadaverine and subjected to histopathological assessment. Each sample was scored using the immunohistochemistry profiler software (Image J, IHC profiler) to determine expression of cadaverine. The results demonstrated significant ($p<0.05$) differences in cadaverine expressions with time in the brain and liver, but not muscle, tissues. These cadaverine expressions in the brain and liver tissues were comparable. There was significant correlation between temperature changes and concentration of brain ($r=-0.898$, $p<0.001$) and liver ($r=-0.958$, $p<0.001$) cadaverine only. Likewise, the post-mortem changes based on routine histopathology correlated well with tissue cadaverine expression. In conclusion, brain and liver cadaverine expressions are potential indicators of PMI.

Keywords: post-mortem interval, immunohistochemistry, cadaverine, dogs

EFFECT OF RED AND BLUE LIGHTS ON STRESS RESPONSE AND GROWTH PERFORMANCE OF JUVENILE RED TILAPIA (*OREOCHROMIS* SP.)

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ABSTRACT

Colour of light was found to have effect on the stress response and growth performance of several species of fish. This study evaluated the effect of visible light with longest (red) and shortest (blue) wavelength on the stress response and growth performance of juvenile Red Tilapia over a period of 23 days. The red and blue lights for treatment groups and white light for the control group were fixed underneath the tank lid and covered with opaque black plastic sheet. Five blood sample were obtained from fish chosen randomly from each tank (n=45) on days 0, 1, 3, 8, 15, and 23. The blood parameters; erythrocyte, leucocyte, and thrombocyte counts, haemoglobin concentration, packed cell volume, mean cell volume, mean cell haemoglobin and plasma protein, triglyceride, glucose, and total serum protein, albumin, globulin and sodium, potassium, and chloride concentrations were determined. Body length and weight were also measured on days 0 and 23 of experiment to determine average length gain, body weight gain, and specific growth and feed conversion rates. The result showed no significant ($p>0.05$) difference in stress response among fish treated with the red, blue and white lights. However, fish treated with blue light showed significantly ($p<0.05$) more gains in length compared to those treated with white light.

Keywords: juvenile Red Tilapia, colour of light, blood parameters, stress response, growth performance

ULTRASONOGRAPHIC IMAGING OF ABDOMINAL ORGANS IN GOATS

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ABSTRACT

Ultrasound imaging is routine conducted in small ruminants as a rapid non-invasive technique to obtain information on the condition of abdominal organs. This study was conducted on the abdominal organ ultrasonography of 5 apparently healthy female goats. The goats were not sedated but restrained in standing position. The area of examination was clipped and shaved. The organs were examined using SonoScape ultrasound scanner attached to linear and convex transabdominal probes with a frequency between 4.0 - 7.0 MHz. The rumen wall could be examined and identified at the left flank area while the spleen the 11th intercostal space (ICS). Splenic vessel was rarely seen. The reticulum wall could be examined at the ventral abdomen at the 6th ICS just caudal to the xiphoid and the abomasum wall at the ventral part of the 7th ICS. The omasum could be examined at the 8th ICS, the liver with the hepatic and portal vein, from the 7th to 12th ICSs. The kidneys could be examined just caudal to the last rib and the gall bladder on the right side at the 10th ICS. In conclusion, ultrasonographic examination is convenient for small ruminant medicine as an aid in disease diagnosis and evaluation of internal organs.

Keywords: ultrasound, intercostal space, probe, goat, organs

A RETROSPECTIVE STUDY ON INDICATIONS AND OUTCOMES OF URETHROSTOMY IN CATS AND DOGS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL UNIVERSITI PUTRA MALAYSIA FROM YEAR 2010 TO 2015

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ABSTRACT

Urethrostomy is the creation of a permanent stoma into the urethra when diversion of urine flow proximal to obstruction, site of narrowing, severely damaged, destruction, or diseased urethra is required. The objectives of this study were to identify the indications for and outcome of urethrostomy in cats and dogs and to determine the prevalence of post-urethrostomy complications in cats and dogs presented to University Veterinary Hospital (UVH), Universiti Putra Malaysia from 2010 to 2015. Twenty urethrostomy cases, comprising of 17 cats and 3 dogs, were identified in this study. Patient clinical data, primarily the signalment, clinical presentation, diagnostic investigation, surgical approaches, complications occurred post-urethrostomy and response to treatment were obtained from UVH records. The most common indications for urethrostomy in feline group were urethral rupture (59%), followed by urethral stenosis (29%), urolithiasis (6%), and urethral fistula (6%). Indications for urethrostomy in dogs were urolithiasis (67%) and urethral stenosis (33%). Post-urethrostomy complications in cats included urine burn (29%), bruises (12%), seroma (12%), euthanasia or death (12%), haemorrhages (6%), and granulomatous lesion at urethral opening (6%). All dogs showed haemorrhages after urethrostomy and one had bruises at the surgical site. All dogs and 47% (8/27) cats showed good response to surgical treatment, while 29% (5/17) cats had the primary problem resolved with occurrence of complication. This study showed that overall response to urethrostomy in cats and dogs were good in spite of several immediate complications.

Keywords: urethrostomy, cats, dogs, University Veterinary Hospital, indications

EFFICACY OF INACTIVATED AVIAN PATHOGENIC *ESCHERICHIA COLI* AGAINST THE BACTERIAL INFECTION IN BROILER CHICKENS

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in poultry manifesting as clinical conditions including colisepticemia and cellulitis. This study was to determine the efficacy of inactivated APEC either singly or combination with virulent associated gene (VAG) 5 and 6 against the bacterial infection in broiler chickens. Eighty-four day-old broiler chickens were divided into 7 groups of 12 chickens each. On day 1, group 1 and 4 were inoculated with inactivated APEC with VAG 5, groups 2 and 5 with VAG 6 and groups 3 and 6 with a combination of VAG 5 and VAG 6, group 7 was not inoculated and served as the control group. On day 14, booster was given to groups 4, 5 and 6. On day 28, the chickens were challenged with VAG 6 either via intramuscular or intranasal routes. On day 35, all the chickens were sacrificed, and the livers and spleens were collected for bacterial identification and the livers and trachea for histopathology. Two chickens from the control group showed stunted growth, dehydration, inappetence and gross perihepatitis and pericarditis after intramuscular challenge. *E. coli* was cultured from one sample only of the same group. The study showed that inactivated APEC either singly or in combination with virulent associated gene (VAG) 5 and 6 gives better protection against the bacterial infection in broiler chickens than in non-inoculated chickens.

Keywords: Avian pathogenic *Escherichia coli* (APEC), inactivated, virulent associated gene (VAG), broiler chickens

ASSESSMENT OF MATING BEHAVIOUR IN BUFFALO BULLS

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ABSTRACT

The objective of this study was to determine the mating behaviour differences among buffalo bulls (*Bubalus bubalis*). The study was conducted at the Buffalo Breeding and Research Centre, Department of Veterinary Services and Animal Industry, Telupid, Sabah, Malaysia. Four buffalo bulls and 16 non-pregnant buffalo cows were selected for this study. The bulls and cows were divided into 4 groups with the ratio of 1 bull to 4 cows. The cows were estrus-synchronised before mixing with the bulls in the paddock. The observation was done over 3 sessions, in morning, afternoon, and evening for 9 days. The mating behaviour parameters such as libido, reaction time and sexual interest were observed and scored. Ten matings by all bulls were observed throughout the study period. The results showed that the highest libido score was 7 of 10 and the lowest was 3 of 10. The reaction time was observed once only, which was 3 minutes and 50 seconds. The Flehmen reaction characterised by the sniffing and licking of genitals were the highest parameter observed at 100% (10 of 10). Tending behaviour was 90% (9 out of 10) and chin-resting behaviour was observed once out of 10 (10%). Of 10 matings, the highest frequency was in the afternoon at 5 times of 10 (50%) followed by 4 times in the evening (40%) and once in the morning (10%). This study showed that mating behaviour vary among bulls with Flehmen reaction and tending behaviour being highest sexual interest behaviour.

Keywords: buffalo, bulls, mating behaviour, libido, sexual interest, reaction time

FELINE AND CANINE VACCINATION PROTOCOLS IN PENINSULAR MALAYSIA AND VETERINARIAN PERCEPTION OF THE RECOMMENDATIONS BY THE WSAVA ASIAN VACCINATION GUIDELINES GROUP

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ABSTRACT

Vaccination in cats and dogs has been one of the most important components in the preventive healthcare. The World Small Animal Veterinary Association (WSAVA) Asian Vaccination Guideline Group (VGG) introduced new recommendations on vaccination protocols that are vastly different from long-established protocols. There were doubts, criticisms and reluctance among veterinary practitioners on the adoption of the new protocols. This study was conducted to determine the current feline and canine vaccination practice in Peninsular Malaysia and to record the perceptions of Malaysian veterinarians regarding the new protocols. A questionnaire adapted from the WSAVA Asian VGG is used and distributed randomly to private clinics in several states of peninsular Malaysia. Forty-two clinics participated in this study and 82% of the respondents were familiar with the guidelines. The majority (53.3%) of clinics recommend starting vaccination at 8 weeks of age and the last puppy series vaccination at 16 week of age. In the clinics 50% of practice vaccination in senior and geriatric dogs, 80% practice annual vaccination while 20% practice triennial vaccination. The recommended age to begin the kitten vaccination series was variable with the majority of veterinarians (85%) starting at 8 weeks of age. The last vaccination for the kitten series was recommended at 16 weeks of age. All clinics practice annual revaccination for adult cats. The perception of veterinarians towards the WSAVA Asian VGG recommendation varied. Fifty-five percent of veterinarians would consider following the recommended guidelines for the vaccination protocols only if suitable vaccine products are available. There is need for veterinarians, researchers and pharmaceutical industries, to further discuss on the adoption of WSAVA Asian VGG recommendations.

Keywords: canine vaccination, Feline vaccination, WSAVA, Peninsular Malaysia, Veterinarian perceptions

USE OF RED AND YELLOW LIGHT-EMITTING DIODES TO PROMOTE GROWTH, PROXIMATE COMPOSITION AND CELL MORPHOLOGY OF THE MARINE MICROALGA, *TETRASELMIS* SP.

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ABSTRACT

Microalgae, *Tetraselmis* sp., are unicellular plants that can manufacture their own food material through photosynthesis. This plant is important in aquaculture development because it is one of the primary food sources for aquatic organisms. In this study, the growth and proximate composition of marine microalga were compared under different wavelengths; red and yellow colour light-emitting diodes (LEDs) with fluorescent light was used for the controls. There was no significant ($p>0.05$) difference in growth rate of the *Tetraselmis* sp. under red, yellow, and fluorescent light during the 11 days. However, fluorescent light produced higher cell density and optical density compared to red LED on days 12 and 13 of the experiment. Lipid and protein contents of microalgae were significantly ($p<0.05$) higher under red LEDs whereas carbohydrate composition was significantly ($p<0.05$) higher under flourseccent light compared to the control. Under scanning electron microscope, the cell morphology and size did not differ among LED treatments. The shape of the *Tetraselmis* sp. Under the three light treatments was broad elliptical at the initial stage and elliptical at the final stage of their development. Dividing cells and flagella on the cells were also observed. During the final stage of development, the cell appeared folded especially under the yellow LED with the size significantly ($p>0.05$) large in final than the initial stage of development. The study showed that *Tetraselmis* sp. had similar growth rate and cell morphology under the red, yellow LEDs, and fluorescent light.

Keywords: growth, proximate composition, *Tetraselmis* sp., light-emitting diodes, morphology

PATHOGENICITY OF ORF VIRUS STRAIN UPM 1/14 MALAYSIA AND UPM 2/14 MALAYSIA IN RATS WITH AND WITHOUT DEXAMETHASONE TREATMENT

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ABSTRACT

Orf virus (ORFV) causes contagious ecthyma which is one of the causes of economic losses in livestock industry. In this study, the pathogenicity of two virus strains, ORFV UPM 1/14 Malaysia and ORFV UPM 2/14 Malaysia was determined rats. Intradermal inoculation of 0.5 mL 1% ORFV UPM 1/14 Malaysia (Group 1) and ORFV UPM 2/14 Malaysia (Group 2) virus suspension were performed in two groups of rat the dorsum, ear pinna, and labial commissure. Dexamethasone-induced immunosuppressed and non-dexamethasone treated rat were given intradermal inoculation of 0.5 mL 1% ORFV UPM 1/14 Malaysia virus suspensions. The rats were observed for clinical signs for 14 days and histopathological changes observed on euthanised rats at the end of the experiment. Mild hyperemia was observed in dorsum, ear pinna and labial commissure in rats of the treatment groups. Group 1 rats had significantly ($p<0.05$) higher mean skin lesion scores than Group 2 rats. Dexamethasone-treated rats treated with the virus had significantly higher ($p<0.05$) mean skin lesion scores than those not treated with dexamethasone. Keratosis, acanthosis and ballooning degeneration were observed in rats that showed skin lesions. Dexamethasone-treated virus infected rats had significantly higher ($p<0.05$) mean thickness of stratum spinosum and stratum basale of labial commissure than those not treated with dexamethasone. Using PCR, the ORFV was detected in the skin tissues of rats with skin lesions. The disease and lesions in rats with ORFV infection are similar to that produced in the normal hosts of the virus. In conclusion, the study showed that ORFV is pathogenic to rats and the rat can be used as an experimental model for OFRV infections.

Keywords: Orf virus, ORFV UPM 1/14 Malaysia, ORFV UPM 2/14 Malaysia, rat, pathogenicity, immunosuppression

RETROSPECTIVE STUDY ON FELINE HEART DISEASE CASES AT UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FOR YEAR 2013 TO 2015

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ABSTRACT

Heart diseases in cat are silent killers. The natural history of this occult disease is variable and difficult to predict with certainty because the majority of cats with heart diseases remain asymptomatic throughout their life and only shows clinical sign when the disease became severe. This resrospective study conducted at the University Veterinary Hospital, Universiti Putra Malaysia on feline heart disease cases presented from 2013 to 2015. The feline heart diseases were stage using the New York Heart Association Classification. The overall prevalence of cats with heart disease was 1% (155/15,493) with an increasing trend over the three study period. The mean age of feline heart disease patient was 5.2 years old and the disease is more often seen in male (57%) than compared to female (43%) cats. The two most common breeds presented with heart disease were Domestic Shorthair (54%) and Persian (26%) cats. Ten of 155 cats diagnosed with heart disease were asymptomatic where the remaining were presented with various clinical signs to include congestive heart failure. Acquired heart disease were more often diagnosed than congenital heart disease. Among acquired heart diseases, hypertrophic cardiomyopathy (HCM) had the highest prevalence at 47%, followed by dilated cardiomyopathy at 18%, restrictive cardiomyopathy at 15% and others such as pericardial effusion, aortic insufficiency, heart base tumor, and feline heartworm disease at 11%, myocarditis at 7%, and congenital heart disease, such as atrial septal defect and ventricular septal defect at 2%. The majority of the feline heart patients in this study were of Class II (n=52), Class III (n=67), and Class IV (n=26). Class I heart disease was of least prevalence (n=10). Echocardiography remains the best diagnostic tool for diagnosis of heart diseases.

Keywords: feline heart disease, prevalence, acquired heart diseases, congenital heart diseases, staging of feline heart diseases

USE OF BLUE AND YELLOW LIGHT-EMITTING DIODES FOR GROWTH, PROXIMATE COMPOSITION, AND MORPHOLOGY ENHANCEMENT OF MARINE MICROALGA *ISOCHRYSIS* SP.

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ABSTRACT

Microalgae are photoautotrophic organisms that need light as the main energy source. In this study, the growth, proximate composition and morphology of marine microalga, *Isochrysis* sp., cultured under blue and yellow light-emitting diodes (LED) with fluorescent light as control were compared. Growth of the microalga was observed for 13 days and the effect of the light sources on cell count, optical density, and specific growth rate were determined. The results showed that *Isochrysis* sp. cultured under fluorescent light had the highest specific growth rate (SGR) while those under yellow LED and blue LED cultures had similar SGR. Yellow LED and fluorescent light produced higher cell density in the *Isochrysis* sp. culture. The cultures were harvested during the stationary phase and subjected to proximate analysis. The results showed that the lipid content of *Isochrysis* sp. was significantly ($p<0.05$) highest when grown under yellow LED, whereas, protein composition was significantly ($p<0.05$) highest under blue and yellow LED. Yellow LED and fluorescent light cultures also had significantly ($p<0.05$) higher carbohydrate composition than blue LED cultures. Under scanning electron microscopy, the fluorescent light culture produced the largest cells among cultures. It can be concluded that yellow LED is optimum for *Isochrysis* sp. growth.

Keywords: microalgae, *Isochrysis* sp., LED light, proximate composition, morphology

A RETROSPECTIVE STUDY ON MILK PRODUCTION AND REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE AT UNIVERSITY AGRICULTURE PARK, UNIVERSITI PUTRA MALAYSIA FOR YEAR 2011 TO 2014

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ABSTRACT

Dairy industry in Malaysia is small and cannot meet the needs of the country. Therefore, Malaysia is heavily dependent on imported milk and milk product. The most significant factor in large scale milk production is extensive and good farming system with optimal reproductive performance. This is a retrospective study conducted at Ladang 16, University Agriculture Park, Universiti Putra Malaysia on milk production and reproductive performance of dairy cattle from years 2011 and 2015. The cattle in the farm were mainly Friesian-Jersey and Friesian-Sahiwal breeds. The animals were on natural breeding at a ratio bull to cows of 1:25. Artificial insemination was used only for only for teaching and research purposes. The parameters examined were annual milk production, calving percentage, culling percentage, feed, disease incidence. The farm did not maintain complete records on pregnancy rate, heat detection, age at 1st calving, and calving interval. However, based on the existing data, the average calving percentage was >60% and the culling percentage was <10%. The average annual milk production for the farm was 44966.6 kg. The average mortality rate was 2 animals/year. There was no significant ($p>0.01$) negative correlation ($r = -0.294$) between annual milk production and disease incidence. The most common cause of mortality in the farm was blood parasite infestation (23.81%). Traumatic injury (25.23%) and lameness (18.32%) were common in the farm. To improve reproductive performance and increase milk production, the farm needs to keep proper performance records and practice appropriate farm and disease management.

Keywords: retrospective study, University Agriculture Park, Universiti Putra Malaysia, reproductive performance, milk production

IN VITRO ANTHELMINTIC ACTIVITY OF NEEM (*AZADIRACHTA INDICA*) LEAF CHLOROFORM EXTRACT AGAINST STRONGYLE THIRD STAGE LARVAE FROM SHEEP

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ABSTRACT

Gastrointestinal parasitism is one of the causes of high mortality and morbidity of sheep in Malaysia. Neem plant, *Azadirachta indica*, has been shown to possess many medicinal properties such as anti-fungal, anti-bacterial, anti-inflammatory and anthelmintic activities. This study was conducted to determine the *in vitro* anthelmintic effect of neem leaves on the third-stage larvae (L3) of strongyles from sheep. Pooled faecal samples from 22 sheep with the history of gastrointestinal parasitism were cultured to harvest the L3. Three thousand L3 were divided into five groups of six petri dishes each containing 100 L3 per dish. The L3 were treated with concentration of neem leaf chloroform extract at either 5, 10 and 15 mg/mL, Levamisole (10 mg/mL), and 0.01% DMSO + deionized water as the negative control. Larvae mortality rate, an indicator for anthelmintic activity, was determined at 2, 4, 6, and 24 hours post-treatment. The results revealed that all concentrations of neem leaf extract showed significant ($p<0.05$) anthelmintic activity against L3 with an average mortality rate of 83% at 10 mg/mL, and 93% at 5 mg/mL concentration of the neem leaf extract. In conclusion, the neem leaves chloroform extract at all concentrations used in this study showed significant anthelmintic effect on L3 of strongyles originated from sheep.

Keywords: neem leaves, chloroform extract, strongyles, L3, sheep

OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPP. ISOLATES IN EGGS OF CHICKEN RAISED UNDER FREE-RANGE AND COMMERCIAL CONVENTIONAL-CAGE FARMS

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ABSTRACT

Free-range and conventional-caged farming systems implement different practices in raising their chicken. This could contribute to differences in the *Salmonella* and other bacterial contaminations of eggs. The objective of this study was to determine the occurrence of *Salmonella* in eggs produced by free-range chickens and commercial conventional-cage layers. Thirty-six chicken eggs were purchased from three free-range chicken farms and 36 from three commercial conventional-cage farms. Swabs samples from eggshells and egg content samples were used *Salmonella* following the method described in Laboratory Protocol of Isolation of *Salmonella* spp. from Food and Animal Feces of World Health Organization. The antibiotic resistance pattern of the bacteria isolates was determined. *Salmonella* spp. occurred in 2.8% (1/36) eggshell samples from conventional-cage farm, and 2.8% (1/36) in eggshell and 5.6% (2/36) in egg content samples of free-range farms. There was significant ($p<0.05$) association between farming systems and level of *Salmonella* contamination in eggs. One isolate from conventional-cage chicken egg was resistant to ampicillin. Two isolates from free-range chicken eggs were resistant to nalidixic acid, and one resistant to tetracycline, streptomycin, and trimethoprim-sulphamethaxzole. These findings showed *Salmonella* isolates from eggs of farms practicing different chicken rearing system showed different antibiotic resistance patterns. This study showed that eggs from these farms may not be wholesome due to the presence of *Salmonella*.

Keywords: *Salmonella* spp., chicken eggs, free-range, conventional cages, antibiotic resistance

IMMUNITY STATUS IN PIGS VACCINATED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME MODIFIED LIVE VIRUS VACCINE

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is a disease characterised by late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. In this study, 240 sera were collected from four farms that practiced various PRRS vaccination regimes for more than a year. Vaccinations were carried out 2 months prior to the study. Fifteen sera from four age groups: sows, growers, weaners and piglets were collected from each farm and analysed using IDEXX PRRS X3 ELISA for PRRSV antibodies. Pooled serum samples were analysed by nested-PCR to differentiate Type I from Type II PRRSV. None of the samples were positive for PRRSV, indicating that the pigs were not viraemic after vaccination. Based on ELISA, all the farms were seropositive for PRRS. Mean sample-to-postive (S/P) ratio for piglets, growers and sows of the four farms were >0.4, the cut-off valued for positive ELISA result. This value did not differ ($p>0.05$) significantly among farms, except for one farm that practiced whole herd US MLV vaccination. In this farm the S/P ratio was low in their piglet, grower and sow groups, suggesting low virus circulation in herd. Another farm that practiced US MLV vaccination in sows had seronegative weaners. Thus, the study shows that PRRS MLV vaccination does not cause viraemia in pigs and whole herd MLV vaccination will help reduce virus circulation in PRRS-endemic farm.

Keywords: Porcine Reproductive and Respiratory Syndrome (PRRS), seroprevalence, vaccination, ELISA, nested-PCR.

HAEMATOLOGY AND SERUM BIOCHEMISTRY REFERENCE VALUES FOR THE BORNEAN SUN BEAR

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ABSTRACT

The sun bear species in Borneo is *Helarctos malayanus euryspilus* while in Asian mainland and Sumatra it is *Helarctos malayanus malayanus*. Blood parameter reference values currently in use are not specific for *Helarctos malayanus euryspilus.* Therefore, there is a need to establish reference values for haematology and serum biochemistry parameters for this sun bear species. Sixty haematology and serum biochemistry sets of values from 44 clinically healthy sun bears were obtained from the records of the Sepilok Bornean Sun Bear Conservation Centre (BSBCC) in Sandakan, Sabah (5˚51'47.9"N 117˚5'57.8"E) for year 2006 to 2015. Statistical comparison between sex, age group, fasting and non-fasting, captive and wild were made. The lower and upper reference limits for parameters with Gaussian distribution were set at mean ± 2 standard deviations. For parameters that were not normally distributed, the lowest and highest values were used to determine the range. The results showed high-density lipoprotein levels in neonates were higher ($p<0.05$) than in sub-adults. Creatinine level in neonates was significantly ($p<0.05$) lower than in sub-adults. Platelet count and phosphate level decreased ($p<0.05$) with age, while total protein and globulin increased ($p<0.05$) with age. Alkaline phosphatase (ALP) in neonates was higher ($p<0.05$) than the other age groups. Captive had higher ($p<0.05$) total cholesterol and ALP levels than wild sun bears. The values obtained from this study differ slightly from values published by ISIS Physiological Reference Intervals 2013 for *Helarctos malayanus*.

Keywords: haematology, serum biochemistry, sun bear, *Helarctos malayanus euryspilus*

A RETROSPECTIVE SURVEY ON COMMON ALLERGEN-SPECIFIC IgE ANTIBODY TITRE IN MALAYSIAN PET DOGS

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ABSTRACT

Allergen is a substance, protein or non-protein, capable of inducing allergy or specific hypersensitivity. Allergens can be either environmental or of food source. In dogs, common environmental source of allergen such as house dust mite has been reported to cause of canine atopic dermatitis. In Malaysian, no study has been conducted to quantify common allergen specific IgE titre in pets. Thus, the objective of the study was to determine the common allergen specific IgE titre in pet dogs with allergic symptoms. Data of 70 pet dogs for year 2012 to 2015, based on clinical symptoms, types and location of lesions, were suspected to suffer from allergy, were subjected to Canine 62 Allergen Specific IgE Serological Test (EBS, Taiwan), were obtained from Rhone Ma (Malaysia) Sdn. Bhd. Information such as breed, age of sample collection, sex, history was recorded. The serology data from the test were classified as follows; Trace; <500, Mild: 500-2000, Moderate: 2000-8000, Severe: >8000. The majority of the dogs included in this study were >3 years old (42%), and males (59%), and medium-small breed (64%). Three environmental allergens that produced the highest number of positive results were mite (52.9%), fungus (38.6%), and pollen (32.9%). Among mites, *Dermatophagoides farinae* appeared to be the most important allergen with 28.5% of affected dogs falling under the category of Moderate to Severe. *Candida albicans* (30%) and *Cynodon dactylon* pollen (25.7%) are among other important allergens for dogs in this cohort. In conclusion, this study showed that environmental allergens, especially house dust mite, are the major cause of elevated specific IgE titre in pet dogs with allergies in Malaysia.

Keywords: *Dermatophagoides farinae*, IgE, allergen, canine, Malaysia

PATHOGENICITY OF ORF VIRUS STRAIN UPM 1/14 MALAYSIA AND UPM 2/14 MALAYSIA IN MICE WITH AND WITHOUT DEXAMETHASONE TREATMENT

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ABSTRACT

Recently, two local Orf virus (ORFV) strains (UPM 1/14 Malaysia; UPM 2/14 Malaysia) were isolated. However, there is no study has been done in mice infected with these virus strains. Thus, aims of this study were to describe the effect and pathogenicity ORFV strains on mice either treated or not treated with dexamethasone. Intradermal injection of 0.2 mL 1% ORFV UPM 1/14 Malaysia and ORFV UPM 2/14 Malaysia were done on each mice at the dorsum, ear pinna, and labial commissure. Intradermal injection of 0.2ml 1% UPM 1/14 Malaysia was performed in dexamethasone-treated and non-dexamethasone-treated groups. Five mice were treated with 5mg/kg body weight Dexamethasone for 5 consecutive days and challenged with ORFV on day 3. All treated mice became hyperaemic. There were no significant ($p<0.05$) difference in lesion score in mice between inoculation sites, dexamethasone and non-dexamethasone treatment, or ORFV strain. However, mice challenged with ORFV UPM2/14 Malaysia showed significantly ($p<0.0\%$) thicker stratum of the ear pinna and labial commissure. The overall histopathology revealed keratosis, acanthosis, and ballooning degeneration in the affected tissues. The skin tissue of mice with skin lesion were positive for ORFV at polymerase chain reaction analysis. In conclusion, intradermal inoculation of local strain ORFV caused mild skin lesions in mice. The study also showed that dexamethasone treatment does not affect pathogenicity of ORFV in mice.

Key words: Orf virus strains, mice, inoculation site, dexamethasone, Polymerase Chain Reaction (PCR)

APPLICATION OF A NOVEL INTRAVAGINAL INSERT AND BEHAVIOURAL RESPONSE TO THE INSERT IN PIGS

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ABSTRACT

The swine industry requires systematic management, particularly in reproduction, to maximise pork production. Swine industry in Malaysia is one main contributor to gross domestic product. To contribute to the knowledge in swine production, this study evaluates the use of novel progesterone-loaded intravaginal inserts for oestrus synchronisation in gilts. The study was conducted at a model pig farm located in Bidor, Perak, Malaysia. Six gilts aged 8 months weighing approximately 120 kg were chosen for the study. The insert was of three types; small wing size with small and large base, medium-size wing with small and large base, and large wing size with small and large base. The ease of application and removal of insert, retention rate, and pig behavioural response to the insert were recorded. Inserts with large base could not be applied successfully. Removal of large wing insert with small base caused discomfort to the pig. Small wing inserts with small base and medium-sized wing inserts with small and large bases were successfully applied and remained in place, before they were finally removed after 13 days. In conclusion, the progesterone-loaded intravaginal insert of appropriate sizes can be used for oestrus synchronisation in pigs.

Keywords: gilts, novel intravaginal insert, oestrus synchronisation, progesterone, swine reproduction

ULTRASTRUCTURAL OF THE SWIFTLET NEPHRON

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ABSTRACT

It has been reported that the structural organisation of the swiftlet nephron resembles that of the reptile, which is "loopless", which is absence of descending and ascending limbs and loop of Henle. Thus, the cortex of the swiftlet kidneys is occupied mainly by the proximal tubules. It is hypothesised that the swiftlet nephron is structurally simple but functionally efficient. This study examined the ultrastructural morphology of the swiftlet nephron. The nephron comprises of numerous microvilli with a high incidence of mitochondria and extensive indentation and infolding of the basal epithelium of the proximal tubule, indicating that it performs enhanced reabsorption of glomerular filtrate and release into peritubular capillaries. The efficiency of the nephron is further augmented by the tubules of the intermediate segment that also have microvilli, mitochondria and basal indentations and infolding, indicating that this section of the tubules also performs considerable reabsorption of fluids. Similarly, the distal convoluted tubules and collecting ducts showed structural evidences of enhanced glomerular filtrate reabsorption and release functions. An interesting observation was the presence of many lipid droplets in the lumen of the nephrons. Microvilli with dilated apical ends seems to be specially designed for the absorption of lipids in the intermediate segment. Thus, the nephron of the swiftlet is simple and loopless, but with highly reabsorption efficiency along the entire length.

Keywords*:* swiftlet, reptilian nephron, intermediate segment, microvilli, glomerular filtrate

EFFECT OF ORAL *PASTEURELLA MULTOCIDA* TYPE B:2 INOCULATION IN MICE

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ABSTRACT

In Malaysia, haemorrhagic septicaemia (HS) is an acute fatal septicaemic disease in cattle and buffaloes caused by *Pasteurella multocida* Type B:2. There is need to develop an easily administered vaccine for cattle and buffaloes. This study describes the effect of oral *P. multocida* treatment on mice. Twenty-six mice were divided into 4 treatments (n=5) and one control (n=6) groups. Treatment group mice were inoculated orally with 0.2 mL of either 10^3, 10^5, 10^7 or 10^9 CFU *P. multicida* while control mice received 0.2 mL phosphate-buffered saline. Surviving mice were re-challenged with 0.2 mL 10^7 CFU *P. multocida* Type B:2 orally. The mice were observed for 7 days for clinical signs and mortality. Mice that survive the 7-day post-challenge were euthanised and the lungs, heart, liver, spleen, kidneys, gastrointestinal tract obtained for bacteriological and histopathological analyses. *P. multocida* was confirmed by gram and wright staining of positive organ cultures. There was no significant ($p > 0.05$) difference in clinical signs between mice treated with 10^3 and 10^5 CFU *P. multocida.* The level of alertness and ocular discharge were high in mice treated with higher doses of *P. multocida*. Presence of inflammatory cells, haemorrhages and congestions were mild to moderate in all treatment groups. However, degeneration and necrosis were observed to be moderate to severe in mice treated with 10^7 and 10^9 CFU *P. multocida*. Bacteria isolation from organs of dead mice were high. Only the heart, lung, liver, spleen, and kidneys of mice that survived showed high bacterial isolations. In conclusion, mice treated with low doses had better survivability and showed milder lesions than those treated with high doses *P. multocida*. Dose of 10^7 and 10^9 CFU *P. multocida* were highly detrimental and caused mortality to mice and should not be used for experimental studies.

Keywords: *Pasteurella multocida* Type B:2, mice, oral inoculation, clinical signs, histological signs, oral vaccine

MILK COMPOSITIONS OF DAIRY COW WITH CLINICAL AND SUBCLINICAL INTRAMAMMARY INFECTIONS

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ABSTRACT

The production and quality of milk can be deteriorated by intramammary infections (IMI) resulting in alteration in milk compositions. This study was done to determine the milk composition parameters of dairy cows during clinical and subclinical IMI and to determine the relationship between somatic cell counts (SCC) and milk composition parameters of cows with and without IMI. Twenty dairy cows from University Agriculture Park, Universiti Putra Malaysia (UPM) and UPM Foster Farms were chosen for the study. The clinical signs in the cows were monitored to determine clinical IMI while California mastitis test (CMT) used to identify subclinical IMI in dairy cows. Ten healthy dairy cows with negative or trace CMT results and withou IMI clinical signs were used as negative controls. The milk SCC, fat, protein, casein, lactose, total solid, solid non-fat (SNF), free fatty acids (FFA), and acidity were determined. Casein, lactose, and SNF were significantly ($p<0.05$) higher and SCC significantly ($p<0.05$) lower in control than subclinical IMI cows. Alteration in milk composition parameters is associated with elevated SCC in the milk of cows with IMI. Casein, lactose and SNF were negatively correlated with the SCC ($r = -0.576$, $r = -0.676$, and $r = -0.722$; $p < 0.01$). Collectively these results were in accordance with previous studies that demonstrated significant changes in the SCC and milk composition parameters during subclinical IMI. In conclusion, significant changes in milk compositions parameter occurs in dairy cows with subclinical IMI and there is strong relationship between the SCC and the milk compositions parameters. The study shows that milk casein, lactose and SNF profile can serve as a marker for IMI.

Keywords: intramammary infections, milk composition parameter, somatic cell counts California mastitis test (CMT), milk quality.

ANTIBACTERIAL EFFECTS OF HYDROMETHANOLIC *SYGYZIUM POLYANTHUM* AND *AQUILARIA MALACCENSIS* EXTRACTS ON ISOLATES FROM MILK OF COWS WITH SUBCLINICAL MASTITIS

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ABSTRACT

Subclinical mastitis can decrease milk production by at least 20%. Malaysia is rich in plants with potential therapeutic properties. In this study, tropical plants, *Sygyzium polyanthum* known as *Salam* and *Aquilaria malaccensis* known as *Karas* were examined for antibacterial effect. Thus, the aims of this study were to identify bacterial isolates from milk of cows with subclinical mastitis and determine the effect of *Salam* and *Karas* on the isolates using the mean zone of inhibition (MZI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests. The hydromethanolic extracts of *Salam* and Karas were prepared as paste. Sixty-eight milk samples were collected from eighteen cows and 20 bacteria species were isolated by blood agar. The isolates were confirmed by biochemical methods to be *Staphylococcus aureus* (6 isolates), *Staphylococcus hyicus* and *Staphylococcus intermedius* (4 isolates each), *Dermatophyte spp*. (2 isolates), *Streptococcus viridans, Streptococcus bovis, Citrobacter freundii,* and *Staphylococcus pseudoint*ermedius (1 isolate each). Antibacterial activity of 100, 50, 25, and 12.5% *Salam* and *Karas* extracts on the isolates were determined using the Kirby-Bauer test. The MZI were 7.12 ± 0.29 to 13.50 ± 0.20 mm for *Salam* and <12.50 ± 0.28 mm for *Karas* extract. The MIC for *Salam* extract was 12.5% whereas for *Karas* extract it was 18.75 to 21.8%. The MBC both for *Salam* and *Karas* extracts was 12.5%. These results show that *Salam* and *Karas* extracts had antibacterial properties that vary with concentration and bacteria isolate.

Keywords: Subclinical mastitis, cows, *Sygyzium polyanthum*, *Aquilaria malaccensis*, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)

EFFECT OF THAWING TEMPERATURE ON THE MORPHOLOGY, MOTION CHARACTERISTIC, AND PLASMA MEMBRANE INTEGRITY OF CRYOPRESERVED BULL SEMEN

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ABSTRACT

Freezing semen is a method of storing and transferring genetic materials. The viability and motility of spermatozoa in frozen semen are highly dependent on the handling techniques. This study was conducted to determine the effect of thawing temperature on the properties of cryopreserved bull semen. Six semen samples were collected from Kedah-Kelantan, Friesian Jersey, and Brangus bulls. The cryopreserved semen straws were thawed for 20 seconds in water baths at 30, 37, and 50°C. The morphology, motion characteristic, and plasma membrane integrity of the spermatozoa were determined. For all breeds, semen thawed at 37°C had higher spermatozoa general and progressive motility than those thawed at 30 and 50°C. Thawing semen at 37°C results in fewer spermatozoal abnormality than at 30 or 50°C. In Kedah-Kelantan cattle semen there were more acrosome abnormalities after thawing at 50 than at 37°C. Although there is no difference between cattle breeds, the plasma membrane integrity was higher at 37 than at 30 or 50°C. The path and progressive velocities of sperm of the Friesian Jersey bull was lowest at 37°C thawing temperature while in the Kedah-Kelantan bull, there was no clear trend. In conclusion, the recommended thawing temperature for cryopreserved bull semen is 37°C.

Keywords: bull semen, cryopreserved, thawing temperatures, motion characteristics, plasma membrane integrity

IDENTIFICATION OF BOVINE MASTITIS-CAUSING PATHOGENS IN DAIRY FARMS IN LABIS, JOHOR, MALAYSIA

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ABSTRACT

Mastitis is one of the important production diseases of dairy animals that can cause economic losses to the farmer. Mastitis is inflammation of the mammary gland affecting all species of domestic animals and is of great concern to the dairy industry. This study was conducted to determine the prevalence of mastitis and assess subclinical mastitis and antimicrobial resistance of bacterial isolates from dairy cows in selected farms in Labis, Johor, Malaysia. One hundred and twenty-eight samples were collected from four farms and subjected to California mastitis test (CMT). Thirty-four (26.56%) milk samples were found to be CMT-positive and subsequently subjected to bacterial culture and identification. Seven bacteria species were successfully isolated from the samples. The most prevalent bacteria were *Staphylococcus aureus* (41.67%), followed by *Staphylococcus intermedius* (27.10%), *Streptococcus uberis* (10.40%), *Staphylococcus shleiferi* (8.33%), *Aerococcus viridans* (8.33%), and *Corynebacterium sp.* and *Chromobacterium sp*., each at 2.10% prevalence rate. In general, the isolates displayed variable susceptibility towards antibiotics. *Staphylococcus aureus* showed highest resistance at 92.60, 88.89, 74.08, 66.67, and 14.82% towards Gentamycin, Streptomycin, Tetracyline, Penicillin G and Oxytetracycline, respectively. *Staphylococcus intermedius* was 100% resistant towards Gentamycin and Streptomycin, 84.62% towards Tetracycline, 54.85% towards Penicillin G, and 30.77% towards Oxytetracycline. Most bacteria isolates were sensitive towards Oxytetracycline, making this antibiotic the most effective amongst those tested in this study. By the results of the study, there is urgent need for effective control of the increasing prevalence of subclinical mastitis and overcoming antimicrobial resistance.

Keywords: Mastitis, prevalence, antibiotic sensitivity test, cattle farms

JAPANESE ENCEPHALITIS ANTIBODY TITRE IN BLOOD SAMPLES OF DOGS AND CATS IN PENINSULAR MALAYSIA

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ABSTRACT

Japanese encephalitis virus (JEV), of the *Flaviviridae* family, is a known cause of acute encephalitis in humans. The virus is transmitted through the mosquitoes, particularly *Culex tritaeniorhynchus.* Japanese encephalitis virus has also been identified in cats and dogs. Thus, the purpose of this study is to determine the presence of JEV antibodies in cats and dogs in Malaysia. Two to 5 mL blood samples were collected from shelter cats (n=45) and dogs (n=45) and 2 mL serum samples, with owner consent, from cats (n=40) referred to the University Veterinary Hospital (UVH), Universiti Putra Malaysia. The age, sex, health status, management data for each animal were obtained from UVH records. ELISA were performed on the serum samples using two commercial kits, Cat JE IgG ELISA kit (SunRed Biotechnology) and Dog JE IgG ELISA kit (MyBioSource). The tests were conducted in duplicates. The results revealed that 15% (6/40) pet cats, 17.7% (8/45) of shelter cats, and 80.4% (36/45) of shelter dogs were positive for JEV antibodies. Shelter dogs were six times more likely to be seropositive than shelter cats. There was no significant ($p>0.05$) relation between JEV antibody titre and sex, health, management, age, or location. Thus, dogs and cats in Malaysia are generally seropositive for JEV antibodies.

Keywords: Japanese encephalitis virus, dog, cat, ELISA, antibody

SELF-RECOGNITION IN A COCKATOO (*CACATUA GALERITA*)

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ABSTRACT

Cognitive function in animals is their ability to perceive sensory information such as visual, auditory, or olfactory, and make judgments about its environment. The mirror self-recognition (MSR) test is a procedure to determine whether animal possesses the ability for self-recognition. Currently, among animals only the great apes have shown mirror self-recognition. Thus, the purpose of this study is to determine the nature of mirror-induced behavior (self-recognition) in the cockatoo *(Cacatua galerita).* One adult Sulfur-crested cockatoo at the National Zoo, Malaysia was chosen for the study. An acrylic 59 × 84 cm mirror was placed outside the observation cage. The test was done over 10 sessions of 15 minutes each over a period of one week. Birds that are able to recognise itself through a mirror typically goes through 4 stages; social response, physical inspection, repetitive mirror-testing behavior, and realisation of seeing itself. Their behaviours were recorded by a video camera mounted in an obscure location and the data analysed using the ethogram. This study revealed that Sulfur-crested cockatoo can recognise its own reflection when scratching and tilting face and preening, which are indicators of positive and successful MSR. Thus, research on self-recognition and cognitive ability will provide information how to improve management and design of captive bird enclosures.

Keywords: mirror, self-recognition, cockatoo *(Cacatua galerita)*, visual perception

EFFECT OF METHANOL BETEL NUT (*ARECA CATECH*) EXTRACT AND LEVAMISOLE ON *IN VITRO* SURVIVAL OF STRONGYLES FROM GOATS

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ABSTRACT

The extensive use of chemical anthelmintics in parasitic gastroenteritis management had caused drug resistance that reduced drug effectiveness. The present study compared the effectiveness of methanol betel nut (*Areca catechu*) extract (BNE) with levamisole on the survival rate of the third stage larvae (L3) strongyles in goat and to determine the effective BNE concentration for *in vitro* strongyle larvacidal activity. Fresh fecal materials of naturally infected goats were collected and cultured for 7 days before harvesting L3 strongyles. The L3 were treated with 300, 600 and 1200 µg/mL BNE. Positive control was treated with 10 mg/mL Levamisole and negative control with 0.1% DMSO in phosphate-buffered saline. The BNE at 1200 µg/mL caused 61.70% L3 mortality by 24 hours while Levamisole caused 100% mortality by 2 hours of post-treatment. The *Oesophagostomum* sp. L3 showed the highest mortality at 43.40%, followed by *Haemonchus* sp at 40.90%, and *Trichostrongylus* sp. 15.70%. Although BNE was not as effective as Levamisole, it still has activity against L3 strongyles from goats and thus can be used as an alternative anthelmintic.

Keywords: betel nut (*Areca catechu*), goats, parasitic gastroenteritis, L3, strongyles, Levamisole.

RELATIONSHIP BETWEEN ULTRASONOGRAPHIC MEASUREMENT OF LONGISSIMUS DORSI, BACKFAT, AND BODY WALL THICKNESS WITH BODY WEIGHT AND TESTICULAR MORPHOMETRY IN BREEDING BOER GOATS

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ABSTRACT

Ultrasound has been used to measure fat and muscle depths in animals for the purpose of genetic selection programs to improve carcass quality. This study was conducted to determine the relationship between ultrasonographic measurements of the longissimus dorsi muscle, backfat and body wall thickness with body weight and testicular morphometry in goats. Sixteen Boer and crossbred goats of ≥2 years of age were used for the study. Ultrasonographic measurements were taken for fat and muscle depths between the 12th and 13th thoracic vertebrae, 3rd and 4th lumbar vertebrae and between 12th and 13th ribs 12.7 cm distal to the dorsal vertebral processes to determine body wall thickness. Vernier caliper was used to measure testicular length (L), width (W), and height (H). Scrotal circumference was measured with a flexible plastic tape. The firmness of the testicles was determined by palpation. Image J (version 1.49) was used to accurately measure the ultrasound images. Testicular volume was calculated using the formula: Volume (cm^3) = 0.5233 × L (cm) × W (cm) × H (cm) while the daily sperm output (10^9/day) = (0.024 x testicular volume) – 1.26, where total testicular volume is the sum of the right and left testicular volume. Pearson's correlation (IBM SPSS version 23) showed that the fat depth of the left thoracic area correlated with the right testicular volume (0.497, p=0.05). The fat depth at the right thoracic area correlated with the right testicular length (p=0.031). Additionally, body weight correlated with thoracic circumference (0.824, $p<0.05$) as well as left (0.722, p=0.02) and right (0.543, p=0.03) longissimus dorsi muscle depth at the area between the 12th and 13th thoracic vertebrae. Overall, the body weight and muscle and fat depths had no correlation with testicular morphometry. Therefore, these measurements can only be used to evaluate carcass traits and not fertility.

Keywords: ultrasound, longissimus dorsi, backfat, body wall thickness, testicular morphometry, scrotal circumference.

PREVALENCE OF GASTROINTESTINAL PROTOZOA IN PET CATS PRESENTED TO VETERINARY CLINICS IN KLANG VALLEY, MALAYSIA AND RISK FACTORS ASSOCIATED WITH INFESTATION

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ABSTRACT

The common gastrointestinal protozoa in cats that cause diarrhoea are *Giardia*, *Isospora*, and *Cryptosporidium* spp., and recently *Tritrichomonas foetus* was recognised as an emerging protozoon that causes chronic diarrhoea in cats. *Entamoeba* spp. is found rarely but present in cats. This study aimed to investigate the prevalence of gastrointestinal protozoa in pet cats presented to selected veterinary clinics in Klang Valley, Malaysia and determine the risk factors associated with these protozoal infections. Rectal swabs were collected from 30 diarrhoeic cats presented to selected veterinary clinics to culture *T. foetus*. Another 30 feacal samples were collected randomly and subjected to staining for the detection of other gastrointestinal protozoa. Two samples were positive for *T. foetus* with a prevalence of 6.7% and both positive samples were from young kittens. *Cryptosporidium* spp. were the only protozoa detected in 3 samples through the staining method with a prevalence of 10%. This study is a first report on the detection of *T. foetus* in cats in Malaysian. The overall prevalence of gastrointestinal protozoa in pet cats in the Klang Valley was low.

Keywords: gastrointestinal protozoa, *Tritrichomonas foetus*, cat, culture, staining

REFERENCE VALUES FOR HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS IN THE BORNEAN ORANGUTAN SUBSPECIES, *PONGO PYGMAEUS MORIO*

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ABSTRACT

A new Bornean orangutan subspecies, *Pongo pygmaeus morio,* that only resides in Sabah, Malaysia and East Kalimantan, Indonesia, was recently classified. Currently there is no published reference intervals for blood parameters for *P. pygmaeus morio.* Therefore, this study was undertaken to develop reference intervals for haematology and serum biochemistry parameters for this subspecies. Data were obtained for years 2009 to 2015. The orangutans comprised of 23 male and 28 female rehabilitated and 9 male and 3 female translocated wild orangutans at the Sepilok Orangutan Rehabilitation Centre, Sabah, Malaysia. The orangutans were classified according to sex, age groups (infant: ≤ 2.5 years, juvenile: 2.5 – 7.5 years, adolescent: 7.5 – 10 years, and adult: >10 years), and captive or wild. The lower and upper reference intervals of the parameter values with Gaussian distribution were established as mean ± 2 standard deviations. For parameters that did not exhibiting Gaussian distribution, the lowest and highest values were used for the reference range. The study showed that the erythrocyte parameters, lymphocyte count, calcium, albumin, albumin:globulin (A:G), alkaline phosphatase, and cholesterol were significantly ($p<0.05$) higher, and creatinine significantly ($p<0.05$) lower in captive than wild orangutans. The erythrocyte parameters, lymphocyte count, albumin, A:G, γ-glutamyltransferase, aspartate transferase, and cholesterol decreased ($p<0.05$) and creatinine increased ($p<0.05$) with age.

Keywords: orangutan, *Pongo pygmaeus morio*, reference intervals, haematology, serum biochemistry

ASSESSMENT OF CHEMORECEPTIVITY IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLINGS FOR THE IDENTIFICATION OF NATURAL FOOD ATTRACTANTS FOR FEED FORMULATION

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ABSTRACT

The African catfish, *Clarias gariepinus,* possesses excellent chemoreception and they locate food source mainly through sense of taste and smell. African catfish has a propensity for carnivorous feeding, suggesting that it has a relatively high dietary protein requirement. This experiment aims to determine the type of protein source preferred by the fish that could be used for the development of feed formulations. Three-compartment maze was used to compare the chemo-attractiveness between commercial fish pellets and natural feed. The natural feeds used in the study were chicken liver, Chironomid larvae, shrimp head, and Artemia cyst. Forty African catfish fingerlings were exposed to 1% solution of commercial and natural feeds placed at the corners of the maze. The behaviour, time and direction of fish swimming were recorded. The result showed that the preferred feed, in order, was shrimp head, Chironomid larvae, chicken liver, Artemia cyst, and fish pellet indicating that African catfish fingerlings preferred natural food over commercially prepared feed.

Keywords: African catfish, maze, chemoattractiveness, feed

SELF-AWARENESS IN A MALAYAN SUN BEAR (*HELARCTOS MALAYANUS*)

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ABSTRACT

Animal cognitive function is the process of understanding and improvement of the animal's memory, stimulus-responses, and attention. In some animals, cognitive function refers to self-awareness, an understanding that one's own mirror reflection represents oneself only. The mirror self-recognition (MSR) test or mark test is a behavioral assessment technique to determine whether an animal possesses the ability for self-recognition or self-awareness. Thus, the objective of this study was to determine the ability of Malayan Sun bear for self-recognition. A Malayan Sun bear (*Helarctos malayanus*) was selected from the National Zoo, Malaysia. A 60 x 84 cm acrylic mirror was placed outside of the bear's cage. The bear was marked on the forehead with a non-toxic white paint. The behavior of the bear was monitored by covered mirror test (CMT), open mirror test (OMT) and mark test (MAT) in 4 sessions of daily observation of 15 minute each, for 5 days. The MSR behaviors were recorded with a video camera and analysed using the ethogram procedure. Animals that exhibit self-recognition undergo 4 typical stages of behavior; social response, physical inspection, repetitive, and realisation behavior. In this study, the MSR behavior of the Malayan Sun bear was inconsistent, with lack of clear display of self-recognition behavior, and thus making the result inconclusive.

Keywords: mirror self-recognition, Malayan sun bear (*Helarctos malayanus*), self-awareness

EFFECT OF CRYOPRESERVATION CONDITION ON VIABILITY OF FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS

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ABSTRACT

Among components of peripheral blood mononuclear cells (PBMCs) are immune cell subsets such as lymphocytes and monocytes. In this study, the effect temperature and composition of cryopreservation media on feline PBMCs viability were examined. Blood samples were obtained from 15 healthy cats and PBMCs isolated using the density gradient centrifugation technique. The PBMCs were stored at -196 °C for 2 weeks in three cryopreservation media: 10% dimethyl sulfoxide (DMSO) with 90% foetal bovine serum (FBS), 10% DMSO with 90% feline serum (FS), and 10% DMSO with 45% FBS and 45% FS. The PBMC recovery was done with medium at room temperature and 4 °C. The cells were thawed and viability determined by trypan blue dye exclusion technique and flow cytometry. Based on the dye exclusion technique, the viability of thawed PBMCs in cryopreservation medium containing FBS was similar to that in medium containing FS. However, flow cytometric analysis showed that thawed PBMC in medium containing FS had higher viability than medium containing FBS. There was no significant ($p>0.05$) difference in recovery of PBMCs between cryopreservation at temperature and 4 °C. The study shows, recovery of feline PBMCs in cryopreservation medium containing FS or FBS were similar, indication cryopreservation of either composition is suitable for feline PBMCs.

Keywords: feline PBMCs, cryopreservation, medium composition, medium temperature, cell viability, flow cytometry

MOLECULAR PREVALENCE OF FELINE MORBILLIVIRUS IN SHELTER CATS

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ABSTRACT

Feline morbillivirus (FmoPV) is an enveloped virus with non-segmented negative strands RNA genomes belonging to the genus *Morbillivirus* and family *Paramyxoviridae*. Domestic cats were not known to harbor morbillivirus infection until a novel FmoPV was discovered in Hong Kong, Japan, and Europe. Subsequently, screening for FmoPV in Malaysia showed the prevalence rate of the virus to be 48.6%. In our study, urine samples collected from 46 cats chosen randomly from three participating shelter organisations were analysed. The samples were subjected to nested polymerase chain reaction assay to amplify the FmoPV L gene to a final product of 401 bp in size. Nineteen samples tested positive for FmoPV. The prevalence of FmoPV in shelter cats in Malaysia is 41.3%.

Key words: Feline morbillivirus, shelter cats, nested PCR assay, L gene, prevalence rate

MICROBIOLOGICAL QUALITY OF *LACTOBACILLUS*-FED AND COMMERCIAL BROILER MEATS AND ANTIBIOTIC SENSITIVITY OF BACTERIA ISOLATES

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ABSTRACT

Antibiotics in poultry feed are gradually being replaced with probiotics. It is believed that probiotics give the same overall health benefits to livestock antibiotics, without the undesirable drug resistance. This study was performed to determine the standard plate count (SPC), coliform plate count (CPC) and antibiotic sensitivity of bacteria isolated from *Lactobacillus*-fed and commercial broiler meats. Twenty *Lactobacillus*-fed and 25 commercial broiler meats were purchased from 7 retail outlets in Selangor and Kuala Lumpur, Malaysia. *Salmonella sp.*, *Escherichia coli,* and *Staphylococcus aureus* were isolated from the meat samples. Eleven (55%) *Lactobacillus*-fed broiler meat samples were positive for *E. coli*, 4 (20%) for *Salmonella* sp., and 1 (5%) for *S. aureus*. Ten samples (40%) from commercial broiler meat were positive for *E. coli*, 1 sample (4%) for *Salmonella sp.,* and 1 (4%) for *S. aureus*. There was no significant ($p>0.05\%$) difference in the occurrence of these bacteria between meats of *Lactobacillus*-fed and commercial chickens. All isolates were subjected to antibiotic sensitivity test. *Salmonella sp.*, from all meat samples were resistant to ceftriaxone and oxytetracycline while *E. coli* isolate were all resistant to ceftriaxone, ampicillin, streptomycin and oxytetracycline. Only one ciprofloxacin-resistant *E. coli* isolate was obtained, which was from commercial broiler meat. *S. aureus* isolate from commercial broiler meat was resistant to oxytetracycline, while that from *Lactobacillus*-fed broiler meat was susceptible to all antibiotics. The antibiotic sensitivity of *Salmonella* sp., *E. coli* and *Staphylococcus aureus* isolates from broiler meats was similar, except the ampicillin sensitivity of *E. coli* was significantly ($p<0.05$) lower in isolates from commercial than from *Lactobacillus*-fed broiler meat. The mean SPC of *Lactobacillus*-fed and commercial broiler meat was 17×10^4 and 44×10^4 CFU/g, respectively while the mean CPC was 23×10^3 and 30×10^3 CFU/g, respectively. The SPC of *Lactobacillus*-fed broiler meat was significantly ($p<0.05$) lower than that of commercial broiler meat. However, there was no significant ($p>0.05$) difference in CPC between broiler meat types suggesting little difference in microbiological quality between *Lactobacillus*-fed and commercial broiler meat.

Keywords: *Lactobacillus*, broiler meat, *Salmonella* sp., *Escherichia coli, Staphylococcus aureus*, antibiotic sensitivity, microbiological quality

ULTRASONOGRAPHIC IMAGING OF THORACIC ORGANS OF GOATS

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ABSTRACT

Ultrasonography is a non-invasive tool that is less commonly used for thoracic examination in goats. In this study, the heart and lungs of goats were examined via ultrasonography to determine their structure and anatomical location. Five animals were selected for the study. The areas for examination from caudal to scapula the last rib of the left and right thoracic regions were shaved. The skin was cleaned with hibiscrub, wiped dry before application of coupling gel. Examination was done with a 5 MHz convex and linear probe. Five imaging planes were obtained from the right side and two from the left side of the 4th intercostal space. Lungs ultrasound image showed hyperechoic horizontal lines with reverberation artifact. In conclusion, in echocardiography can be performed at the 4th intercostal space approximately 2 cm above the olecranon whereas lung ultrasound at a location between the 7th to 11th intercostal space.

Keywords: ultrasound, goat, heart, lung

ANTIBIOTIC RESISTANT *SALMONELLA* SPP. IN PET AND STRAY CATS

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ABSTRACT

Salmonellosis is an important zoonotic disease. The organisms commonly reside in the gastrointestinal tracts. Cats are popular pet animals, yet the risk that these animals pose in the transmission of *Salmonella* to humans is unclear. The objectives of this study were to determine the occurrence and antimicrobial resistance of *Salmonella* spp. in stray and owned cats. Thirty rectal swab samples each was collected from pet and stray cats. The stray cats were in residential and food stalls areas. Only 3 (5%) cats, all strays, tested positive for *Salmonella* spp. The *Salmonella* spp. isolated were tested against six antibiotics, ampicillin, chloramphenicol, sulfamethoxazole-trimethoprim, ciprofloxacin, gentamicin, and nalidixic acid. All isolates were resistant to ampicillin, but susceptible to ciprofloxacin, and one (33%) isolate was susceptible to chloramphenicol and gentamicin and. This is expected since stray cats are more prone to intestinal *Salmonella* infection than household pet cats. Stray cats may pose a potential threat to public health and their faecal materials may play significant roles in the bacterial contamination of the environment.

Keywords: cats, *Salmonella* spp., antibiotic resistance, faeces

SALMONELLA AND *ESCHERICHIA COLI* IN EDIBLE BIRD'S NEST RANCHED IN HOUSED-SYSTEM

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ABSTRACT

The swiftlet industry in Malaysia is growing very fast due to the high demand of edible bird's nest (EBN). The EBN can be contaminated by bacteria that reduces quality and causes foodborne diseases. The aim of this study was to determine the presence of Salmonella and *Escherichia coli* in EBN and faeces of swiftlets ranched in housed system. In this study, 64 samples were collected from unharvested EBN and faeces in three swiftlet houses in Terengganu. All samples were pre-enriched and enriched before culturing on Brilliant Green agar and xylose lysine deoxycholate for isolation of *Salmonella* spp. The isolates were propagated in nutrient broth before culturing on eosin methylene blue Agar. Standard plate count (SPC) and coliform count using petrifilm were done to determine microbial content. No *Salmonella* was isolated from EBN. The prevalance of E. *Coli* in EBN was 3.13% and of *Salmonella* and *E. Coli* in faecal samples was 12.5 and 68.75%, respectively. The average SPC of EBN was 3.2×10^5 CFU/g and CPC was $\leq$ 100 CFU/g. Hence, the absence of *Salmonella* and low number of *E. coli* in EBN shows that this Malaysian product is of very good quality.

Keywords: swiftlet, edible bird's nest, housed-system, *Salmonella, E. Coli*

PREVALENCE OF RESPIRATORY DISEASES IN RACING THOROUGHBRED HORSES IN PERAK TURF CLUB, MALAYSIA AND THEIR PERFORMANCE AFTER SURGICAL CORRECTIONS

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ABSTRACT

In equine athletes, respiratory diseases affecting either the upper or lower airways or both are common and they have been identified as important causes of poor performance in racehorses. The prevalence of upper airways disorders is difficult to be determined since these conditions may not be evident during examination. This study focuses mainly on the prevalence of respiratory diseases in Thoroughbreds racing in Perak Turf Club, Malaysia for year 2011 to 2015. The performance of racehorses was compared between those that underwent surgery corrections and those that did not. The most prevalent respiratory diseases were induced pulmonary haemorrhage (EIPH) Grade 1 or respiratory distress (38.2%), EIPH Grade 2 (30.2%), recurrent airway obstruction (17.6%), epiglottic entrapment and other conditions (4.0%), and dorsal displacement of soft palate and respiratory noises (3.0%). There was no significant association between racing performance and surgery. Thus, it can be concluded that surgical corrections do not assure improvement in equine performance.

Keywords*:* Thoroughbreds, racehorse, respiratory disease, prevalence, performance, surgical correction

AN ULTRASTRUCTURAL STUDY ON THE FORMATION OF SECRETORY GRANULES AND MODE OF SALIVARY GLAND SECRETION IN SWIFTLETS

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ABSTRACT

The swiftlets, *Aerodramus fuciphagus* and *Aerodramus maximus*, are the only avian species known to build their nests from salivary gland secretions. During the breeding season, especially during nesting, the salivary glands of swiftlets are markedly enlarged and hypertrophied to produce salivary secretion that hardened to become the edible bird's nest (EBN). The EBN has been reported to be highly nutritious and fortified with immunoprotective and antiviral properties. The present study examined the unique salivary gland of *A. fuciphagus* at the ultrastructural level, focusing on the formation of secretory granules and mode of secretion. The study also intended to provide some plausible explanation to the origin or source of immunoprotective and antiviral properties of EBN. Samples of swiftlet salivary glands were processed and examined under transmission electron microscope. Formation of the secretory granules appeared to originate from a coalescence of pinched-off dilated ends of rough endoplasmic reticulum. These were carried to the apical cytoplasm where they again coalesce to form larger secretory granules. The secretory granule appeared to released its content into the lumens of acinus by rupture of the apical cell membrane, thus classifying the mode of salivary gland secretion in swiftlets as the merocrine type. Ultrastructural evidences indicated that the cytoplasmic ribosomes of the acinar cell are the contributors to the immunoprotective and antiviral properties of the EBN.

Keywords: swiftlet, ultrastructure, secretory granules, merocrine, ribosomes

BOCAVIRUS IN MALAYSIAN CATS AND DOGS

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ABSTRACT

The number of novel groups of feline bocavirus (FBoV) and canine bocavirus (CBoV) discovered in on the increase. Currently, there is a lack of information on the prevalence of FBoV and CBoV in Malaysia. Thus, this study aimed to determine the presence of bocavirus in cats and dogs in Klang Valley, Malaysia. Four cats and 37 dogs were chosen from animal shelters. Tissue samples consisting of submandibular lymph node, lung, kidney, mesenteric lymph node, intestine, and inguinal lymph node were obtained from necropsy, processed, and subjected to conventional polymerase chain reaction (PCR) using primers targeting the conserved nonstructural protein 1 (NS1) gene. Based on PCR analyses, 100% (4/4) and 24.3% (9/37) of cats and dogs, respectively, were positive for bocavirus. Partial nucleotide sequencing of the NS1 gene were performed on 2 PCR FBoV products and compared with published sequences. Preliminary phylogenetic analysis revealed that the Malaysian FBoV isolates are distinctly different from reference isolates. Further studies on the prevalence and pathology of bocaviruses should be conducted to determine their pathogenesis. By this study, Malaysia became the fifth country to report presence of FBoV and CBoV.

Key words: feline bocavirus, canine bocavirus, PCR, sequencing, phylogenetic analysis

A SURVEY ON PET OWNER PERCEPTION REGARDING NEUTERING IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Neutering is surgical removal of reproductive organs, primarily done to prevent unwanted breeding and bad behaviour and reproductive diseases in animals. Failure of pet-owners to neuter their pets results in increase in stray animal population. This study was conducted to determine pet-owners' perception on and awareness of neutering and their decision to subject their pets to the procedure. A cross-sectional study on pet-owners was conducted by questionnaire. One hundred and forty-six pet-owners responded to the survey. The result was classified according to pet-owner perception that influences decision to neuter or not to neuter. The results showed that 38% of pet-owner were aware, 37% moderately aware, 25% not aware of neutering. Their knowledge on cat and dog reproduction was poor (63%). However, very few pet-owners who were aware of neutering (20.9%) and had knowledge on cat and dog reproduction (17.9%) decided to neuter their pets.

Keywords: neutering, Klang Valley, Malaysia, cats, dogs, perception

EFFECT OF FEED FORMULATION ON BODY WEIGHT GAIN, FEED INTAKE, AND STRESS PARAMETER OF GOATS

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ABSTRACT

Balanced nutrition is essential for health and performance of livestock. It is best when feed is formulated according to the production stages of goat. However, as the goats grow, they undergo transition in nutritional requirements. In this study, body weight gain, feed intake haematological parameters were used to determine the effect of feed formulation on goat does. Eighteen adult goat does with similar body condition score and weighing approximately 20 kg were divided into three groups; group 1 was give the normal farm diet, group 2 were fed a diet formulated for maintenance, and group 3 fed diet formulated for flushing. The study was conducted over 4 weeks. The body weights of does were obtained before implementation of diets and at weeks 2 and 4 of the experiment while blood samples were obtained before implementation of the diets and at Week 4 of the experiment. The body weight gain and feed intake was highest in does fed flushing diet, followed in order by those fed maintenance diet and normal farm diet. Does given flushing diet showed the highest body weight gain ($p<0.05$) while those given normal farm diet the lowest. Leucocyte count was highest in does given maintenance diet; however the neutrophil:lymphocyte, as an indicator of stress, did not differ ($p>0.05$) among diet groups. Thus, the diet formulations used in this study did not cause stress in goat does.

Keywords: feed formulation, body weight gain, feed intake, stress leucogram, N:L ratio.

EFFECT OF ORAL TREATMENT WITH IMMUNOGENIC LIPOPOLYSACCHARIDE EXTRACTED FROM *PASTEURELLA MULTOCIDA* TYPE B:2 ON MICE

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ABSTRACT

Pasteurella multocida serotype B:2 is the causative agent for haemorrhagic septicaemia (HS), a highly fatal disease of buffaloes and cattle, in Malaysia. Lipopolysaccharide (LPS) is one of the major immunogens of *P. multocida* that can that can be used as subunit vaccine for HS. This study aimed to determine the protective effect oral inoculation of *P. multocida* B:2 LPS towards HS. Twenty-five healthy mice were divided into five equal groups; control group was inoculated orally with 0.2 mL phosphate buffer saline (PBS) pH 6.8, groups 1, 2, 3, and 4 with 0.2 mL LPS extracted from 10^3, 10^5, 10^7 and 10^9 cfu of *P. multocida* serotypes B:2, respectively. The experimental animals were observed for clinical signs for 17 days. On day 17, all mice were challenged with 0.2 mL of 10^7 wild type *P. multocida* B:2 and observed for clinical signs for another 7 days. Surviving mice were euthanised and the organs were collected for bacterial isolation and identification and histopathological examination. Before death, 60% of all mice had diarrhoea, 38.5% had severe ocular discharge, and 100% had laboured breathing. All mice developed mild to moderate histopathological lesions in the heart, lungs, liver, spleen, kidney, small intestine, large intestine, small intestine, and stomach. Mice treated with LPS from 10^3 CFU bacteria showed mild presence of inflammatory cells in the spleen and small intestine, whereas those treated with LPS from 10^9 CFU bacteria developed mild to moderate degeneration and necrosis in the kidneys and stomach. *P. multocida* was isolated from the heart, lung, liver, spleen, kidney, and gastrointestinal tract of all mice. The rate mortality did not differ significantly ($p>0.05$) among treatment groups with mortality of the control mice at 40%, LPS from 10^3 and 10^5 bacteria-treated mice at 33.33% mortality, LPS from 10^7 and 10^9 CFU bacteria-treated mice at 66.67%. The study showed that oral treatment with LPS extracted *P. multocida* failed to protect mice from the lethal effect of the organism.

Keywords: haemorrhagic septicaemia, *Pasteurella multocida* serotypes B:2, mice, lipopolysaccharides, histopathological lesions

AWARENESS, KNOWLEDGE AND UNDERSTANDING OF FELINE PREVENTIVE HEALTH CARE AMONG CLIENTS OF THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Many pet-owners do not have the fundamental knowledge required for provision of basic health care to their pets. Thus, some animal management practices instituted by the owners can have adverse effects on their pets. The purpose of this study was to determine the level of awareness, knowledge and understanding of Feline Preventive Health Care (FPHC) among clients of the University Veterinary Hospital, Universiti Putra Malaysia. Among pet management practices of concern in this study are vaccination, parasite control, neutering, and nutrition. Sixty-seven clients participated in a questionnaire survey. The survey showed 95.52% of respondents agreed that vaccination is important, while 4.48% were unsure. Only 81.25% of those who agreed on the importance of vaccine actually had their cats vaccinated. Among respondents who did not agree, the majority were unsure of the diseases they were preventing with vaccination. Although 97.01% of cat owners agreed that parasite control and prevention in cats are important, only 84.62% adopted the practice. Only 71.64% owners agreed that it is important to neuter cats, with 20.9% unsure and 7.46% disagreeing. Among the 48 owners that were in favour of neutering, only 68.75% had their cats neutered. 53.73% of owners fed their cats commercial diets only whilst the remainder fed a mixture of commercial diet and other foods. Based on the survey, 80.6% of respondents relied on veterinarians for information and guidance on FPHC, with the internet being the second most common source of information. This study demonstrates that while the majority of respondents were aware and agreed that basic preventive healthcare was required, they had lesser understanding of how it impacts their cats. There is dire need for owner education on pet healthcare and veterinarians can play a major in the disbursement of relevant information to pet-owners.

Keywords: preventive medicine, cat owners, Feline Health Care

PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE *BOVIDAE* AT THE NATIONAL ZOO, MALAYSIA

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ABSTRACT

Parasitic diseases constitute one causes of mortality in captive *Bovidae*. This study was conducted to determine the prevalence of gastrointestinal parasites in animals of the *Bovida*e family at the National Zoo, Malaysia. Fourteen faecal samples were collected from 8 species of animals of Genus *Bos* (n=6), *Hippotraginae* (n=2), *Tragelaphus* (n=2), *Oryx* (n=2), and *Kobus* (n=2). All samples were processed using direct wet mount preparation, formalin ethyl acetate concentration technique and stained with trichrome and giemsa. Intestinal parasites found were *Strongylids* (21.4%), *Moniezia sp.* (14.9%), *Capillaria sp.* (7.1%), *Cryptosporidium sp.* (7.1%) and *Entamoeba sp.* (7.1%). All samples tested positive for helminths or protozoa were from asymptomatic animals with low parasitic loads. Monitoring the gastrointestinal parasite of wild *Bovidae* in captivity is therefore imperative in assisting zoo management to formulate and implementation of preventive and control measures for parasitic diseases.

Keywords: gastrointestinal parasite, *Bovidae*, formalin ethyl acetate concentration, helminths, protozoa

COST OF MASTITIS TREATMENT IN A GOAT

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ABSTRACT

In Malaysia, among constraints in goat production are poor husbandry, reproductive disease, and poor nutrition. Reproduction diseases lead to production losses and additional costs due to treatment. The objective of this study is to estimate the cost of mastitis in a doe. The inputs for estimation of cost from a survey of four intensive goat farms included information on expert opinion, mastitis treatment protocol, and the associated costs. The costs of clinical mastitis treatment were calculated with the assumption that the doe is cured of mastitis within 3 days. On the first day, treatment cost is RM30 for one visit by the veterinarian. The doe is treated with intra-mammary antibiotics and analgesics at RM60.30/treatment. Assuming that the mastitis is cured by the third day, caring for the doe and kid by worker cost RM48.60 for 3 days that includes labour cost of RM5/hour. Milk production loss to allow for a 33-day antibiotic withdrawal period totals to RM412.00. Therefore, the total cost for mastitis treatment is estimated at RM 550.90 for the doe. If the doe is not cured by 3 days of treatment, it will be kept for 33 days at a cost of RM534.60 before culling and selling at RM600. It is assumed that the doe is replaced by a new one a cost of RM1,300. Loss of revenue from milk for 33 days is estimated at RM 412.25. Thus the total cost of mastitis for the doe if treatment fails is RM1,646.85. The study shows that treatment costs for mastitis are expensive, particular when doe failed to cured of the disease, need to be replaced. Our study also discovered that farmers did not treat mastitis in does. It would be much more economical had the farmer practiced mastitis preventive measures in their animals.

Keywords: costing, mastitis, reproduction, doe

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