15th PROCEEDINGS of the SEMINAR on VETERINARY SCIENCES

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Content

Preface

Most academic members of the Faculty of Veterinary Medicine, Universiti Putra Malaysia are young and vibrant scientists and researchers. These young researchers are not only well-trained but also driven to thrive in their professions. They have great ability, current knowledge, and research skills to conduct frontier researches. Their abilities are reflected in the quality researches conducted by postgraduate students under their charge. Undergraduate veterinary students had also benefited from the research knowledge and skills of the young academics. In the early days of the Faculty, the final year projects mostly centred on farm evaluations. Now, these students are exposed to projects using current technologies including cell cultures, molecular techniques, and gene analyses. There were also more studies on wildlife behaviour, primarily a benefit from the presence of several wildlife experts in the Faculty. Certainly, the information gained from these wildlife studies will serve the country well in the attempt to conserve the dwindling exotic animal species.

The term for the final year project is short. Students are required to complete research work within a period of one month. We are amazed by the quality of results obtained by some projects within such a short period. The editors wish to congratulate the students and supervisors for jobs well done.

The conduct of the majority of the final year projects required the use of Faculty laboratory facilities. Our utmost gratitude to all laboratory coordinators and staff for your assistance in the conduct of the projects. Without your cooperation, these projects would not have come to successful conclusions.

Finally, the editors wish to express gratitude to Faculty Management for facilitating the final year projects and publishing of the 15th Proceedings of the Seminar on Veterinary Sciences.

Editors

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ANTIMICROBIAL PROPERTIES OF ETHANOLIC EXTRACT OF *MORINGA OLEIFERA* TOWARDS *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS PYOGENES*

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ABSTRACT

Mastitis in ruminants is a common herd health problem causing economic losses to the dairy industries. In the treatment of mastitis, the use of antibiotics has been the mainstay. However, with the emergence of antimicrobial resistance (AMR) bacteria, antimicrobial has become ineffective. Thus, there is need for the development of alternative strategies for treatment of bacterial infections. This study was conducted to evaluate the antimicrobial properties of the ethanolic extract of *M. oleifera* leaves towards *Staphylococcus aureus* and *Streptococcus pyogenes*, the two most common causative bacteria for mastitis. The antimicrobial effect of ethanolic extract of *M. oleifera*, was determined in vitro using disk diffusion and minimal inhibitory concentration (MIC) methods. This study revealed that 100 mg/mL ethanolic extract of *M. oleifera* produced the largest zone of inhibition against both bacteria. Based on MIC, ethanolic extract of *M. oleifera* showed complete inhibition for *S. aureus* and *S. pyogenes* at 12.5 mg/mL. In conclusion, ethanolic extract of *M. oleifera* has antimicrobial properties towards *S. aureus* and *S. pygenes*.

Keywords: *Moringa oleifera*, *Staphylococcus aureus*, *Streptococcus pyogenes*, disk diffusion, minimal inhibitory concentration

INTRODUCTION

Staphylococcus aureus and *Streptococcus pyogenes* are versatile bacteria and have the ability to develop resistance against antibiotics, making these drugs ineffective to combat the infections. There is need to discover alternatives to antibiotics for *S. aureus* and *S. pyogenes* infections. The antimicrobial properties of plants have been intensively investigated by numerous researchers worldwide (Angiolella et al, 2018; Joshi, 2018).

The extract of *Moringa oleifera*, is also known as *Pokok Kelor* in Malaysia, evidently have variable antibacterial activities towards *Escherichia coli*,

Pseudomonas aeruginosa and *Staphylococcus aureus* (Singh and Tafida, 2014). The extract was shown to be more effective than available antibiotics against human pathogenic bacteria. However, there is limited information of the effect of *M. oleifera* extract on infections in animals. Thus, the objective of this study was to determine antimicrobial activity of *M. oleifera* leaf extract on *Staphyloccocus aureus* and *Streptococcus pyogenes*, the bacteria that common cause infections in animals.

MATERIALS AND METHODS

Extraction of Ethanol Leaf Extract

The leaves of *Moringa oleifera* were air-dried in an oven at 40°C. The leaves were pulverised and sieved through a 60 Mesh. Three concentrations of 10, 50, and 100 mg/mL *M. oleifera* extracts were prepared and allowed to stand for 24 h with intermittent shaking at 30 min intervals. The suspensions were filtered using Whatman No.1 filter paper and the extract concentrated using a rotary evaporator at 40°C under reduced pressure. The extract was kept in universal bottle and stored at 4°C until use.

Bacterial Cultures

Staphylococcus aureus and *Streptococcus pyogenes* were obtained from Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The *S. aureus* and *S. pyogenes* strains were revived and cultured on nutrient agar and blood agar, respectively. Biochemical tests were performed to verify the bacterial strains before sub-culturing on nutrient and blood agar, respectively for 24 h.

Preparation and Standardization of Inoculum Suspension

Isolated colonies were obtained from the agar plates using inoculating loop and suspended in a sterile test tube containing phosphate buffered saline. The turbidity was adjusted equivalent to 0.5 McFarland standard to achieve approximately 1.5×10^8 CFU/mL (Hudzicki, 2009) and suspensions of the bacteria prepared.

Antimicrobial Activity

The antimicrobial activity of *M. oleifera* extract was determined using the disc diffusion method. To determine the zone of inhibition (ZOI), the Mueller Hinton Agar (MHA) was used for *S. aureus* and blood agar for *S. pyogenes*. Sterile swabs were dipped into the inoculum suspension and used to inoculate the agar by streaking thrice over the entire surface. Filter paper blank discs were autoclaved and impregnated with 25 uL each with *M. oleifera* extract. The discs were placed onto the agar surface using sterile forceps. Pen-Strep (10 mg/mL) was the control. The plates were inverted and incubated at 37°C for 24 h. The ZOI was measured in triplicates using Vernier calipers.

Minimal Inhibitory Concentration

The minimum inhibitory concentrations (MIC) of *M. oleifera* extract on Fresh *S. aureus* and *S. pyogenes* inoculum suspensions were determined in 96-well sterile microtitre plates (Gupta and Negi, 2016). Two-fold serial dilutions of the extract was done to obtain concentrations ranging from 0.391 to 50 mg/mL in 1% dimethysulfoxide (DMSO). Twenty-five microlitre 10 mg/mL Pen-strep was used as the positive control. The plate was incubated at 37°C for 24 h. Since the MIC cannot be determined by visual inspections for bacterial growth because of the colour of the extract, 10 uL of aliquot from wells of the *S. aureus* and *S. pyogenes* plates were inoculated onto agar plates and incubated at 37°C for 24 h. Minimal inhibitory concentration was taken as the minimum concentration of the extract that inhibited bacterial growth.

Statistical analysis

Datasets obtained were subjected to Kruskal-Wallis Test to determine the differences across extract different concentrations at 95 % confidence level.

RESULTS AND DISCUSSION

The results showed that ethanol extracts of *M. oleifera* leaves had antimicrobial effect on *S. aureus* and *S. pyogenes* with mean MIC of 12.5 mg/mL extract. It has been suggested the antibacterial activity of *M. oleifera* extracts are attributed to their 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy)-benzylisothiocyanate component (Olson and Fahey, 2011). The mechanism of action of this compound involves the inhibition of essential cellular membrane enzymes of bacteria.

S. aureus was more susceptible to the effect of *M. oleifera* extract than *S. pyogenes* (Table 1). The antibacterial effect of the extract appeared to be concentration-dependent, with greatest inhibition of bacterial growth at 100 mg/mL, the highest concentration of extract used in this study.

Ethanol was the solvent used in the preparation of the *M.oleifera* leaf extract. Ethanol on its own is also known to have antimicrobial activities. Thus, preparing extracts using this organic solvent will enhance the antimicrobial activities of the natural compounds.

CONCLUSION

Moringa oleifera leaf extract has antimicrobial properties against *S. aureus* and *S. pyogenes*. Thus, the extract has potential to be developed into an antimicrobial agent or nutraceutical to be used as an alternative to known antibiotics in the treatment of infections. The use of these plants products as antimicrobial compounds will not only be safe but contributes to economy of the country.

Table 1. Inhibition of *Staphylococcus aureus* and *Streptococcus pyogenes* growth by *M. oleifera* extract.

Bacteria	Treatment	Concentration (mg/mL)	Zone of inhibition (mm)
S. aureus	*M. oleifera*	100	9.81 ± 0.63
		50	8.43 ± 0.53
		10	7.57 ± 0.28
	Pen-Strep	10	580 ± 0
S. pyogenes	*M. oleifera*	100	8.53 ± 0.02
		50	7.74 ± 0.18
		10	0
	Pen-Strep	10	520.00 ± 34.64

Values are mean ± standard deviation.

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HISTOPATHOLOGICAL CHANGES IN PREGNANCY-RELATED TISSUES AND FOETUS OF GOATS WITH PREGNANCY TOXAEMIA

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ABSTRACT

Pregnancy toxaemia is a common metabolic disorder affecting goats at late gestation. This condition is associated with negative energy balance. The objectives of the study were to determine the histopathological changes in organs of goats with pregnancy toxaemia and foetus, and the association between severity of lesions and serum β-hydroxybutaric acid (BHBA) concentration. Six pregnant goats were from a previous study induced to develop ketosis by reducing their diet by 50%. Two non-treated pregnant goats served as the controls. The serum BHBA were analysed in these goats. Aborted and nonaborted goats were sacrificed and the formalin-fixed. Thirty-one formalin-fixed organ samples, comprising of 5 umbilical cord, 6 placentas, and 6 uteri and from the foetuses, 6 hearts, 5 livers, and 3 cerebrums were used in this study. Histological lesions in the organs were described, and scored. The goats with pregnancy toxaemia showed haemorrhage and congestion in umbilical cord, congestion in placenta, absence of endometrial gland secretory phase in uterus, congestion in foetal heart, necrosis and haemorrhage in foetal liver. The was a significant positive correlation between umbilical cord histological lesions and serum BHBA concentration.

Keywords: goat, foetus, pregnancy toxaemia, histopathological changes

INTRODUCTION

Goat industry in Malaysia is gaining economic importance with the increase in national demand for goat milk and meat. However, in Malaysia, the local goat industry is still infantile with respect to animal husbandry, feed and feeding practices, reproduction and breeding, and control and prevention (Jamaluddin et al., 2012).

Ketosis is a metabolic condition characterised by increase in ketone body production in the body. In ruminants, ketosis usually occurs as the result of inappropriate feeding regimen leading to a state of negative energy balance (González et al., 2011). This condition is often observed in pregnancy, leading to economic losses. In Australia losses in pregnancy toxaemia in small ruminants, due to increased cost of production loss, prevention, and treatment had amounted to

A$15.82 million (Meat & Livestock Australia, 2015). In seriously affected animals, the disease can cause 20% morbidity and 80% mortality.

Pregnancy toxaemia systematically affects dams and foetuses. This study was undertaken to examine histopathological changes in does and foetus of goats with pregnancy toxaemia.

MATERIALS AND METHODS

The study used samples from a previous study where 6 goats at late-pregnancy (> 3 months) were induced to develop pregnancy toxaemia ketosis by 50% reduction of caloric intake. Two pregnant goats given standard feeding requirement, enough to meet nutrient requirement energy for pregnant goats (602.5 kJ ME/body weight kg/day), were used as controls. Records of serum serum β-hydroxybutaric acid (BHBA) concentrations estimated in the study were retrieved and recorded. Thirty-one formalin-fixed organ tissue samples from 5 umbilical cords, 6 placentas, and 6 uteri and from the foetuses, 6 hearts, 5 livers, and 3 cerebrums were obtained.

Histopathology

The tissues were subjected to routine preparation of histopathology slides using the standard paraffin-embedded technique and examined microscopically (Olympus Moticam 285A). The tissues on the slides were examined over 8 fields. The lesions were scored based on severity using either 4 or 5 distinct semi-quantitative grades (Mann et al, 2012).

Serum beta-hydroxybutyrate

Data collected from histological scoring for type of lesions was compared with serum BHBA concentrations of the goats.

Statistical analysis

Data collected from histological scoring for type of lesions were analysed statistically using the Mann-Whitney-U and Kendall's Tau Correlation.

RESULTS AND DISCUSSION

The goats with pregnancy toxaemia showed haemorrhage and congestion in the umbilical cord, congestion in the placenta, absence of the endometrial gland secretory phase in the uterus while in the foetus, there were congestion of the heart, necrosis and haemorrhage of the liver. The correlation between severity of histological lesion in organs and serum BHBA concentrations of goats with pregnancy toxaemia is shown in Table 1. The umbilical cords showed strong while other organs showed weak positive correlations between severity of lesions and serum BHBA concentrations. The results suggest that serum BHBA concentration could be an indicator of severity of histological lesions in organs of goats with

pregnancy toxaemia. It is postulated that the increase in serum BHBA concentrations may have caused the inhibition of hepatic gluconeogenesis resulting in maternal hypoglycaemia, leading to severe clinical effects on the animal.

Table 1. Correlation between severity of organ histological lesions and serum BHBA concentrations in goats with pregnancy endotoxaemia

Organ	Correlation	p value
Umbilical cord	0.949	0.011
Placenta	0.258	0.248
Uterus	0.577	0.072
Foetal heart	0.258	0.256
Foetal liver	0.316	0.224

CONCLUSION

Pregnancy toxaemia, a metabolic disorder and can adversely affect both does and the fetuses they carry. The condition can cause lesions in the organs of does and foetuses and contributing to in utero foetal death and abortion. The severity of lesions in organs, especially the umbilical cords, in pregnancy toxaemia in goats is correlated with the serum BHBA concentrations.

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PREVALENCE AND RISK FACTORS ASSOCIATED WITH RUMINANT FASCIOLIASIS IN SELECTED FARMS IN PAPAR, SABAH, MALAYSIA

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ABSTRACT

Fascioliasis from *Fasciola hepatica* and *Fasciola gigantica* infestation, besides being zoonotic, can cause significant economic losses to the livestock industry. The prevalence and associated risk factors of fascioliasis in ruminants in Sabah, Malaysia is not known. Therefore, this study determined the prevalence of fascioliasis in selected livestock farms in Papar, Sabah, Malaysia and the risk factors associated with the infection in ruminants. Faecal samples from 352 cattle, goat and sheep of 14 farms were obtained. Sedimentation and Flukefinder® methods were conducted to determine presence of the *Fasciola* sp. The sedimentation method showed an overall prevalence of 1.4% (5/352) while Flukefinder® technique showed a prevalence of 1.99% (7/352). Animal reared under semi-intensive showed higher risks of acquiring fascioliasis than those under intensive and extensive farming. The risk of being infected with *Fasciola* was higher among farms practicing symptomatic treatment than those without the practice or with scheduled practice. This study showed that the overall prevalence of fascioliasis among ruminants in Papar, Sabah is relatively low. Fascioliasis was found to be associated with type of management and deworming practices.

Keywords: fascioliasis, ruminant, risk factors, Sabah.

INTRODUCTION

Fascioliasis caused by *Fasciola hepatica* and *Fasciola gigantica. F. hepatica* that commonly occurs in sheep of temperate regions while that caused by *F. gigantica* is common in cattle and buffalo in tropical Asia, Southeast Asia, and Pacific regions.

Fascioliasis imposes direct and indirect economic impact on livestock production causing weight loss.

Diagnosis of fascioliasis is mostly based on the detection of liver fluke eggs in faeces. The occurrence of fascioliasis in farm animals is associated with the environmental temperature and humidity, age of animals, deworming programmes, management of farm animals and presence of snails, as the intermediate host. Deworming is effective in reducing fluke burden in farm animals.

Currently, the prevalence and risk factors of associated with ruminant fascioliasis in Malaysia is not clear. Thus, study was conducted to determine the prevalence of ruminant fascioliasis and the risk factors associated with the infection in ruminants.

MATERIALS AND METHODS

Fourteen farms in Papar, Sabah, Malaysia was recruited in the study. The study was approved by Institutional Animal Care and Use Committee (IAUCC), Universiti Putra Malaysia. Fresh faecal samples collected per rectum from 352 animals (299 goats, 5 sheep, and 48 cattle) at 14 farms. Information on sex and age of animals, management, and deworming practices were collected through questionnaires distributed to the farmers.

Fasciola sp. detection

The faecal samples were analysed in laboratory by the standard sedimentation technique and Flukefinder® method for the detection of *Fasciola* spp. eggs. The eggs identified microscopically based on their characteristics; operculated, thin-walled and yellow-brown staining features. Faecal samples with even one *Fasciola* spp. egg were considered positive for fascioliasis.

Sedimentation technique

Four grams of faecal sample was homogenised with 60 mL water and sieved through a strainer into a conical flask, which was then filled with water. The filtrate in the conical flask was allowed to sediment for 3 min. The supernatant was carefully removed without disturbing the sediment. The flask was again filled with water and again allow to sediment. The step was repeated until a clear sample was obtained. The sediment was collected, and three drops of methylene blue added. The sample was examined under a dissecting microscope.

Flukefinder® method

Flukefinder® is a commercially available kit comprising a unit of two 2-inch wide sieves of approximately 125 nm and 30 nm mesh. Two grams of faeces was mixed with water, the suspension poured into the Flukefinder® unit, and washed well with water. Larger materials retained by the large diameter sieve were discarded. Material retained in the smaller diameter sieve was back-washed into a 2-inch beaker and allowed to settle for 2 min before pouring off the supernatant. The remaining material was poured into a 2-inch petri dish. More water was added to fill the dish, and the

contents allowed to settle for 20 s before pouring off the supernatant. Three drops of methylene blue solution were added to the sample and examined under a dissecting microscope.

Statistical analysis
Prevalence of fascioliasis was calculated using the following formula:

$$P(\%) = \frac{\text{Number of positive samples}}{\text{Total number of samples examined}} \text{ x } 100$$

Other parameters; management, sex, deworming practice and age were subjected to Chi Square test to determine their associations with prevalence of fascioliasis.

RESULTS AND DISCUSSION

Using the Flukefinder® method, it was shown that 1.99% (7/352) while the sedimentation technique showed 1.42% (5/352) of samples were positive for fascioliasis. The Flukefinder® method was shown to be more sensitive and specific than the sedimentation method (Faria et al., 2008). The prevalence fascioliasis in Papar, Sabah is low compared to that recorded in other states of Malaysia; Kedah at 5.55% and Terengganu at 41% (Rita et al., 2017; Nur Alia Diyana et al., 2019). The differences in prevalence of fasciolasis among these regions in Malaysia is attribute to the differences in environmental conditions, management, and production systems.

There is significant association ($p<0.05$) between prevalence of fascioliasis and type of farming. The prevalence of fascioliasis is higher in semi-intensive than in intensive or extensive farming. Under semi-intensive farming, with grazing near stagnant water or drinking the water highly infected with *Fasciola* sp., the animals had greater probability of acquring the infection.

There is significant association between deworming practice and fascioliasis ($p<0.05$). The prevalence of the infection is higher in farm practicing symptomatic treatment than those not practicing treatment or practicing scheduled deworming. Animals receiving symptomatic treatment for fascioliasis are highly associated with the infection.

In the treatment of fascioliasis, animals were given albendazole. This drug affects the adult stages of *Fasciola* sp. (Shokier et al., 2013) while the immature stages of the parasite can still develop to become adults. Thus, in spite of the treatment, fluke eggs were still present in the faeces. The frequent use of same drug over an extended period may lead to resistance of *Fasciola* sp. towards albendazole.

CONCLUSION

The study showed that the prevalence of fascioliasis in ruminants in farms in Papar, Sabah is low. There is association between management type and deworming practice with prevalence of fascioliasis in ruminants.

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PREVALENCE OF MANGE AND IDENTIFICATION OF MITES IN RABBITS OF A COMMERCIAL FARM, SELANGOR, MALAYSIA

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ABSTRACT

Rabbits are commonly infested with various ectoparasites including tick, fleas, lice, and mites causing dermatological problems. This study aimed to provide preliminary information on the status of mite infestation and determine its prevalence in a commercial rabbit farm in Malaysia. A total of 200 rabbits comprising of 110 breeder and 90 grower rabbits were sampled and examined for any clinical signs associated with mite infestation and skin scrapings. Microscopic examination was carried out to determine the presence and the identification of mites based on morphology. The results showed that the prevalence mite infestation was 40.5%. There was an association between the mite infestation causing mange and age of rabbits. Higher prevalence of mange was observed in the grower than breeder rabbits. It is suggested that the prevalence of mange is due to the naiveness of immune system and high-density stocking of rabbits in cages that had facilitated transmission of the disease. Based on morphological identification, the most prevalent species of mite in skin scraping is *Sarcoptes scabiei* at 99%.

Keywords: rabbits, mite infestation, mange, prevalence, morphological study

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) farming is gaining popularity in Asian countries mainly for meat production. One of the factors contributing to the increasing interest in rabbit production is the limitation of land. Rabbit farming usually requires smaller land areas that ruminant or poultry farming and the cost of their feed is lower compared to that for ruminants and poultry.

In Malaysia, there are only a few rabbit farms and these farms focus on breeding replacement and grower rabbits. The turn-around time in rabbit farm is short and growers and farmers usually sell their rabbits at 3 months of age or 2 kg in body weight. Breeding of rabbits for replacement can be as young as 5 months old.

Rabbits are usually breed in close proximity, at two to three rabbits per cage. High stocking density predisposes rabbits to contagious diseases like mange. The most common mange affecting rabbits are psoroptic, notoedric mange, and srcoptic mange. Although *Psoroptes* spp. and *Notoedres* spp. mites have lesser zoonotic potential than *Sarcoptes spp*, their presence is important because these parasites can still cause health and production problems and decrease the aesthetic and economic values of the rabbits (Wall and Shearer, 2001).

MATERIALS AND METHODS

The study was carried out in a commercial rabbit farm, in Sepang, Selangor, Malaysia. The rabbits, New Zealand White, were kept in cages and separated based on age; grower and breeder.

Sampling method and sample size
Systemic sampling method was used where every second cage was selected from each row. The sample size was determined by taking 30% of the total population of breeder (n=366) and grower (n=300) rabbits. The rabbits used in the study, 110 breeders and 90 growers, were examined for mange lesions.

Prevalence
The overall prevalence of mange in rabbits was determined using the following formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of rabbits with mange lesions}}{\text{Total number of rabbits sampled}} \times 100$$

Data Analysis
Data was entered into Ms Excel and exported to SPSS statistical software. Chi-square test was used to determine association between mite infestation causing mange and age.

Evaluation of mange
The records for the rabbits including history of mange infestation and previous treatments were recorded. Only rabbits that manifested clinical lesions associated with mange infestation were subjected to thorough physical examination and lesions that suspected to be associated with mite infestation were recorded. Clinical signs observed include hair loss, severe itch and crusty or scaly skin lesions. The mange-like lesions were observed mainly on the ear pinnae, eyes, nose, mouth, and paws.

Sample collection and mite identification
Affected skin with lesions was scrapped with a #15 sharp, clean and sterilised scalpel blade dipped in liquid glycerol until blood oozed. The affected area was also softened with liquid glycerol to facilitate the procedure. Direct smears of the samples were

made on a glass slide and observed light microscopy to determine presence of mites. The mites were identified based on morphological characteristics.

RESULTS AND DISCUSSION

The result showed that the overall prevalence of mange in rabbits in the farm was 40.5%. A total of 81 rabbits with mange lesions were identified in the study. There is a significant association between the mange-causing mite infestation and the age of rabbits ($p < 0.05$), that is, mange was more prevalent in growers (52.2%) than breeders (30.9%).

The predominant mite that caused mange in these rabbits was *Sarcoptes scabiei* and they were present both superficially and in deep skin scrapings. Only one rabbit showed *Psoroptes cuniculi* at the superficial skin scraping (Figure 1).

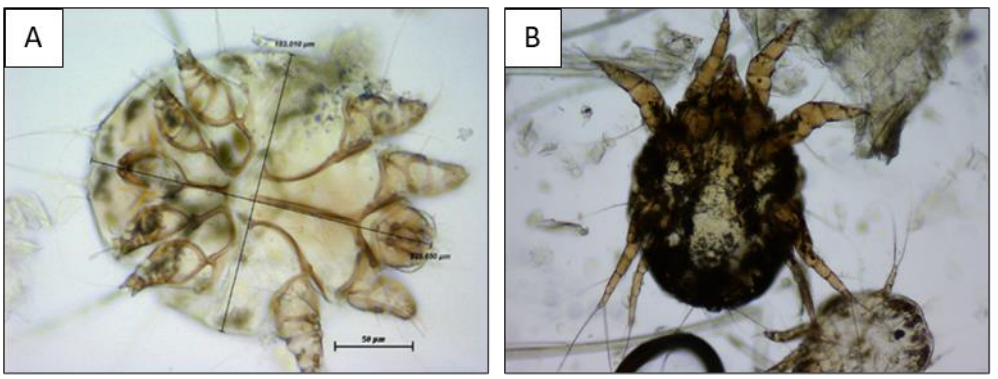

Figure 1. Mites identified in skin scapings in rabbits with mange. (A) *Sarcoptes scabiei*, (B) *Sarcoptes scabiei*

CONCLUSION

There was a high prevalence of mange in the commercial rabbit surveyed. The study showed that mange was more prevalent in grower than breeder rabbits. The predominant mite causing mange is rabbits was *Sarcoptes scabiei*.

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KNOWLEDGE, ATTITUDE, AND PRACTICES ASSOCIATED WITH BRUCELLOSIS AMONG SMALL RUMINANT FARMERS

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ABSTRACT

Brucellosis is an important zoonotic disease and an occupational health hazard for livestock farmers in Malaysia. Understanding the knowledge, attitude and practice (KAP) levels is crucial to address misconceptions on the disease among farmers. A cross-sectional study was conducted to investigate the KAP levels of small ruminant farmers on brucellosis in Selangor, Malacca, Negeri Sembilan and Johore, Malaysia. Twenty-six small ruminant farmers were recruited by convenience sampling. A pre-tested questionnaire was provided to the farmers via 'pen-and-paper' or online survey. The questionnaire consisted of close-ended questions on demographic information, farm backgrounds and farmers' KAP towards brucellosis. The mean score for knowledge, attitude and practice for the farmers were 57, 77, and 86%, respectively. Most farmers (89%) acknowledged that brucellosis can be transmitted between animals and infect humans. The majority of the farmers believed that brucellosis is preventable. Fifty-eight percent of farmers recognised that direct contact with aborted animal foetuses and placenta is an important mode of transmission of the disease to humans. All farmers agreed on the importance of wearing gloves to avoid infections while 96% practiced good personal and farm hygiene practices. The study showed that the KAP levels among small ruminant farmers was acceptable. However, some farmers could not recognise symptoms of brucellosis in humans or the risks associated with consumption of raw goat or sheep milk. Therefore, the small ruminant farmers in Malaysia are in dire need for education on brucellosis and its public health threats.

Keywords: brucellosis, knowledge, attitude, practice, small ruminant farmers, public health

INTRODUCTION

Brucellosis is a highly contagious zoonotic disease caused by bacteria of the genus *Brucella*. *Brucella melitensis* is the most virulent *Brucella* spp. that mainly affects cattle and small ruminants (Yahaya et al., 2019). In humans, the infection is mainly acquired through consumption of unpasteurised milk and milk products and direct contact with aborted foetuses and reproductive secretions from infected animals

(Bekele et al., 2011). Animals may acquire the infection from other infected animals through direct contact and vertical transmission.

Small ruminant production has improved the livelihood of farmers in Malaysia. However, infections from zoonotic pathogens like *Brucella* spp. had posed threats to animals causing economic losses. This problem is compounded by the lack of sufficient knowledge among farmers on the disease, high-risk practices, and absence of effective preventive and management strategies. The improvement of knowledge, attitude and practices (KAPs) among livestock farmers will greatly impact small rumination production by reducing zoonotic brucellosis infections at the farm. This study was conducted to determine the knowledge, attitude and practices on brucellosis among small ruminant farmers in Malaysia.

MATERIALS AND METHODS

Study population and procedure

A cross-sectional study was conducted using structured questionnaire to assess the KAP associated with brucellosis among farmers in Peninsular Malaysia. The study population comprise of 26 small ruminant farmers from Selangor, Malacca, Negeri Sembilan and Johore. Convenience sampling was used, based on availability, willingness, and consent of participants.

Questionnaire design

The questionnaire consisted of 48 close-ended questions in both Bahasa Malaysia and English. The questions were in the form of multiple choice or 'yes-or-no' questions. The questionnaire comprised of 5 sections; (A) demographic background, (B) farm characteristics, (C) knowledge related to brucellosis, (D) attitude related to brucellosis, and (E) practices in brucellosis prevention.

Data collection

The farmers were contacted via phone calls and emails to seek their consent to participate in the survey. Hardcopy or online Google self-assessment forms were given to the farmers with instructions.

Statistical analysis

The data were analysed using the Microsoft Excel 2013 and IBM SPSS Statistics Version 25. The test performed were descriptive analysis, percentage analysis, binomial test and Chi-square test with α=0.05.

RESULTS AND DISCUSSION

The mean score for farmers on knowledge, attitude, and practice level was 57 ± 15, 77 ± 13, and 86 ±16 %, respectively. The majority of the farmers (89%; 23/26) have heard of brucellosis. Most farmers (89%; 23/26) acknowledged that brucellosis can

be transmitted among animals and 81% (22/26) were aware that brucellosis can also infect humans. Fifty-eight percent (15/26) of the farmers recognised that an important transmission route for brucellosis to humans are via direct contact with an animal aborted foetuses and placentas. Sixty-nine percent (18/26) of farmers believed that brucellosis is preventable. Knowledge on brucellosis are crucial for prevention and control of the infection in animals and humans.

All farmers in the survey agreed on the importance of wearing gloves. Most farmers (69%; 18/26) buried aborted foetuses and retained placenta, while 12% (3/26) and 19% (5/26) discarded and burned, respectively. Seventy-three percent (19/26) of the farmers sought veterinary assistance for treatments, 50% (13/26) did their own treatments, 19% (5/26) slaughtered, and 31% (8/26) isolated aborting animals.

Ninety-six percent (25/26) of farmers practiced good personal and farm hygiene by washing hands after touching uterine, placenta, and aborted materials and disinfected area vacated by infected animals. Twenty-three percent (6/26) of farmers consumed unpasteurised milk or milk products on regular basis and 8% (2/26) sold their dairy products. Consuming and selling unpasteurised milk or dairy products are risk factors for human brucellosis (Garcell et al., 2016).

In general, lack of sufficient knowledge on the disease, health risk practices, misconceptions about brucellosis (Lindahl et al., 2015: Musallam et al., 2015), and absence of effective prevention and management strategies had resulted in the spread of the disease, causing economic losses and posing public health risks.

CONCLUSION

Small ruminant farmers in Malaysia showed moderate knowledge, good attitude and good practices on brucellosis. However, some of the farmers could not recognise the symptoms of brucellosis in humans. They also generally failed to identify health risks associated with consumption or selling of raw goat or sheep milk. Therefore, there is need to educate small ruminant farmers in Malaysia on brucellosis for the safety of humans and animals.

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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* FROM DAIRY FARMS IN KENINGAU, SABAH, MALAYSIA

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ABSTRACT

Staphylococcus aureus is a gram-positive opportunistic pathogen responsible for a variety of infections in humans and animals. In dairy cattle, *S. aureus* is frequently associated with clinical and subclinical mastitis. The emergence of methicillin-resistance *S. aureus* had posed a health threat to public. The aims of the study were to determine the prevalence of *S. aureus* in milk and environment samples from farm environment and to profile its antimicrobial susceptibility. Forty milk samples, comprising of 36 composite milk and 4 bulk tank milk and 20 environmental swabs from milkers' hands, udder, milking equipment, and floor were obtained. Fourteen samples (23.3%; 14/60) than yielded *S. aureus* strains were 11 from cow and 3 from bulk tank milk samples. The environmental samples did not yield any *S. aureus.* Most isolates were equally sensitive to tetracycline and erythromycin (92.9%) and least to penicillin G. A high isolation of *S. aureus* from bulk tank milk meant for public consumption is a public health concern.

Keywords: *Staphylococcus aureus*, dairy farms, antibiotic susceptibility test, milk samples, environmental swabs.

INTRODUCTION

Staphylococcus aureus is a gram-positive bacteria and versatile opportunistic pathogen than can cause a variety infections, from superficial skin infections to severe invasive diseases (Papadopoulos et al., 2019). Currently, the emergence of methicillin-resistance *S. aureus* (MRSA) has become a public health issue. The emergence of MRSA is primarily due to the presence of alternative penicillin-binding protein, known as PBP2a, which is encoded by the mecA gene located in the mobile genetic elemented Staphylococcus cassette chromosome (SCCmec) (Vanderhaeghen et al, 2010). The resistant strain *S. aureus* or livestock-associated MRSA (LA-MRSA) were isolated from various animals including dogs, pigs, horses, poultry, and cattle (Leonard and Markey, 2008).

In dairy cows, *S. aureus* can cause intra-mammary infections, accounting for 10 to 40% of all mastitis cases worldwide. The emergence of *S. aureus* is also associated

with raw milk and food contamination with the bacteria (Rola et at., 2016). *S. aureus* can potentially cause toxic shock syndrome from the effect of its toxin-1 (TSST-1), staphylococcus enterotoxin (SE), and Panton-Valentine leucocidin (PVL). The SE is the major cause of food poisoning in humans (Bergdoll et al., 1981).

To our knowledge, no research has been done in East Malaysia on the antimicrobial susceptibility of *S.aureus* in dairy farms. Thus, the objectives of this study were to determine the prevalence and antimicrobial susceptibility of *S.aureus* isolated from dairy farms in Sabah.

MATERIALS AND METHOD

Study population

The study was conducted at on four consenting dairy cow farmers in Keningau, Sabah, Malaysia. The study recruited 36 Sabah Friesian-Sahiwal dairy cows managed intensively and fed cut-and-carry Napier grass. Four cattle dairy farms from Keningau, Sabah, Malaysia were included in the study. All cows were milked twice daily and the milk yield ranged from 4 to 11 litres/cow/day. Thirty-six composite and 4 bulk tank milk and 20 environmental swab samples were obtained for analysis.

Composite and bulk-tank milk

Ten lactating cows from each farm were sampled on the day of the visit. The teat quarters were washed clean water to remove dirt and followed by chlorhexidine before rinsing with water and dried with clean tissue paper. Then, teats were pre-dipped in iodine. Composite milk samples from four quarters of each cow were collected in one sterile disposable tube. Bulk-tank milk was stirred and approximately 80 mL milk samples were aseptically collected into sterile tubes.

Udder skin

Five udder skin swab samples were collected using sterile swabs (Sterile transport media, Cary Blair Medium, Labchem Sdn Bhd) and immediately placed in transport media swabs.

Milker's hand

Hand samples from consenting individuals were collected using sterile saline-moistened swabs (Sterile transport media, Cary Blair Medium, Labchem Sdn Bhd). One swab was used for both hands. The swabs then placed in transport media.

Environment

Three environmental samples from the floor, milk buckets, and milking machine were collected using swabs (Sterile transport media, Cary Blair Medium, Labchem Sdn Bhd), which were placed in transport media.

Laboratory
All samples were transported on ice to the Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Isolation and detection of Staphylococcus aureus
Bacterial isolation from milk samples were performed according to the procedures described in the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019). A loopful of milk samples was cultured on blood agar plate enriched with 7% sheep blood. The plates were then incubated aerobically as 37°C for 24 h and observed for growth and colony morphology. The bacteria were sub-cultured on nutrient agar to obtain pure culture with circular, golden yellow and white colonies. *S. aureus* was finally identified as gram-positive cocci and catalase test-positive. Suspect *S. aureus* were screened on Mannitol Salt Agar. Yellow colonies indicate positive while pink colonies indicate negative for *S. aureus*. Biochemical assays used for preliminary identification of *S.aureus* were haemolysin, Voges-Proskauer (VP), maltose, mannitol and arginine dehydrolase tests. A milk or swab sample is positive if it contained at least one *S. aureus* isolate.

Antibiotic susceptibility testing
The isolate suspension was prepared and turbidity determined using the 0.5 McFarland standard. Each isolate suspension was streaked onto Mueller Hinton Agar by sterile swab. Antibiotic susceptibility testing was performed using the Kirby-Baur diffusion method on Mueller-Hinton agar, according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019) using the following antimicrobial susceptibility disks (Oxoid, Basing Stoke, and UK); Erythromycin (15µg), Vancomycin (30µg), Chloramphenicol (30µg), Tetracycline (30µg), Kanamycin (30µg), Sulphamethoxazole (25µg) and Penicillin (10units), Ciprofloxacin (1µg) and Gentamicin (10µg). The areas of inhibition were recorded as the diameter of the area around the individual disk without bacterial growth.

RESULTS

Composite and bulk-tank milk samples

The rates of isolation of *S. aureus* from composite and bulk tank samples are shown in Table 1. Eleven (30.6%) milk specimens were positive for *S. aureus* and two (5.56%) for *S. pseudointermedius. S. aureus* from 5 (13.9%) specimens were resistant to antibiotics, particularly penicillin.

Table 1. Composite and bulk tank milk samples from dairy farms in Keningau, Sabah, Malaysia positive for *S. aureus*

Farm	Composite milk			Bulk tank		
	No. of samples	*S. aureus*-positive sample	Prevalence of *S. aureus* (%)	No of Samples	*S. aureus*-positive sample	Prevalence of *S. aureus* (%)
A	10	2	20	1	0	0
B	10	3	30	1	1	100
C	9	2	22.2	1	1	100
D	7	4	57.1	1	1	100
Total	36	11	30.6	4	3	75

Prevalence of *S. aureus* in milk sample

Swab and Milker's hand samples
All samples were negative for *S. aureus.*

Environment
Only one sample, that is from the floor, was positive for *Staphylococcus intermedius*.

Antimicrobial susceptibility

All were subjected to antimicrobial susceptibility test. Most *S. aureus* isolates were equally sensitive to tetracycline and erythromycin at 92.9%. *S. aureus* showed lowest sensitivity to penicillin G.

DISCUSSION

The study showed composite and bulk tank milk from the four dairy farms surveyed in this study were positive for *S. aureus*. This finding is similar to that reported in dairy farms in Brazil (Lee, 2012), and Iran (Jamali et al., 2015), and the USA (Amico and Donnelly, 2010). However, comparing the rate of isolation of the *S. aureus* in milk between our study and others is difficult, because the occurrence of the bacteria is dependent on factors such as region, animal management practices, and milking hygiene (Lee et al., 2012). It seems that milk from bulk tanks are more prone to contamination with bacteria than individual milk. Among cause of bacterial contamination are improper cleaning of bulk tank, dirty udder and milking equipment, unsanitary milk handling technique, and improper milk storage (Thaker et al., 2013).

The study did not isolate any bacteria from the environment. This is surprising because others have shown that hand swabs, ropes, and the farm gate also harbour the *S. aureus* (Monte et al., 2018). It is possible that these farms in Sabah practice good hygiene for the farm environment.

The *S. aureus* isolate from milk samples in this study show high resistance to penicillin G. This observation was also shown by Papadopoulos et al., (2019) and Jamali et al., 2015. Conventional small dairy farms are usually contaminated with bacteria with lower antibiotic resistance (Suriyasathaporn et al., 2012). The presence of penicillin resistant *S. aureus* in these dairy farms in Sabah indicates indiscriminate use of the antibiotic for the treatment of mastitis (Matallah et al., 2019) and other infections in humans and animals (Akanbi et al., 2017).

CONCLUSION AND RECOMMENDATION

There is a high prevalence of *S. aureus* in milk of dairy farms in Keningau, Sabah. However, based on the samples in this study, the environments of these farms were generally free of the bacteria. The *S. aureus* isolates from milk in these farms were resistant to penicillin G, which is a public health concern. The *S. aureus* isolated from milk were sensitive to tetracycline and erythromycin.

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SEROLOGICAL DETECTION OF *LEPTOSPIRA* SP. INFECTION IN A DEER FARM IN SERDANG, SELANGOR

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ABSTRACT

Leptospirosis is important zoonotic diseases worldwide with high incidence in tropical countries. This serological study was conducted to determine the presence of *Leptospira sp*. infection in deer farm in Serdang, Selangor, Malaysia. The farm practices good management and biosecurity farm to ensure the animal free from infective agent. One hundred and eleven serum samples were collected via jugular vein venipuncture from deer aged between one to 12 years. The serum samples were tested using microscopic agglutination test (MAT) for the detection of *Leptospira* sp. infection. Twenty live *Leptospira* spp. serovar were selected as antigen for MAT. Nine samples were showed evidence of agglutination with a titre of 1:50. Five serum samples showed agglutination against *Leptospira* serovar Hebdomadis, two serum were positive for *Leptospira* serovar Ballum while two were positive for *Leptospira* serovar Coppenhageni. The presence of low antibody titre to *Leptospira* sp. in the deer could be due to past infections or early exposure to the *Leptospira* spp.

Keywords: leptospirosis, microscopic agglutination test (MAT), zoonosis, deer

INTRODUCTION

Leptospirosis is an emerging zoonotic disease that can infect and cause illness in various mammalian hosts, including cattle, dogs, swine, horses and even humans. The infection is a common occurrence in tropical and subtropical regions of the world (Bharti et al., 2003). Leptospirosis is caused by spirochetes of the genus *Leptospira*, which belong to the family Leptospiraceae, order Spirochaetales. The characteristics of the bacteria are highly motile, thin, flexible, and filamentous, and with fine spirals and hook-shaped ends. The genus *Leptospira* consists of 20 species which includes 9 pathogenic, 5 intermediate, and 6 saprophytic species. Currently, there are more than 250 pathogenic serovars worldwide. In Malaysia, 37 serovars of Leptospira had been isolated from wildlife and humans (Bahaman, 1988).

Transmission of the leptospirosis from infected animals to human usually from direct or indirect exposure with urine or other body fluids of infected animals. Indirect exposure occurs via contact with contaminated water and soil. Leptospirosis

can enter body through breaks in skin, intact skin, mucosa, including the respiratory tract, and conjunctiva. In Malaysia, leptospirosis remains to be an occupational hazard for those in high risk groups, such as workers in agricultural sectors, search and rescue workers in high risk environments, sewerage workers, and livestock handlers. In humans, leptospirosis causes febrile sickness with combination of chills, conjunctiva suffusion, severe headache, myalgia, lower back, legs and abdominal pain, vomiting, and diarrhea. When clinical progresses rapidly, the infection causes icterus, renal failure, cough, dyspnea, and death.

In Southeast Asia, the most common *Leptospira* serovars associated with the disease in domestic animals and humans are Icterhaemorrhagiae, Autumnalis, Canicola, Pomona, Patoc, Grippotyphosa, Australis, and Poi (Victoriano et al., 2009). The *Leptospira* serovars are adapted to specific reservoir or maintenance host (Levett, 2001).

In Malaysia, the *Leptospira* spp. were first isolated in 1928 from black rats (*Rattus rattus*) (Fletcher, 1928) and serovar Hebdomadis from dogs (Bahaman, 1988). The seroprevalence in human leptospirosis in Malaysia is approximately 9.7%.

Wildlife species are important epidemiological carriers, mainly because of their frequent exposure to *Leptospira* spp. native to their habitat. Currently, little is known about the prevalence of leptospirosis in deer in Malaysia. Therefore, the objectives of this study were to determine the seroprevalence of *Leptospira* spp. infections and to identify the infective *Leptospira* spp. serovars in deer in a farm in Malaysia.

MATERIALS AND METHODS

Study design and samples

Serum samples were collected from a deer farm in Serdang, Selangor, Malaysia. The deer were captured and blood sampled in a dark room at the farm. One hundred and eleven serum samples were collected via jugular vein venipuncture from deer aged one to 12 years. Whole blood samples were collected into 5-mL plain tubes, centrifuged at 250 × *g* for 10 min, and sera separated and stored refrigerated until use.

Microscopic agglutination test

The microscopic agglutination test (MAT) was performed according to the protocol described by World Health Organisation in the OIE Terrestrial Manual 2008 for leptospirosis (OIE, 2008). Live antigen of the following *Leptospira* serovars were used: *Autumnalis, Australis, Ballum, Bataviae, Cellodoni, Cynopteri, Canicola, Copenhageni, Djasiman, Grippotyphosa, Hardjo Bovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Lai, Malaysia, Pomona, Pyrogenes, Patoc* and *Tarassovi*. The antigens were cultured in Ellinghausen, McCullough, Johnson and Harris (EMJH) medium containing 1% bovine serum albumin and Tween 80 at 30°C for 7 to 10 days. The MAT was conducted in level II biosafety cabinet. Hyperimmune serum serving as the positive control and negative serum as negative

control were obtained from the Bacteriology Laboratory, Veterinary Laboratory Services Unit, Universiti Putra Malaysia. The positive and negative control samples was placed in the first and second column of the plate, respectively. Fifty microlitres of sterile phosphate buffer saline (PBS) was added to each well. Another 46 µL of PBS followed by 4 µL diluted serum were added to the third column. Sera were serially diluted to give concentrations of 1:50 to 1:151200. Equal volumes of live antigen were added to each diluted serum and mixed in shaker incubator for 5 min before incubating for another 90 min. The mixtures were examined under dark field microscope to observe for agglutination. Serum was considered positive when ≥ 50% of the leptospires agglutinated at cut-off titer of 1:100. Sera that did not show reactivity below this cut-off titer were considered MAT-negative.

RESULTS

A total of 111 deer serum samples were tested using *Leptospira* spp.serovar antigen and MAT. All deer were appeared clinically healthy during sampling. The result showed that 8% (9/111) of serum sample were reacted to *Leptospira* spp.serovar Ballum, Copenhageni and Hebdomadis at 1:50 titre. Two (2%) serum samples was reacted to serovar Ballum, whilst two (2%) and five (5%) serum samples reacted to serovar Copenhageni and Hebdomadis, respectively. All sera were reported negative to *Leptospira* spp.infection because the titer was below cut-off titer of 1:100.

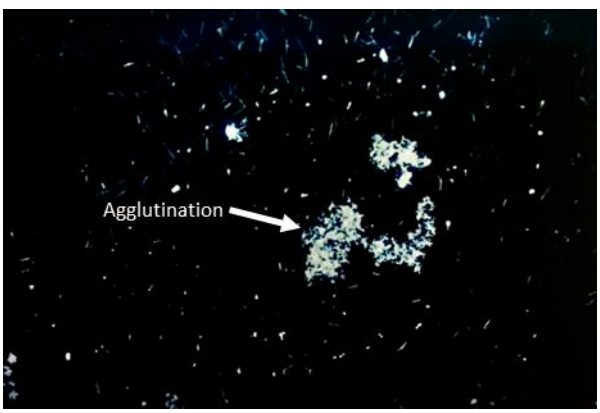

Figure 1. End-point microscopic agglutination test titre showing 50% agglutinations and 50% free antigen of *Leptospira* spp. Magnification 40×.

DISCUSSION

The deer in this study were healthy and so far the farm has not reported any case of leptospirosis. Based on the cut-off titre of 1:100, all deer samples were negative to *Leptospira* spp.infection. However, 9 deer showed a titer of 1:50 for serovar Ballum,

Copenhageni and Hebdomadis. The low leptospira titer in these deer may suggest that leptospirosis is endemic in Malaysia (Benacer et al., 2013). In asymptomatic infections the antibody titres will decrease over time to below the cut-off value of 1:100 until not detectable (OIE, 2008). It should be note, however, serological negativity may occur in the early stages of infection. Therefore, it is recommended that serum analysis for current and past leptospirosis should be done on paired serum sample. Paired serum analysis will show that the antibody titers increase current and decrease in past infections (Benacer et al., 2013). The lack of significant antibody titre shows that this farm practices good management and implements biosecurity to prevent infections in the deer.

The MAT is sensitivity and specificity and a reference standard test for serological diagnosis of leptospirosis (Adler and de la Peña Moctezuma, 2010). The non-reactive serum in this study indicates that the deer were negative for antigens of the serovars *Autumnalis, Australis, Ballum, Bataviae, Cellodoni, Cynopteri, Canicola, Copenhageni, Djasiman, Grippotyphosa, Hardjo Bovis, Habdomadis, Icterohaemorrhagiae, Javanica, Lai, Malaysia, Pomona, Pyrogenes, Patoc* and *Tarassovi*. It has been reported that there are 38 serovar of *Leptospira* spp.present in Malaysia (Bahaman et al. 1987). Since only 21 of the 38 leptospiral antigens were examined in this study, the true picture of *Leptospiral* spp.infection in these deer cannot be fully ascertained.

CONCLUSION

This study showed using MAT and cut-off titre of 1:100, that the deer in a farm in Serdang Selangor, Malaysia, were serological negative to the panel of 20 leptospiral serovars. Nine deer showed a titre of 1:50 titer for serovar Ballum, Conphengani, and Habdomadis. It is recommended that similar studies in the future should consider using the complete panel of leptospiral serovars on paired serum samples to ensure a true presentation of leptospirosis in farm animals.

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OCCURRENCE OF FASCIOLIASIS AT SABAH MEAT TECHNOLOGY CENTRE AND VETERINARY PUBLIC HEALTH LABORATORY, SABAH, MALAYSIA

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ABSTRACT

Fascioliasis is a neglected yet important zoonotic disease that may affect both livestock and humans. The fascioliasis is distributed worldwide, and Malaysia is one of the countries facing problems related to this parasite. The purpose of this study is to investigate the occurrence of fascioliasis at the slaughterhouse, Sabah Meat Technology Centre (SMTC), Kinarut, Papar and Veterinary Public Health Laboratory (VPHL) Kepayan, Sabah, Malaysia using retrospective data collected over an 11-year period (2008-2018). A total of 2297 cattle, buffaloes and goats were slaughtered at SMTC during this period, and 21 livers were condemned due to fascioliasis, giving a total occurrence rate of 0.91%. In VPHL, among 760 faecal samples from cattle and buffaloes submitted, 189 were positive for fasciola, with an occurrence rate of 24.87%. Twenty-four adult fasciolids were collected from livers at SMTC that were positive for fasciola and used for morphometric analysis. The analysis was based on the measurement of 8 parameters; body length (BL), body width (BW), cone length (CL), cone width (CW), distance between ventral sucker and the posterior end of the body (VS-P), body area (BA), BL to BW ratio (BL/BW) and BL to VS-P ratio (BL/VS-P). The results show that all adult fasciolids were *Fasciola hepatica*-like species.

Keywords: *Fasciola* spp., morphometric analysis, fascioliasis, Sabah

INTRODUCTION

Fascioliasis or trematode infection is an important parasitic infection caused by two *Fasciola* species, *Fasciola hepatica* and *Fasciola gigantica*. In one study conducted by Phalee and Wongsawad (2014), the rate of occurrence of fascioliasis at 3 slaughterhouses in Chiang Mai was 67.27%. However, the rate is lower than that recorded in 58 farms in Guangxi, China, which was 87.35% (Zhang et al., 2019). In Peninsular Malaysia, based on a retrospective study at 5 Veterinary Regional Laboratories, fascioliasis was of relatively low occurrence at 1.76% (Nur Alia Diyana et al., 2019)

The *Fasciola* spp. have a unique life-cycle with snails as the intermediate host. Adult flukes reside in the bile duct of the definitive host and shed unembryonated eggs in the faeces. The eggs hatch under the influence of multiple factors including egg maturity, temperature, and light intensity. Thus, in ruminants, the liver and gall bladder from slaughtered ruminants are potential sources of adult *Fasciola*. Generally, liver flukes have a flat and leaf-like body shape. The adult *F. hepatica* is much smaller than the adult *F. gigantica*, with body length of 20 to 30 mm and 25 to 75 mm, respectively.

In Malaysia, fascioliasis is due to infection by *F. gigantica* (Ann Zainalabidin et al., 2015). In subtropical regions, *F. hepatica* and *F. gigantica* co-exists. These parasites sometimes appear in form of intermediate fasciolid, which makes species classification challenging (Mas-Coma et al, 1997).

In this study, the morphometric method is used to compare the morphological characteristics of the liver flukes collected from a slaughterhouse in Sabah.

MATERIALS AND METHODS

A total of 2297 cattle, buffaloes and goats were slaughtered at Sabah Meat Technology Centre (SMTC) during an 11-year period (2008-2018) and livers condemned due to fascioliasis used in this study. At post-mortem examination, the livers were observed for abnormalities. The meat inspector may condemnation of livers based on damaged tissue, adhesion, hemorrhage or thickened bile duct. The presence of adult liver flukes indicated positive fascioliasis. Three livers of bovine species from the Veterinary Public Health Laboratory (VPHL), Kepayang, Sabah, condemned and suspected to be infected with fascioliasis were isolated and stored in individual containers filled with 75% ethanol. The livers were dissected and adult liver flukes were extracted using forceps, and transferred to vials containing normal saline, to remove tissues and debris. The adult liver flukes were placed in containers filled with 75% ethanol before transporting to Faculty of Veterinary Medicine, Universiti Putra Malaysia for morphometric analysis. Cattle and buffalo faecal samples were also obtained from VPHL for parasite examination.

Morphometric analysis

The flukes were subjected to gross morphologic examination and measurement. Eleven flukes were deformed and exclude from examination. The parameters used in the measurement of flukes were body length (BL), maximum body width (BW), cone length (CL), cone width (CW), distance between the ventral sucker and the posterior end of the body (VS-P), BL/BW ratio, BL/VS-P and body area (BA). All measurements were made using a digital caliper. The mean, standard deviation and range of all measurements of specimens were compared with those of *F. hepatica* and *F. gigantica* obtained from environment where these parasite species do not co-exist (Periago et al., 2008).

Statistical analysis

All data, including the occurrence of fascioliasis were validated and calculated using Microsoft Excel Version 2015. Chi-square was used to determine the association between the occurrence of fascioliasis and host species using SPSS software Version 23.

RESULTS AND DISCUSSION

Occurrence of fascioliasis at SMTC and VPHL

During period of the study, 21 livers from SMTCwere condemned due to fascioliasis, giving an occurrence rate of 0.91% (21/2297). Year 2008 recorded the highest occurrence of fascioliasis at 8.42% (17/202), followed by year 2018 and 2011 with the occurrence of 1.13 and 0.55%, respectively.

The data showed that at VPHL, among 760 faecal samples 189 were positive for *fasciola* spp. ova, giving an occurrence rate of 24.87%. The highest occurrence was recorded in 2009 at 48.45% (125/258), followed by 2011 and 2014 at 43.75 (14/32) and 30.77% (8/26) respectively ($p<0.05$).

The overall occurrence of fascioliasis at SMTC was at 0.91% (21/2297). This is relatively low compare to the occurrence of fascioliasis at the Maiduguri abattoir, Nigeria for 2015 and 2016, which was 13.67%. The cattle population in Nigeria is larger than in Malaysia, therefore, abattoirs in Nigeria receives larger number of animal (Adedipe et al, 2014) than abattoirs in Malaysia, which accounts for the differences.

In VPHL, the overall occurrence of fascioliasis in cattle and buffaloes was 24.87% (189/760), which is much higher than that reported for five Veterinary Regional Laboratories in Peninsular Malaysia, which was 1.76% (35/1988) (Nur Alia Diyana et al., 2019). The mean annual rainfall in Sabah and Peninsular Malaysia is 2,630 and 2,420 mm, respectively. The heavy rainfall provides a good environment for the breeding of *Lymnaea spp*, the intermediate host for *Fasciola* spp. (Halimi et al., 2015).

The study showed SMTC recorded lower occurrence of Fascioliasis that VPHL. This is probably due to the fact that in slaughterhouses, meat inspector may occasionally misinterpret the condition of the livers, especially at the early stage of parasitic infection. At the early stage of infection, liver pathological changes due to fascioliasis is still not manifested. In addition, the immature liver flukes, being very small, are not readily detectable at early infection.

Morphometric analysis of adult liver flukes collected at SMTC

The mean, standard deviation and range value of 8 parameters used in the morphometrically is shown in Table 1. The results show that BL, BW, BL/BW ratio,VS-P and BA of the specimens overlap with those of *F. hepatica*.

Among adult liver flukes collected from three livers, two were of animals from the Desa Cattle Sdn. Bhd (Ranau) and one from a local farm. Morphologically, the adult flukes from both livers of animals from Desa Cattle were morphologically

similar to *F. hepatica*. However, the bodies of these parasites were shorter and broader than *F. hepatica*. In contrast, the adult flukes obtained from the liver of animal from the local farm were more like *F. gigantica* in appearance, since they were longer and narrower (Periago et al., 2008). Furthermore, *F. hepatica* has V-shaped posterior end while *F. gigantica* has a posterior end that narrows only towards the tail-end.

Table 1. The mean, standard deviation and range value of morphological parameters of adult flukes, and standard of *F. hepatica* and *F. gigantica* population

Parameters	*Fasciola spp*	*F. hepatica*	*F. gigantica*
	Mean ± Std Dev (*Range*)	Mean ± Std Dev (*Range*)	Mean ± Std Dev (*Range*)
Body length (BL) (mm)	20.61±4.34 (*12.4-28.9*)	23.73±0.33 (*15.48–28.71*)	44.65±1.15 (*35.25-48.71*)
Body width (BW) (mm)	8.03±2.05 (*5.2-12.2*)	10.54±0.15 (*8.21-14.27*)	10.36±0.46 (*8.23-13.60*)
BL/BW ratio	2.75±0.96 (*1.36-4.40*)	2.27±0.03 (*1.65-2.76*)	4.37±0.17 (*3.43-5.50*)
Cone length (mm)	2.33±0.53 (*1.4-3.7*)	2.23±0.04 (*1.36-2.98*)	3.16±0.11 (*2.61-3.68*)
Cone width (mm)	2.59 ±0.34 (*2.0-3.3*)	3.18±0.04 (*2.05-3.99*)	3.81±0.10 (*3.25-4.34*)
VS-P (mm)	17.83±4.07 (*10.4-25.6*)	20.79±0.31 (*12.40-25.08*)	41.02±1.21 (*31.01- 45.39*)
Body area (mm^2)	163.57 ±44.26 (*79.36-235.46*)	180.92± 4.70 (*92.73-303.96*)	359.20±19.05 (*226.16-475.95*)
BL/VS-P	1.16±0.05 (*1.04-1.27*)	1.14±0.004 (*1.05-1.28*)	1.09±0.01 (*1.06-1.14*)

VS-P - Distance between the ventral sucker and the posterior end of the body

The values of BL, BW, BL/BW ratio, VS-P and BA of specimens overlap with those of *F. hepatica*. It was suggested that BL and BW are specific and sensitive indices in comparison with *Fasciola* sp. (Periago et al., 2008)

Thus far, only *F. gigantica* were found in the livers of buffaloes and cattle and this was during screening for fascioliasis in abattoirs in Perak (Zainalabidin et al., 2015).

There are several factors the contribute to findings of this study, First, the Desa Cattle farm is located in the highlands in Kundasang, Ranau, Sabah. It appears that *F. hepatica* is more abundant at high altitudes while *F. gigantica* is dominant at low altitudes (Howell et al., 2012; Ashrafi et al., 2006). Second, Malaysia practices importation of replacement heifer from Australia and New Zealand, where *F. hepatica* is prevalent. Thus, these cattle may have been previously infected.

CONCLUSION

In conclusion, the rate of occurrence of fascioliasis in Sabah is high. However, from the data obtained, VPHL showed higher occurrence of fascioliasis that SMTC. It is suggested that the occurrence of *Fasciola* spp. in animals in Malaysia may be the result of transportation of infected animals. Therefore, there is need to impose strict restrictions on the importation of animals from area endemic with fascioliasis. In addition, there is need to raise awareness among farmers regarding this disease to ensure that infections by parasites do not lead to significant economic losses.

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NICKEL (II) COMPLEXES OF AMINO ACIDS AND DITHIOCARBAMATE SALT AS POTENTIAL ALTERNATIVE TREATMENTS FOR BACTERIAL INFECTIONS

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ABSTRACT

There is need to develop alternative treatments for bacterial infections because most pathogens can develop resistance to antibiotics. In this study, nickel (II) bis (N-methylglycine) diaqua dihydrate (Ni-S), nickel (II) bis (glycine) diaqua dihydrate (Ni-G) and potassium N, N-bis (hydroxyethyl) dithiocarbamate (KL3) were screened for their potential antibacterial activity against 4 bacteria; *Streptococcus canis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli*. Disc diffusion method was used to determine the zone of inhibition (ZOI) and the broth microdilution to determine the minimum inhibitory concentration (MIC). The antimicrobial effect to these bacteria were further assessed by inoculation onto Mueller Hinton agar to determine the minimum bactericidal concentration (MBC). Ni-G and Ni-S at 0.1M produced ZOI towards *E. coli* growth while 0.01M and 0.05M KL3 towards *S. canis* and *S. aureus*, respectively. The MIC and MBC for *E. coli* were observed with 0.013M Ni-G and 0.025 Ni-S treatments, respectively. The study showed that Ni-G and Ni-S possess antibacterial effects towards *E. coli* but not *S. aureus, S. canis* or *P. aeruginosa*.

Keywords: Ni-G, Ni-S, KL3, disc diffusion method, MIC, MBC, antibacterial

INTRODUCTION

Almost all infectious agents develop multidrug resistance (Tanwar et al., 2014). There are now numerous researches to discover alternative compound for the treatment of bacterial infections. Among the potential antibacterial agents are metal compounds. Nickel (II) complexes are currently being studied for their potential antibacterial activities. According to Chohan (2000), nickel ions can penetrate microbial cells, inactivate their enzymes and eventually kill them. It was also shown that amino acids in complexion with metal ions, such as nickel, inhibit growth of bacteria (Chohan et al., 2006).

Dithiocarbamate, is a function group and analogue of carbamate, has strong chelating ability with metal ions. Dithiocarbamates in complex with metal ion as ligands was shown to produce larger zones of inhibition for *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* growth in culture than ligand-free dithiocarbamates (Yousif and Hasan, 2017).

The objectives of this study were to determine the effectiveness of water-soluble amino acid- and dithiocarbamate-nickel ion complexes as antibacterial agents.

MATERIALS AND METHOD

Preparation of molar concentration of Ni-G, Ni-S and KL3

The stock solution (0.1M) was prepared by diluting 86 mg of nickel (II) bis (N-methylglycine) diaqua dihydrate (Ni-S) and nickel (II) bis (glycine) diaqua-dihydrate (Ni-G) powder in 3 mL distilled water and 75 mg of potassium N, N-bis (hydroxyethyl) dithiocarbamate (KL3) powder in 3 mL distilled water. The stock solution was diluted with distilled water to give concentrations of 0.05, 0.01, 0.005, and 0.001 M.

Bacteria isolation

Field strain Gram-positive bacteria, *S. aureus* and *S. canis,* and gram-negative bacteria *P. aeruginosa* and *E. coli* were used in this study. Standard biochemical tests were performed to confirm the bacterial species. The bacteria were then sub-cultured on nutrient and blood agars and incubated at 37ºC for 24 h.

Inoculum suspension

Colonies of *S. aureus, S. canis, P. aeruginosa,* and *E. coli.* from pure culture was transferred aseptically to test tubes containing 3 to 4 mL sterile distilled water and mixed thoroughly. The turbidity of the inocula were compared against 0.5 McFarland standard. The final suspension contains 1.5×10^8 colony forming unit per mL (CFU/mL) of bacteria.

Disc diffusion method

S. aureus, P. aeruginosa, and *E. coli* inocula were spread uniformly using sterile swab over the entire Mueller Hinton agar while *S. canis* inoculum onto blood agar. Ten microlitres of 0.1, 0.05, 0.01, 0.005 M Ni-G suspension were dropped onto the empty discs using micropipettes. Sterile forceps was used to adhere the discs onto the agar. The culture plates were incubated at 37°C for 24 h. Amoxicillin-clavulanic acid (30 µL/mL) was used as a control. The process was repeated for all metal suspensions. To ensure reproducibility, the whole procedure was done in triplicates. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (ZOI).

Minimum inhibitory concentration and minimum bactericidal concentration

Only metal complexes produced zones of inhibition were used to determine minimum inhibitory concentration (MIC) for the respective bacteria. Eight 2-fold serial dilutions of the metal complexes were used in the study. Metal compound suspensions in nutrient broth were used as negative control and bacteria inocula in nutrient broth as positive control. Bacterial colonies from pure culture plates were inoculated into brain heart infusion broth (BHI) and incubated at 37ºC for 6 h. Between 10 to 20 uL of inoculum was added into nutrient broth and adjusted to 0.5 McFarland standard, which was then added to 10 mL nutrient broth to make a final bacteria concentration of 1×10^5 CFU/mL. Freshly prepared inoculum suspensions at concentration of 1×10^5 CFU/mL was added to the wells of a microplate. The microplate was sealed with aluminium foil and incubated overnight at 37 °C. The MIC was determined by comparing the turbidity with that of the control. The minimum bactericidal concentration (MBC) was determined by plating all non-turbid well with one turbid well on nutrient agar and incubating at 37ºC for 24 h. The MBC and MIC values were compared. MBC values 4-fold higher than MIC, indicates bactericidal effect.

RESULTS

The study showed that 0.1M nickel (II) bis (glycine) diaquadihydrate (Ni-G) and 0.1M nickel (II) bis (N-methylglycine) diaquadihydrate (Ni-S) produced zone of inhibition (ZOI) for *E. coli* growth. However, the compound did not produce apparent ZOI with *S. canis, S. aureus,* and *P. aeruginosa.* Potassium N, N-bis (hydroxyethyl) dithiocarbamate (KL3) produced ZOI for *S. canis* and *S. aureus* concentration of 0.1 and 0.05 M, respectively. There was no ZOI in the agar plate when *P. aeruginosa* and *E. coli* were used as the test bacteria.

The MIC was determined based on the turbidity of the well plate. However, because the colour of the solution was too dark blue, the turbidity cannot be visually differentiated. However, one loop of suspension from wells, including the positive and negative control were plated onto Mueller Hinton Agar (MHA). The MIC and MBC were then determined based on colony counts.

E. coli started to grow with treatment with 0.013M Ni-G and Ni-S producing colonies of 37×10^5 and 36×10^5 CFU/mL. At 1.56×10^{-3} M and higher, the colonies were too numerous to count (TNTC) with an estimated value of $> 200 \times 10^5$ CFU/mL. There was no *E. coli* growth at treatment concentrations of $\geq$0.025 M.

The KL3 compound at $\leq$ 0.025 M inhibited growth of *S. canis* and *S. aureus*. Both bacteria only began to grow at 0.013M KL3 reaching 43×10^5 CFU/mL of colonies for *S. aureus.*

The MBC was obtained with 0.025 M and MIC with 0.013 M of all metal compounds. The pHs for all metal complex solutions with and without nutrient broth were shown to be between pH 7 and 8.

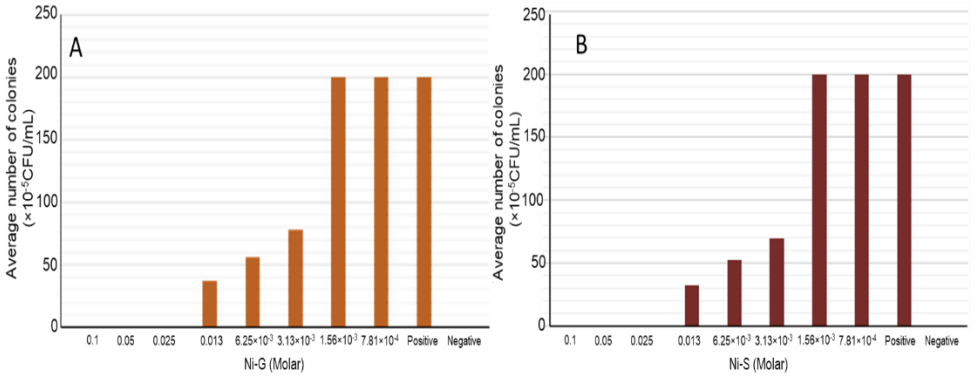

Figure 1. Effect of (A) nickel (II) bis (glycine) diaquadihydrate (Ni-G) and (B) nickel (II) bis (N-methylglycine) diaquadihydrate (Ni-S) on growth of *Escherichia coli*.

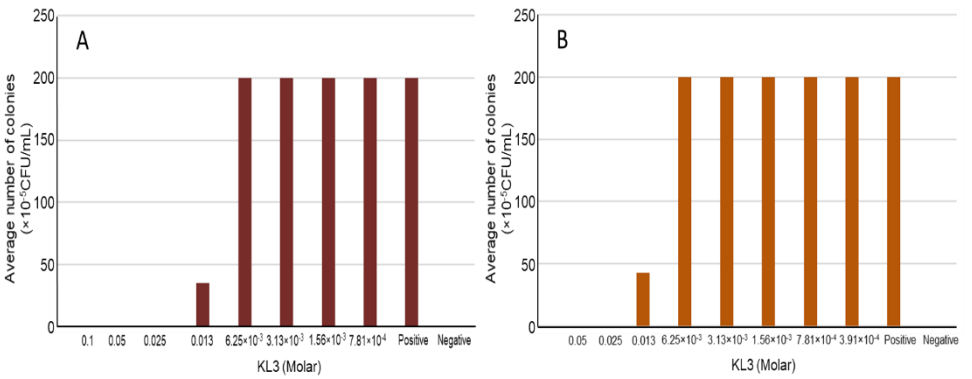

Figure 2. Effect of potassium N, N-bis (hydroxyethyl) dithiocarbamate on growth of (A) *Streptococcus canis* and (B) *Staphyloccocus aureaus*.

DISCUSSION

Complexing with ligands increases the liposolubility and decreases the polarity of metal ions through the sharing of positive charges. This also enhances the penetration of the complexes into the lipid membrane of the bacteria. Metal, including Co (II), Cu (II), Ni (II) and Zn (II) when complexed with amino acids showed antimicrobial properties (Chohan et al., 2006). It was shown than Zn (II) complexed with histidine had antimicrobial effects towards *E. coli* and *P. aeruginosa*.

The amino acid, glycine, is known to inhibit the cell wall synthesis of peptidoglycan and the effect varied between gram-positive and gram-negative presumable due to the differences in thickness of their cell walls. In our study, we showed that the Ni-G inhibited growth of *E. coli* only and not *P. aeruginosa.* Incidentally, *P. aeruginosa* is known to have developed resistance to antibiotics,

including aminoglycosides, quinolones, and β-lactams. Thus, it is imperative to develop alternative antimicrobial compounds, other than nickel complexes, for infections caused by this bacterium.

The study showed that KL3 inhibited growth of *S. canis* and *S. aureus* at concentrations beginning at 0.013M suggesting that it is a good antimicrobial agent.

CONCLUSION

The study showed that Ni-S and Ni-G had antimicrobial properties towards *E. coli* and KL3 towards *S. aureus* and *S. canis*. The MIC for all metal compounds was 0.013 M whereas the MBC was 0.025 M.

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DETECTION OF FUNGI IN CLAWS AND SALIVA OF COMMUNITY CATS IN SRI SERDANG, SELANGOR, MALAYSIA

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ABSTRACT

Fungi are generally classified as either mould or yeast. Some are known classic pathogens, whereas others are environmental saprobes. Cat claws and saliva are frequently contaminated with fungi especially those with access to outdoors. The aims of this project were to determine the presence of fungi in the claws and saliva of cats from 25 community cats caught in Sri Serdang, Selangor, Malaysia. All samples were inoculated onto Sabouraud dextrose agar (SDA) supplemented with cycloheximide and chloramphenicol. The species of the moulds were determined by macroscopic and microscopic examination, while the yeasts were identified using the API 20 C AUX test kit. A total of 20 different fungi were identified; all are known to cause infections in humans. The prevalence of cats harbouring fungi in their claws and saliva are 48 and 60%, respectively. Overall, the prevalence at least one species of fungus in their claws or/and saliva of cats in Sri Serdang is 80%. Thus, it can be concluded that a large proportion of community cats in Sri Serdang harbour at least one type of fungus in their claws and/or saliva. Thus, cautioned should be taken when handling community cats to minimise the risk of fungus infections.

Keywords: community cats, claws, fungi, moulds, Sabouraud dextrose agar, saliva, yeasts

INTRODUCTION

Domestic cats (*Felis catus*) have very long relationships with humans. Scientists believe that cats are the only animals that domesticated themselves because they prefer to live near humans and feed on the mice, rats, and other rodents. Community cats do not have owners and live outdoors.

Fungi are among the most widely distributed organisms on earth and are of great environmental and medical importance. The fungi kingdom comprises of a distinct group of eukaryotic organisms that absorb nourishment from living or dead organisms or organic matter. Fungi can be divided into three main groups; multicellular filamentous (moulds), unicellular (yeasts), and dimorphic fungi.

Dimorphic fungi are capable of changing their growth characteristics to either multicellular or unicellular (Boyce and Andrianopoulos, 2015).

Human to companion animal contact and animal bites are contributing factors to the transmission of zoonotic fungal diseases. To date, in Malaysia, there is lack of data regarding common fungus present in cat claws and saliva and whether humans can acquire fungal infections through scratches and bites. This study determined the presence of common fungus in the claws and saliva of community cats.

MATERIALS AND METHODS

A total of 25 community cats were captured through cluster sampling from several areas in Sri Serdang, Selangor, Malaysia. The cats were captured and restrained manually for sampling. The project was approved by the Institutional Animal Care Unit Committee (IACUC), Universiti Putra Malayia (Reference number: UPM/IACUC/AUP-U020/2019).

Sample collection

Saliva samples were taken from each cat by swabbing the buccal cavity on both sides of the mouth and placed in Amies transport medium. The claw samples were collected from both forelimbs using a nail clipper that was cleaned and disinfected with 70% alcohol solution before each use. The claw samples were transported in separate paper envelope for each cat.

Sample processing

Isolation and identification of fungi were conducted at the Bacteriology Laboratory, Veterinary Laboratory Services Unit, Universiti Putra Malaysia. The buccal swab samples were directly cultured on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and cycloheximide. The nail samples were inoculated into the SDA broth for 4 to 5 days for fungal enrichment before culturing onto SDA supplemented with chloramphenicol and cycloheximide. The cultured SDA medium were incubated at 25°C for 4 weeks. Moulds were stained with lactophenol cotton blue and the species determined by macroscopic examination of the colonies. The identification of yeast was done with gram-staining and microscopic examination. The yeast species were determined using API 20 C AUX test kit.

Data analysis

The fungus was classified into moulds and yeasts. The number of samples positive for specific fungi was counted and was used to determine their prevalence. Chi-square test was also applied to determine statistical association between saliva and the nails samples at α=0.05.

RESULTS AND DISCUSSION

A total of 20 fungi species were isolated from the claws and salivas of community cats. Surprisingly, the fungi isolated form nails were different from that from saliva. Forty eight percent and 60% of community cats harboured at least one type of fungus in their nails and saliva, respectively. There was no significant association ($p>0.05$) the presence of fungus between nail and saliva samples.

All 20 isolates were fungus that can cause diseases or infections in humans. Fifty five percent of isolates were moulds and 45% yeasts. Moulds are 3 types; dermatophyte and superficial fungi, fungi causing subcutaneous mycoses, and opportunistic fungi. Our findings revealed that 55% were the opportunistic fungi, 36% were those capable of causing subcutaneous mycoses and 9% were fungi capable of causing superficial mycoses.

The natural behaviours of cats are to bury faeces in the soil and sharpen their claws on wood. Thus, it is highly likely that cats may have acquired the fungi from the environment. This is particularly true for community cats because they roam freely and like all cats, groom regularly. Thus, these cats could have taken up the fungus on their paws, furs, and in saliva. Consumption of fungus-contaminated food may be another means of cat acquiring the organisms.

CONCLUSION

Most community cats in Sri Serdang, Selangor harboured at least one species of fungi in their claws or saliva. Therefore, caution should be exercised when handling these cats to avoid from acquiring pathogenic and opportunistic fungal infections.

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SEROLOGICAL DETECTION OF *LEPTOSPIRA* SPP. IN WILD RATS FROM *PASAR BORONG*, SELANGOR, MALAYSIA

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ABSTRACT

Leptospirosis is one of the zoonotic diseases that causes a wide range of manifestations in human and animals. In Malaysia, 12,325 cases of leptospirosis in human with 338 deaths were reported from 2004 to 2012. Wild rat was reported to be the most important sources of *Leptospira* infection in humans and animals. This study aimed to determine the occurrence of *Leptospira* spp.infection and to identify the infective *Leptospira* serovar in wild rats from *Pasar Borong*, Selangor, Malaysia. Thirty-two rats were trapped and blood was taken for serological detection of *Leptospira* spp. infection by using microscopic agglutination test. Twenty live antigens of *Leptospira* spp. comprised of serovar Hardjobovis, Hebdomadis, Celledoni, Malaysia, Pomona, Tarassovi, Pyrogenes, Australis, Gruppothyposa, Cynopteri, Canicola, Lai, Icterohaemorrhagiae, Bataviae, Javanica, Autumnalis, Ballum, Djasiman, Patoc and Copenhageni were used in this study. Antibody against *Leptospira* spp. was detected in 12.5% (4/32) of rat serum samples, which are comprised of serovar Bataviae (6.25%), Javanica (3.13%) and Copenhageni (3.13%). Rats from the *Pasar Borong* were seropositive for *Leptospira* spp. and can be a source of *Leptospira* infections in humans and animals.

Keywords: rats, *Leptospira* spp, MAT, *Pasar Borong* Selangor Malaysia

INTRODUCTION

Leptospirosis, caused by pathogenic *Leptospira* spp., is a zoonotic disease transmitted from animals to humans causing significant public health issues worldwide. The disease is responsible for an estimated 500,000 human cases occurring annually, with fatality as high as 70%. Humans usually get infected by this bacterium through direct contact with the infected animal urine, via mucous membrane and exposed skin or by indirect contact through exposure to the contaminated soil, water, and food (Haake and Levett, 2015). In humans, the disease has variable onset, ranging from 1 day to 4 weeks after exposure, and in survivors, the infection can last months. Malaysia, with its hot and humid climate and frequent rainfall, is favourable for the survival and the transmission of the infection. Rain

tends to clean up rat holes causing *Leptospira* to emerge in surface soil and water bodies.

Rodents, including rats and mice, are an asymptomatic reservoir, but infections in other animals can triggered a broad variety of manifestations including abortion, acute kidney injury, liver failure, loss of milk production, pulmonary haemorrhage syndrome, uveitis, and weight loss (Mori et al. 2017). Malaysia has a high population of rats and the infestation is compounded by unsanitary and indiscriminate trash disposal from homes, eateries, and wet markets. Markets are especially suitable for rodent infestation because of the humid environment and abundance of food.

Rats carry various pathogenic serovars of *Leptospir*a spp.that can cause disease in humans and other animals. The *Leptospira* serovars in rats are of the highly pathogenic strains, Bataviae and Javanica. The objectives of the present study were to determine the seroprevalence of *Leptospira* spp.infection and identify the infective *Leptospira* spp.serovar in wild rats.

MATERIALS AND METHODS

Rat trap cages were placed overnight at various spots around *Pasar Borong*, Selangor, Malaysia. A total of 32 rats were trapped and brought to the post-mortem laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The rats were anesthetised with diethyl ether and blood collected via intracardiac puncture and transferred to plain tubes. Clotted blood was centrifuge at 250 × *g* for 10 min and serum collected into 1.5-mL micro centrifuge tubes. The study was approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-U017/2019).

Twenty live antigens of the *Hardjobovis*, *Hebdomadis*, *Celledoni, Malaysia, Pomona, Tarassovi, Pyrogenes, Australis, Gruppothyposa, Cynopteri, Canocola, Lai, Icterohaemorrhagie, Bataviae, Javanica, Autumnalis, Ballum, Djasiman, Patoc* and *Copenhageni* serovars were prepared. Antigen preparation and microscopic agglutination test on the serum samples followed the protocol described in the World Animal Health Organization (OIE) Terrestrial Manual 2014 for Leptospirosis (OIE, 2008). The antigen and serum were mixed in the wells and examined under dark field microscope to observe for agglutination. Serum was considered positive when approximately 50% or more of the leptospires were agglutinated and show a cut-off titer ≥1:100.

RESULTS AND DISCUSSION

The result showed that 12.5% (4/32) of rats from *Pasar Borong* were seropositive for *Leptospira* spp. Antibody to *Leptospira* serovar Bataviae was detected in 6.25% (2/32) of the sera with a titer of 1:200. *Leptospira* serovar Javanica was detected in 3.13% (1/32) of sera with a titer 1:400 whilst *Leptospira* serovar Copenhageni was detected in 3.13% (1/32) in of sera with titers of 1:100 and 1:200.

Leptospira survives well in wet and moist conditions of environments with stagnant water and wet soils. Rats thrives in environments where there is abundance of either raw or dry foods. These environmental conditions typify the conditions of wet markets like *Pasar Borong*, Selangor. Thus, the rats of the *Pasar Borong* are infected and carry bacteria that can potentially be transmit to humans and animals.

CONCLUSION

This study showed that rats from *Pasar Borong* Selangor, Malaysia are seropositive for *Leptospira* spp., especially of the serovars Javanica, Conphengani and Bataviae. The infected or carrier rats from the *Pasar Borong* can be a source of infections to other animals and human through indirect contact with rat urine-contaminated environments. This study shows that the public should aware of the risk of contracting leptospirosis from rats.

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FACTORS INFLUENCING ANTIMICROBIAL USAGE AMONG SMALL ANIMAL PRACTITIONERS IN KLANG VALLEY, MALAYSIA

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ABSTRACT

Information regarding the usage of antimicrobials in small animal practices is limited. Hence, the purpose of this study was to identify the factors driving choice of antimicrobial usage among small animal practitioners, to analyse source of information in determining antimicrobial agents, and to evaluate perceptions and concerns regarding antimicrobial resistance (AMR). A questionnaire survey was conducted on 51 small animal veterinarians in Klang Valley, Malaysia. Among 51 respondents, convenience and cultural reasons were the most and least important factor, respectively, influencing their antimicrobial prescription. Textbooks, drug handbooks, and peer-reviewed scientific literature were the most important sources of information for the respondents. The study also showed that small animal veterinary practitioners with more than 5 years of experience have the same level of concern about AMR infections as those with less than 5 years of experience. There is need to improve awareness on judicious antimicrobial usage among small animal practitioners.

Keywords: antimicrobial, antimicrobial resistance, small animal, survey, questionnaire

INTRODUCTION

In 2017, the Center for Disease Control and Prevention defined antimicrobial resistance (AMR) as the ability of microorganisms to defeat the drugs that were designed to kill them. Antimicrobial resistance is a threat to public health. The development of AMR will cause major health crisis as common infections such as pneumonia, tuberculosis, and food poisoning may be difficult to treat.

The frequency of antiobiotic-resistant bacterial infection in companion animal veterinarians are on the increase (Frey, 2018). It was shown sick pets contribute to clinically relevant antibiotic-resistant bacteria and play a role its spread (Hartantyo et al., 2018). In addition, humans may acquire antimicrobial-resistant bacteria via physical contact with their pets (Guardabassi et al., 2004; Pomba et al., 2016).

This study aimed to identify factors influencing choice of antimicrobials and the source of information used by small animal practitioners on antimicrobials. The study also evaluated their perceptions and concerns regarding AMR.

MATERIALS AND METHODS

The respondents were certified small animal practitioners. Convenient sampling was done where all veterinary clinics or hospitals in 7 districts of Klang Valley, Malaysia, listed under Malaysian Small Animal Veterinary Association (MSAVA) are contacted to respond to the questionnaire. Twenty-seven clinics were approached from 5th to 30th of August 2019 through face-to-face meeting in practice settings, while another 21 clinics received the questionnaire via email. Demographic information such as age, gender, area of practice, year of graduation, experience in clinical practice, and the factors that influenced their antimicrobial prescriptions were obtained.

Survey questionnaire

The survey questionnaire was developed based on previous studies (De Briyne, (2013; Ekakoro and Okafor, 2017). The questionnaire comprised of 30 questions in four sections; demographic data, factors influencing antimicrobial usage, source of information regarding antimicrobials, and antimicrobial awareness. The Likert scale was used and the data analysed using the Statistical Package for the Social Sciences (SPSS) software program for Windows version 2.2 (SPSS, Chicago, IL, USA). The Mann-Whitney U test was applied to determine the differences in level of concern on AMR, between small animal practitioners with less than five years of experience and those with more than five years of experience. Demographic data collected include age, gender, town/area of practice, year of graduation, experience, country where veterinary degree was obtained (Malaysian or non-Malaysian) and specialty board certification. The study was approved by the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia.

RESULTS AND DISCUSSION

Overall, 54% of active registered clinics in Klang Valley participated in the survey. The study showed the small animal practitioners used textbooks or drug handbooks and peer-reviewed scientific literature as the most important sources on antimicrobials and their uses.

Perceptions and concern regarding antimicrobial resistance

The survey showed that 60.8% (31/51) small animal practitioners were aware of the guidelines of veterinary antimicrobial usage, 7.8% (4/51) were not aware, and 31.4% (16/51) unsure.

Table 1: Respondents scores for the factors influencing antimicrobial prescribing behaviour.

Factor	Element	Score
Responsible use	Risk of antimicrobial resistance	3.51 ± 0.674
	Antimicrobial sensitivity test	3.55 ± 0.702
	Legal restriction	3.33 ± 0.887
	Product usage label	3.53 ± 0.703
Convenience	Ease of administration	3.49 ± 0.644
	Drug availability	3.71 ± 0.502
Culture	Practice policy	3.39 ± 0.750
	Owner demand	1.51 ± 1.206
	Advertisement	1.33 ± 1.211
	Societal influence	1.59 ± 1.117
Economics	Price	2.73 ± 0.827
	Duration of prescription	3.37 ± 0.662
	Profit margin	1.84 ± 1.027
	Marketing offers	1.39 ± 1.150

Values are mean ± standard deviation (n=51).
Score scale: from 0 (not important) to 4 (most important).

Table 2: Respondent score for source on information on antimicrobials

Source of information	Score
Peer-reviewed scientific literature	3.27 ± 0.750
Peers or clinicians within clinic	3.08 ± 0.778
Peers or clinicians outside clinic	2.86 ± 0.800
Textbooks or drug handbooks	3.51 ± 0.674
Pharmaceutical company representatives	2.25 ± 1.129
Veterinary information network (VIN)	3.12 ± 0.799
Online	2.67 ± 0.931

Values are mean ± standard deviation (n=51).
Score scale: from 0 (not important) to 4 (most important).

Only 58.9% (30/51) of small animal practitioners in Klang Valley either always or often refer to the guidelines on antimicrobial usage and prescription. Unfortunately, 3.8% (2/51) had never used the guidelines when prescribing antibiotics (Figure 1).

Most respondents (96.1%;49/51), irrespective of duration of practice experience, were either quite or very concerned about AMR infections

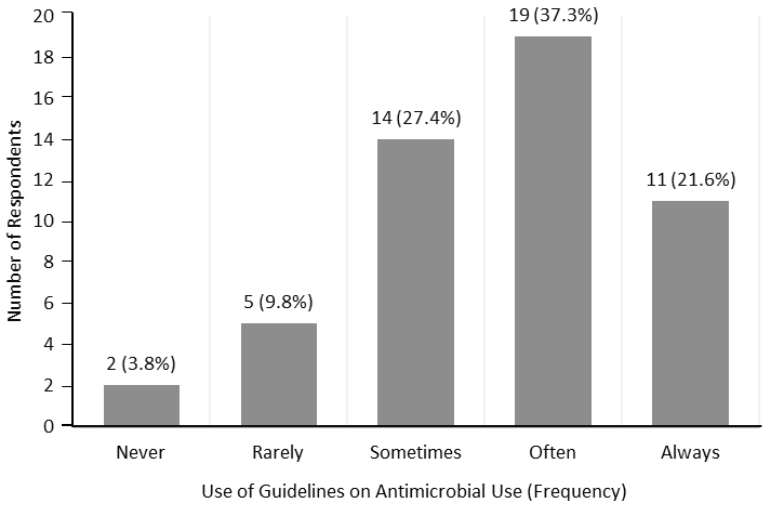

Figure 1: Frequency of using the guidelines on veterinary antimicrobial use among respondents.

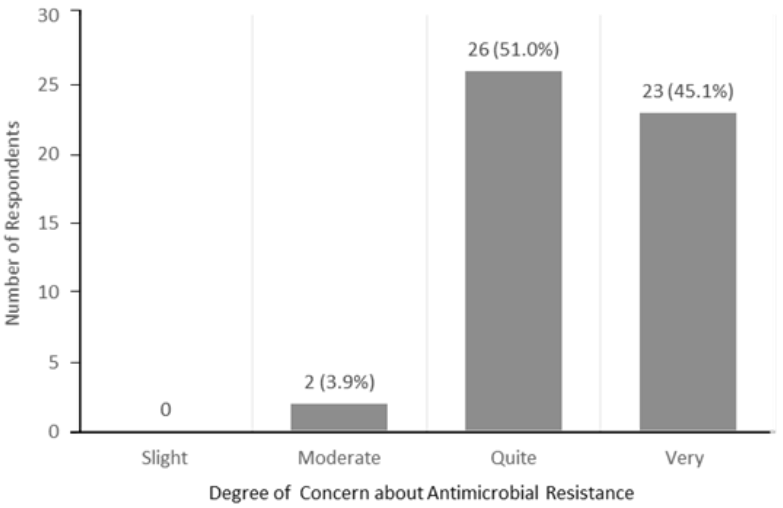

Figure 2: Degree of concern of respondents on development of antimicrobial resistance infections

Among small animal practitioners in Klang Valley, 86.3% (44/51) indicated that the cost and turnaround time of diagnostic tests as the main obstacles to the implementation of the antimicrobial stewardship plan (Table 3).

Table 3: Factors posing challenge to implementation antimicrobial stewardship plan by respondents.

Factors	Respondent (%) (n=51)
Cost of diagnostics (i.e, bacterial culture or sensitivity tests)	86.3
Time to results of diagnostics (i.e, bacterial culture or sensitivity tests)	70.6
Practice culture	64.7
Client expectations of receiving antibiotics	62.7
Time required for adequate client education	52.9
There are no barriers to antimicrobial stewardship plans in small animal practice	11.8
Antimicrobial stewardship plans are not needed in small animal practice	0.0

CONCLUSION

Convenience were the main factors influencing small animal practitioners in Klang Valley when prescribing antimicrobials. Textbooks, drug handbooks, and peer-reviewed scientific literature were their most important sources of information on antimicrobials. All small animal practitioners, irrespective of duration of practice experience have the same concern AMR infections.

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COMPOSITION OF MILK FROM FRIESIAN-JERSEY AND FRIESIAN-SAHIWAL CROSSBRED CATTLE IN KENINGAU, SABAH, MALAYSIA

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ABSTRACT

Milk is an important source of nutrition for humans and animals. However, the nutrient content of milk varies with breed of cattle. Milk composition is influenced by many factors including dietary intake, environment, management system, health status, genetics, breed, stage of lactation, and age of cattle. Currently, the milk composition of Friesian-Jersey and Friesian-Sahiwal bred in Malaysia is not known. Hence, this study determined and compared the milk composition of Friesian-Jersey and Friesian-Sahiwal crossbred cattle in a commercial farm in Keningau, Sabah, Malaysia. The milk fat, protein, lactose, total solids and non-solid fat compositions were determined using the Master Pro Milk Analyzer calibrated for cows' milk. The results showed that the fat, total solid and non-solid fat contents were significantly ($p<0.05$) higher while protein and lactose were significantly ($p<0.05$) lower in Friesian-Jersey than Friesian-Sahiwal milk. Thus, it can be concluded that milk produced by Friesian-Jersey is of better quality than that produce by Friesian-Sahiwal cows.

Keywords: milk composition, Friesian-Jersey, Friesian-Sahiwal, cattle

INTRODUCTION

Developed countries provided most of the world supply of milk. In these countries milk one of the main sources of nutrition and sustenance for humans. The reproductive and milk production traits of cows, sheep and goats are determined by factors such as genetics, age, lactation stage, parity and management, including the method of milking (El-Tarabany et al., 2018).

There are differences in the milk composition among cows. Genetics is one of the factors that determine whether one breed produces milk with high protein content while another rich in fat (MGI). An earlier study showed that the milk of Jersey had significantly higher fat and protein contents than that of the Holstein cows (White et al, 2001).

Currently, there is limited information on nutrient composition of cattle milk in Malaysia. Thus, this study determined the milk composition of Friesian-Jersey and Friesian-Sahiwal crossbred cows in Sabah.

MATERIALS AND METHODS

Farm and animals

This study was conducted in August 2019 at a commercial dairy farm in Keningau, Sabah, Malaysia. This farm had a population of approximately 5000 Friesian-Sahiwal, Friesian-Jersey and Holstein-Sahiwal crossbred cattle. Forty-four Friesian-Sahiwal and 22 Friesian-Jersey, both 6.5% cross, were chosen to determine and compare their milk compositions. The Friesian-Jersey and the Friesian-Sahiwal cows aged between 2 to 5 years were at second and 2 to 9 years at fourth stage of lactation, respectively.

Milk collection

Milk collection was done in the milking parlour at 1600 to 2000 h. The udders and teats were cleaned using clean water to remove dirt. Milk was stripped and first few streams of milk discarded. Approximately 15 mL milk samples were collected from all quarter into falcon tubes and stored chilled at 3ºC until used.

Milk analysis

Master Pro Milk Analyzer (Milkotester, Bulgaria) was used to determine the fat, protein, lactose, solid-not-fat (SNF), total solid, density, freezing point, salt and water content in the milk samples according to manufacturer's recommendation.

Data analysis

The data were organised and analysed in Statistics SPSS IBM® version 25. Independent sample T-test was used to compare the mean of milk composition between two independent groups.

RESULTS AND DISCUSSION

The milk yield and compositions are shown in Table 1. The mean milk yield, fat, solid non-fat, and total solid contents were significantly ($p<0.05$) higher and the protein and lactose contents significantly lower ($p<0.05$) in Friesian-Jersey than Friesian-Sahiwal cows.

There were breed differences in milk composition of cows. One study showed SNF to fat ratios were highest for Ayrshire followed by Jersey, Guernsey, Brown Swiss, and Holstein. The Jersey and Guernsey cows' milk have among the highest percentages of total protein, casein, and whey among cattle (Rolleri et al., 1956).

The Friesian-Jersey cattle although a temperate breed still perform well in the tropical country like Malaysia, producing good quality of milk. The performance of

the dairy cattle is dependent on complete nutrient intake, good farm management and environment, complete, and animal welfare. The farms in this study practices good management and husbandry system and the animals were healthy.

Table 1. Mean values for milk yield and compositions between two different breeds.

Milk parameter	Breed	
	Friesian-Jersey	Friesian-Sahiwal
Yield (L/cow)	$19.65^{a} \pm 1.14$	$19.08^{b} \pm 0.53$
Fat (%)	$3.89^{a} \pm 0.11$	$3.51^{b} \pm 0.06$
Solid non-fat (%)	$9.50^{a} \pm 0.24$	$8.61^{b} \pm 0.14$
Total solid (%)	$13.39^{a} \pm 0.34$	$12.12^{b} \pm 0.20$
Protein (%)	$2.96^{a} \pm 0.19$	$3.62^{b} \pm 0.08$
Lactose (%)	$3.47^{a} \pm 0.42$	$5.15^{b} \pm 0.15$

Value are mean ± standard deviation.
[a,b]Means within row with different superscripts are significantly different at $p<0.05$.

CONCLUSION

The study showed the quality of milk from Friesian-Jersey was better than that from Friesian-Sahiwal cows.

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OCCURRENCE OF TUBERCULOSIS IN WILD BOAR POPULATION IN SELECTED AREAS IN SELANGOR, MALAYSIA

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ABSTRACT

Tuberculosis is a zoonotic disease that has serious economic impact on livestock industry and poses conservation problems among wildlife and major public health issues. Wild boar in is known to be a true reservoir for *Mycobacterium tuberculosis* complex (MTC) causing tuberculosis in other animals. To date, there were no study on wildlife tuberculosis in Malaysia. Therefore, this study was aimed to determine the occurrence of tuberculosis in wild boars in selected area in Selangor, Malaysia. Serum and tissue samples were collected from 30 wild boar carcasses. The organs were examined grossly for tuberculosis-like lesion (TBLL), and serum samples were subjected to antibody determination using commercial IDEXX *M. bovis* Ab test ELISA and in-house purified derivatives (bPPD) ELISA. The Ziehl-Neelsen staining and PCR methods were used for antigen detection in tissues. Gross pathology showed that the proportion of samples with TBLL was 30% (9/30). The proportion of seropositive samples using the in-house bPPD ELISA was 16.7% (5/30). PCR detected DNA of MTC in 9 out of 12 (75%) wild boar lymphoid organs or organs with TBLL only. DNA-sequenced positive samples showed 100% identity with *M. tuberculosis* variant *bovis* strain 1. In conclusion, this preliminary study reports the occurrence of MTC in the wild boar population in Selangor. There is need for further examination to substantiate the findings.

Keywords: *Mycobacterium tuberculosis* complex, tuberculosis, detection, wild boar, wildlife, Selangor

INTRODUCTION

Bovine tuberculosis (TB) is an important re-emerging zoonotic disease caused by *Mycobacterium bovis*. It is one of the most important disease in animals with huge economic impact on the livestock industry. To control the infection, some countries, including Australia and Canada implemented successful eradication programmes for

their cattle (de la Rua-Domenech et al., 2006). Similar test and slaughter programs in other countries had also shown success in reducing of the incidence of bovine TB. However, the disease still persists with sporadic outbreaks. These outbreaks is suspected to be associated with the presence of wildlife that acts as a reservoir host for *M. bovis* (Kovalev, 1980).

In Australia, feral and domestic swine are known as dead-end hosts for *M. bovis* infection (McInerney et al., 1995). However, from epidemiological, pathological and microbiological evidences, in Mediterranean Spain, the Eurasian wild boar was identified as the natural reservoir for the infection (Naranjo et al., 2008). Currently, it is not clear how wildlife in the Southeast Asian (Che-Amat and Ong, 2018).

This study examined evidences of tuberculosis in wild boar carcasses in Malaysia.

MATERIALS AND METHOD

Animal sampling

With the official permit (B-00156-15-19) from PERHILITAN Selangor, 30 hunter-harvested wild boars were sampled in Selangor from May to August 2019. Necropsies for 20 of the wild boars were performed in the field. Blood and submandibular, tracheobronchial, mediastinal, and mesenteric lymph nodes, and tonsil, lung, spleen, liver, and kidney tissue samples were collected from each of 20 wild boars. Tissue samples from carcass were obtained within 1 to 3 h of death. Organ samples from 10 wild boars were submitted to the laboratory by the hunters within 3 to 20 h of hunt. Blood that leaked from organs were collected for analyses.

Gross pathology

Necropsy was performed on site on 20 animals. Gross pathology examination for tissue parts collected was done in the Post-mortem Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Selected tissue was sectioned serially to 3 to 5 mm thickness for histopathological examination.

Ziehl-Neelsen staining

Contact smear on clean glass slides was made from all tissue samples and stained with using the Ziehl-Neelsen method. The slides were examined for acid fast bacilli.

Serology

Serum samples were subjected to in-house ELISA test that adopted from Boadella et al. (2011) and Che-Amat (2015) using an ELISA plate reader at wavelength 450nm. Since there is no control available for the test, the cut-off was determined by plotting a vertical graph of the net-OD. Positive value (OD>X) was chosen from the most evident jump in OD.

Polymerase chain reaction

DNA extraction from the tissue samples was done using the QIAGEN® DNeasy blood and tissue kits. Initially the PCR analysis was done for the pooled organ extracts and lymphoid organs only. The primer 16Myc pair was used for the detection of *Mycobacterium* sp. and TB1-F and TB1-R for *Mycobacterium* sp. in the Mycobacterium Tuberculosis Complex (MTC).

DNA Sequencing

The amplified fragments of DNA products from positive lymphoid tissues or organs with tuberculosis-like lesion (TBLL) were sent for sequencing (FIRST BASE laboratories, Malaysia). The sequence was analysed using NCBI GenBank.

RESULTS AND DISCUSSION

Gross pathology of selected tissue samples showed that 30% (9/30) of the wild boars were positive for TBLL. No acid-fast bacilli were detected by Ziehl-Neelsen method. The in-house ELISA detected 16.67% (5/30) seropositive individuals.

Polymerase chain reaction analysis of pooled organ samples were negative. However, among 12 lymphoid tissues or tissues with TBLL analysed, 75% (9/12) were positive with PCR. The sequenced PCR product was subject to the BLAST analysis tool by the NCBI GenBank. The organism showed an identity index of 100% with *M. tuberculosis* variant *bovis* strain 1 and query coverage of 99%.

CONCLUSION

The result of this preliminary study showed that the wild boar populations in selected areas in Selangor are positive for *M. bovis*.

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A SURVEY ON *SALMONELLA* SPP. CARRIAGE ON FOOTPADS AND RECTUM OF OWNED AND STRAY CATS

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ABSTRACT

Salmonellosis in cats usually manifests as latent infection with raw feed diet implicated as an important risk factor. This study determined the occurrence of *Salmonella* spp. in the rectum and on the footpads of owned and stray. Rectal and footpad digital, metacarpal, and metatarsal swabs were obtained from 30 owned and 30 stray cats by convenience sampling. The swabs were subjected to xylose lysine deoxycholate (XLD) agar and Brilliant green agar (BGA) to isolate presumptive *Salmonella* colonies and biochemical and polyvalent *Salmonella* antisera test for bacteria identification. Eight *Salmonella* isolates were obtained from 7 cats, of which 4 were owned cats. Three of the owned cats were managed fully indoors and 1 semi-roamer. The majority of isolates were from the rear footpads (n=5), followed by rectum (n=2) and 1 was from the forelimb footpads. One stray cat caught near a wet market had *Salmonella* both in the rectum and on left hindlimb footpads. Thus, cat can potentially spread salmonellosis to humans. Pet owners should be educated on the importance of maintaining good personal hygiene, responsible pet ownership, and safe interaction with cats to prevent transmission of zoonotic diseases like salmonellosis.

Keywords: *Salmonella* spp., risk factors, stray, pet cats, public health

INTRODUCTION

Salmonella is a gram-negative, lactose fermenter, facultative anaerobic bacteria that possess rod or coccobacillus shape. This organism can survive in the environment for a few weeks and in water for several months (WHO, 2018). Typhoidal *Salmonella* is a human-specific pathogen. Non-typhoidal *Salmonella* has a broader range of hosts and can potentially be zoonotic. *Salmonella enterica* ser. Enteriditis and *S. enterica* ser. Typhimurium are of public health concerns worldwide and animals can serve as their reservoir and potentially transmit the disease to humans.

Companion animals such as cats can have latent infections and intermittently shed the organism (Demirbilek, 2017). Raw feed diet is getting more popular for pet owners because it is claimed to be nutritious to cats. In fact, raw feed is one of the main dietary factors in the development of salmonellosis in cats. Raw feed is often

contaminated with enterobacteriaceae, including *Salmonella* (Fredriksson-Ahomaa et al., 2017). Cats can be infected if they come in contact with carrier animals and faeces, and consume faeces-contaminated food and water (Demirbilek, 2017).

It is a natural behaviour for cats to bury their faeces. This habit increases the chance their foodpads being contaminated with *Salmonella* spp. The cats also frequently clean themselves by licking their paws, genital organs, and anus. Thus, humans in contact with cats are at risk of acquiring the infection.

This study was conducted to determine the presence of *Salmonella* spp. in the rectum and footpads of owned and stray cats.

MATERIALS AND METHODS

Footpad and rectal swab collection

Sixty cats comprising of 30 owned and 30 stray cats from Bangi, Kajang, Serdang, and Shah Alam, Selangor, Malaysia were recruited in the study based on convenience sampling. Stray cats were captured at the markets and eateries. Samples were collected from the rectum and digital, metacarpal and metatarsal footpads using sterile swabs moistened with sterile distilled water. Personal protective equipment was used for the procedure.

Bacteria isolation and identification

The swabs were placed in 10 mL buffered peptone water as pre-enrichment broth for *Salmonella* spp.and incubated at 37°C for 18 to 24 h. Then, 1 mL sample was transferred in 9 mL Rappaport-Vassiliadis broth as enrichment and incubated overnight at 42°C. A loopful of the overnight suspension was spread on Brilliant Green agar (BGA) and xylose lysine deoxycholate agar (XLD) and incubated overnight at 37°C. Presumptive colonies of *Salmonella* spp. on XLD were red colonies with black centers while on BGA the suspected colonies were pink surrounded by red zones. The presumptive colonies were then isolated in nutrient agar and incubated overnight at 37°C to produce pure cultures, which were subjected to gram-staining. Gram-negative coccobacillus or rod bacteria were then subjected to citrate, triple sugar iron (TSI), urease, and oxidase tests. For the TSI agar, the presence of *Salmonella* spp. was indicated by the acid butt, alkaline slant, and positivity for gas and hydrogen sulphide. The polyvalent O and H serotyping tests were used to determine *Salmonella* spp. phenotype.

Questionnaire survey

A questionnaire, comprised of 13 close- and open-ended questions was distributed to participating cat owners (n=30) to obtain information on management, behavior, and lifestyle of their pet cats.

Statistics

All data collected was tabulated in Microsoft Excel® to generate descriptive statistics on the isolates and occurrence of *Salmonella* spp. in the cat samples.

RESULTS AND DISCUSSION

Seven of 60 cats (11.66%), 4 owned and 3 strays were positive for *Salmonella* spp. isolates. Three *Salmonella* spp.-positive cats were managed fully indoors and 1 was a semi-roamer. Among fully indoor cats, the right forelimb, left hindlimb and rectum were positive for *Salmonella* spp. The same was true for the right hindlimb in the semi-roamers. The Salmonella spp.-positive stray cats were from the wet markets. *Salmonella* spp. was found on both the left hindlimb and rectum of one cat and on the left hindlimb only of two others.

DISCUSSION

Cats habitually scavenge rubbish and hunt birds and rodents. Birds and rodents may be carriers for the *Salmonella* spp. Cockroaches too were shown to harbour Enterobacteriaceae including *Salmonella* (Wahab et al., 2015). It is through contact with these animals and pests that cats, whether managed indoors or allowed to roam can became positive for the bacteria.

The faeces of apparently healthy animals may also harbour *Salmonella* spp. This is due to the fact that *Salmonella* resides in the intestinal tract and are shed intermittently in the faeces (Demirbilek, 2017). Stray and semi-roamer cats can acquire the bacteria from direct contact with faeces of *Salmonella*-positive animals.

CONCLUSION

Overall, 7 cats were positive for the *Salmonella* spp. Among these, 3 were stray cats, 1 semi-roamer and 3 fully indoor cats. The carriage rate of *Salmonella* in these cats may be influenced by their lifestyle such as access to the outdoor environment, presence of house geckos and healthy carriers and consumption of food waste. This is a first report on isolation and identification of *Salmonella* from the footpads of the cats in Malaysia.

Veterinarians can play an important role in educating pet owners on the importance of maintaining good personal hygiene, responsible pet ownership and safe interaction with cats to prevent transmission of zoonotic diseases, like salmonellosis.

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DISEASE INVESTIGATION IN CAGE CULTURED ASIAN SEABASS (*LATES CALCARIFER*) IN MARANG, TERENGGANU, MALAYSIA

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ABSTRACT

Asian seabass is a hardy, euryhaline fish species suitable for mariculture. In aquaculture, infectious and non-infectious diseases remain the cause significant financial losses to fish farmers. The main objective of this study was to determine the cause of fish mortality in a floating net-cage Asian seabass farm in Marang, Terengganu, Malaysia. In this study, ten moribund fish of various culture ages and sizes were observed for clinical signs. The fish were examined for parasites in skin scrapings, gills, and intestine. Swabs of the skin, gills, kidney, intestine, and trash fish used as feed were streaked on non-selective and selective agars for bacterial isolation and identification and biochemical tests. All ten fish exhibited abnormal swimming behaviour and marked skin lesions. The bacteria successfully isolated and characterised include *Streptococcus porcinus, Lactococcus raffinolactis, Oerskovia sp., Vibrio alginolyticus* and *Serratia marcescens* were isolated from the swabs. The presence of bacteria in cultured fish suggests potential anthropozoonosis.

Keywords: Asian seabass, cage-cultured, fish mortality, bacteria, anthropozoonosis

INTRODUCTION

Aquaculture in Malaysia has grown rapidly, providing food security, job opportunities, and export revenues. In Malaysia, there was an increase aquaculture production from 79,699 metric tonnes in 1995 to 525,000 metric tonnes in 2012 and decreasing again to 427,015 metric tonnes in 2015. Brackish water aquaculture is practiced in Malaysia, contributing 139,129.51 metric tonnes in 2012 (Yusoff, 2015).

During the last 20 years, cage aquaculture had gain popularity in Malaysia, filling cage cultures at sheltered coastlines and estuaries with Asian seabasses (*Lates calcarifer*), snappers (*Lutjanus johni*), and groupers (*Epinephelus taurirna*). In fact, the Asian seabass, because of its hardy and euryhaline nature makes it a most common cultured marine species (Yusoff, 2015).

Malaysia had suffered a USD1.3 million loss due to fish disease outbreak affecting cage-cultured groupers, snappers, and seabasses in 1989. The outbreak of vibriosis 1990 resulted in severe Asian seabass mortality causing the industry in Malaysia to loss another USD7.4 million (Lee and Wee, 2014). Due to the significant economic losses as a result of disease outbreaks, a study was conducted at an aquaculture farm in Marang, Terengganu, Malaysia to create awareness on fish diseases among researchers and farmers and formulate preventive measures for disease outbreaks in aquaculture.

The objectives of this study were to record clinical signs associated with infected fish, isolate, and identify infectious agents in fishes.

MATERIAL AND METHODS

History and clinical signs

The study was conducted at a seabass farm in Marang, Terengganu, Malaysia. Management data from the farm and 2 adjacent seabass farms were obtained through one-on-one interviews with owners of aquaculture facilities. Ten moribund fish were selected from the sampling farm and clinical signs of diseases recorded.

Ectoparasite examination

Ectoparasite examination was done on skin scrapings and gill biopsies. Skin scrapings were obtained from each fish using the blunt side of scalpel blade #20. The mucous was spread onto a clean glass slide, covered with cover slip and examined microscopically. The gills were first observed for lesions. The operculum was raised to expose the gill filaments and a portion of the secondary lamellae cut using a pair of iris scissors, the section was placed in a drop of saline water on a slide, covered with cover cover slip and examined microscopically.

Endoparasite examination

Blood was obtained from the caudal vertebral vein of diseased fish using a 1 mL syringe and a 23 G needle. A drop of blood was thinly spread on a clean glass slide and a smear made. The thin blood smears were air-dried, fixed in 100% methanol for 1 min and stained with Giemsa stain for 45 mins. The air-dried blood smear was examined microscopically. The posterior intestine content was scraped with the blunt side of scalpel blade #20 onto a clean glass slide and stained with methylene blue and examined microscopically.

Bacterial isolation and identification

Each fish was examined for external lesions. The skin and gills were swabbed for bacterial culture using a sterile inoculation loop. Each fish was dissected by making a linear incision beginning from cranial to the anus region up to the level of the pectoral fin using sterile mayo scissors. The contents of the abdomen were exposed, and the internal organs examined for lesions and other abnormalities. The kidneys and the intestinal walls were swabbed using a sterile inoculation loop and sample

streaked on trypticase soy agar (TSA) supplemented with 1.5% NaCl and *Thiosulfate-citrate-bile salts-sucrose (*TCBS) agar before incubating for 24 to 48 h at room temperature for bacterial growth. Pure cultures from each colony were subjected to catalase, oxidase, indole, and motility tests. The API 20 E and BBL Crystal ID kits were used to identify gram-negative and gram-positive bacteria, respectively.

Kidney squash smear
The anterior part of the kidney of each fish was scraped using scalpel blade #20 and the scraping transferred to a glass slide for squash smear. The smear was fixed with 100% methanol for 1 min, stained with May-Grunwald stain for 5 min and with Giemsa stain for 10 min. Upon drying, the slides were cleared with xylene, and mounted with DPx, before examining microscopically.

In-situ water quality
The temperature, pH, salinity and dissolved oxygen of the water of cultured fish were determined using a YSI probe meter. Nitrate, nitrite and ammonia levels were also measured using the Aquarium Pharmaceuticals Nitrate (NO^{3-}), Nitrite (NO^{2-}) and Ammonia (NH_3) test kits.

Nitrate and nitrite
A 5-mL test tube was filled with water. Ten drops of nitrate test solution #1 was added to the test tube with vigorous mixing for 30 s. Then 10 drops of nitrate test solution #2 was added into the test tube with vigorously mixing for 1 min and left to stand for 5 min. The suspension was observed for colour change and either the Nitrate or Nitrite Colour Card for saltwater used to determine the nitrate level.

Ammonia
A 5-mL tube was filled was filled with water. Eight drops of ammonia test solution #1 followed 8 drops ammonia test solution #2 to the test tube with vigorous shaking to mix thoroughly. The tube was allowed to stand for 5 min and the colour of the solution compared with the ammonia colour card for saltwater to determined ammonia level.

RESULTS AND DISCUSSION

History and clinical signs
The management and performance data from the farms are presented in Table 1.

Management history, information on feeding and rearing are essential to determine the cause of disease in aquaculture. Based on harvested fish per cycle, the study showed that the sampling farm had better performance the adjacent farms. However, the sampling farm showed a high mortality per cycle of 50 to 70%. No information on mortality per cycle was obtained from the adjacent farms. Based on the data, the probable predisposing factors to vibriosis in the sampling farm were

high stocking density and feeding with trash fish. Cage-cultured grow-out Asian seabass commonly adapted a stocking density of 10 to 35 fish/m³. Since the sampling farm had a cage size of 57 m³, the number of fish that is suitable is from 570 to 1,995 tails per cage, with moderate stocking density of only 900 juvenile Asian seabass per cage post-grading. High stocking densities could result in inter-individual contact, competition for food, and stress, which directly affect growth performance of fish.

Table 1. Descriptive results of fish sampling and farmed Asian seabass management in Marang, Terengganu.

Variables	Mean value		
	Sampling farm	Farm A	Farm B
Net-cages size (m^3)	57	57	57
Number of net-cages/ farm	35	14	20
Fingerling size at stocking (cm)	11.43	11.43	11.43
Stocking density (Tails/farm)	10,000	4,500	4,500
Grow-out period (Months)	10 (8-12)	12	12
Fish harvested (Tails)	4,000	900	900
Harvest weight (kg/tail)	1.5	1	1
Mortality rate/cycle	50-70%	-	-

The second predisposing factor for the development of vibriosis may be the use of trash fish for feeding in aquaculture. In Cambodia for example, because of cost and inconsistent supply, formulated feed represents only approximately 1% of the diet of the fish (Sorphea et al., 2019). In Malaysia, aquaculture farmers resorted to low quality agricultural by-products and waste to reduce cost of feeding fish. Low quality feeds are moist and unstable in water and contributes to the introduction pathogens to the farm.

In this study, common manifestations of vibriosis such as skin ulceration and haemorrhages were not seen in the fish. However, the fish showed circling and incoordination, breathing difficulties, lethargy, skin erosion with sloughed-off scales, fin and tail rot, cutaneous haemorrhage, and body pigmentation. It was reported that in vibrosis, gross pathological lesions were usually limited, since death preceded manifestations of gross lesions (Mohamad et al., 2019).

Parasites

The gills of the fish from the sampled farm were infested with *Dactylogyrus* sp. and *Icthyophythirius multifilis*. The intestinal contents of these fish also showed presence of endoparasite eggs and cysts. Parasite-infested fish are under prolonged stress, which rendered them immunocompromised and susceptible to diseases like vibriosis.

Bacteria

Both the gram-positive and gram-negative bacteria were identified in the kidney squash smears of the Asian seabass from the farm.

Serratia marcescens, an opportunistic, gram-negative, nosocomial pathogen of the family *Enterobacteriaceae* (Khanna et al., 2013) was isolated from trash fish. *S. marcescens* is an opportunistic pathogen for fish and humans (Baya et al. 1992). The bacteria can survive in a highly polluted environment.

Water quality

The water quality parameters in the farm were at the optimum level. However, this did not prevent the fish from acquiring vibriosis. Vibrosis in fish is independent of the host and the *Vibrio spp.* can survive in an aquatic environment temperature of >17°C and salinity of 30 to 35 ppt (Pridgeon and Klesius, 2012). In fact, vibriosis has been reported in many aquaculture farms in Malaysia (Mohamad et al., 2019).

Heavy rain and typhoons adversely affect salinity of water in aquaculture facilities. The Marang district is known for its heavy rainfall and floods, particularly in November and December of each year that contribute to the deterioration of water quality and susceptibility of fish to infections. Therefore, constant monitoring of water quality parameters is crucial to control and prevention outbreaks of vibriosis in the farms.

CONCLUSION

The farm in this study was affected by vibriosis causing a high fish mortality. The risk factors to the infection include age of fish, stoking density, and feeding with trash fish. To prevent future vibriosis outbreaks, the farm should adopt prevention and control measures including strict biosecurity, good hygiene, monitoring of abnormal behaviours and clinical signs, and quarantine of fish. Oxytetracycline treatment is effective to control infections by the *Vibrio* species.

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EFFECT OF FOWL ADENOVIRUS ON HUMORAL IMMUNE RESPONSE OF CHICKS

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ABSTRACT

Outbreaks of fowl adenovirus (FAdV) infections can cause huge economy losses to the the poultry industry. The objective of this study was to determine the effect of inactivated FAdV (UPM1137) on humoral immune response in commercial broiler chickens. Forty day-old chicks were divided into 5 equal groups. Groups A, B, C and D day-old chicks were inoculated subcutaneously with 0.2 mL inactivated FAdV of virus titre of 10^6, 10^7, 10^8, and 10^9 $TCID_{50}$, respectively. Group E was nontreated and served as the control. The chicks were given feed and water *ad-libitum* and were monitored twice daily for abnormal clinical signs. On day 0, 4 chicks from Group E were sacrificed by cervical dislocation. On days 7 and 14 post-inoculation (pi) 4 chicks from each group were sacrificed. The body weights of and blood samples were then taken from the chicks prior to necropsy. At necropsy, gross liver lesions and liver weight were recorded. Liver samples were fixed in 10% buffered formalin for histological examination, while the blood samples were subjected to ELISA to determine anti-FAdV antibody titre. The study showed no abnormal clinical sign or gross or histological liver lesion in the chicks. On day day 7 pi, the liver to body weight ratio of Group C was significantly higher ($p<0.05$) than that of Groups A (0.043±0.012) or D (0.043±0.001). At day 7 pi, Group B (6170±1805) showed higher antibody titre than either Group A (3043±1559), C (1353±542), D (2880±1076) or E (1589±709). At day 14 pi, the antibody titre of Groups A (2504±1680) and B (2506±1072) was apparently higher than that of other groups. The study showed inactivated FAdV can induce antibody response in chicks, suggesting that it is safe to be used as a vaccine.

Keywords: fowl adenovirus, commercial broiler chickens, virus titre, antibody titre

INTRODUCTION

Fowl adenovirus (FAdV) classified under family Adenoviridae and genus Aviadenovirus. There are 5 genotypes (Zsak and Kisary, 1984) and 12 serotypes of FAdV. Fowl adenovirus Group I has been identified as a causative agent of inclusion body hepatitis (IBH), hydropericardium syndrome (HPS), and gizzard erosion in

chickens and causing a major economic impact to poultry industry worldwide (Adair and Fitzgerald, 2008).

Inclusion body hepatitis was first reported in USA by Helmboldt and Frazier (1963) and then identified for the first time in Malaysia by Hair-Bejo (2005). The Malaysian FAdV isolates were characterised as FAdV-8b species E, a highly pathogenic serotype (Juliana et al, 2014). Broilers from farms with the virus, with history of high mortality and poor growth, showed enlarged, pale and friable liver with areas of haemorrhages and congestion. Histologically, IBH show numerous eosinophilic and basophilic, round or irregular shaped intranuclear inclusion bodies. Vaccination against IBH is difficult due to the presence of various serotypes and the unclear primary role of the virus (Hair-Bejo, 2005).

The efficacy of a vaccine like inactivated FAdV vaccine can be determined by degree of humoral response (Qing et al, 2017). In virus infections, the factors affecting the production of neutralising antibodies include viral dosage and route of administration. Inactivated FAdV-2 vaccine can induce high anti-Hexon IgG response, mean sample-to-positive (S/P) ratios with high virus titres of $10^7 \mathrm{TCID}_{50}$ and $10^6 \mathrm{TCID}_{50}$ (Junnu et al, 2015).

Currently, there is minimal information on the effect of the Malaysian inactivated FAdV on immune response of chicken. Therefore, this study determined the effect of inactivated FAdV titre on the antibody response of broiler chicken.

MATERIALS AND METHODS

UPM 1137, a FAdV isolate was obtained from a IBH and gizzard erosion outbreak in commercial layer chickens. All diluted seeds were combined with aluminium potassium sulfate as adjuvant with ratio 1 in 10 dilution.

Day-old commercial broiler chicks were obtained from the Linggi Poultry Farm (M) Sdn. Bhd., Rembau, Negeri Sembilan, Malaysia. The chicks were housed in the Animal Research Facility, Faculty of Veterinary Medicine, Universiti Putra Malaysia, throughout the 14-day study.

Forty-one-day-old commercial broiler chicks (DOC) were divided into five groups. The chicks in groups A, B, C and D were inoculated subcutaneously with 0.2 mL inactivated FAdV of virus titre of 10^6, 10^7, 10^8, and 10^9 TCID_{50}, respectively. Group E remained nontreated and served as the control.

Four chicks at day 1 of age from the control group were sacrificed by cervical dislocation for first sampling. The chicks from the other groups were inoculated with the inactivated FAdV. The chicks were given feed and water *ad-libitum* and monitored twice daily for any abnormal clinical signs. On days 7 and 14 of age, 4 chicks from each group were weighed, sacrificed, and blood collected intra-cardially. The blood samples were used to determine FAdV antibodies titre using the enzyme linked immunosorbent assay (ELISA) technique. At necropsy the chicks were examined for gross lesions and the livers collected, weighed, and fixed in 10% buffered formalin for histological examination.

Statistical analysis

The IBM SPSS Statistics version 25 was used to analyse the data. The data analysed with parametric test (One-way ANOVA) with α=0.05 used to determine significant differences among means.

RESULTS AND DISCUSSION

No mortality was observed among the chicks in the study. The body and liver weights of chickens of all groups increased significantly ($p<0.05$) from day 0 to day 14 pi. However, there was no significant difference ($p>0.05$) in body and liver weights among groups at each sampling.

The liver to body weight ratio of day-old chicks was 0.031 ± 0.001. On day 14 pi, the liver to body weight ratio of the chicks of Groups A, B, C, D and E were 0.025±0.001, 0.028±0.001, 0.026±0.002, 0.025±0.001, and 0.030±0.002 respectively. On day 7 pi, the liver to body weight ratio of Group C was significantly higher ($p<0.05$) that those of other groups.

The livers of chicks from Groups A, B, C, D and E appeared dark red, glistening with sharp edges, indicating normal livers. Histologically, the liver tissues also appeared normal.

The FAdV antibody titer of the control chicks on day 0 was 6459±1520 and valued decreased to 1742±609 on day 7 and 1970±1195 on day 14 pi. On day 7 pi, Group B chicks showed the highest antibody titre at 6169 ± 1805 among treatment groups but it decreased 2506±1072 on day 14 pi. Similar decreases of antibody titre from day 7 to 14 pi were observed in Groups A and D. However, for Group C chicks, the antibody titre increased from 135±3542 on day 7 to 1681±843 on day 14 pi.

FAdV isolates if not fully attenuated or inactivated can cause infections in the chickens. In addition, the side effects of chemicals including formalin and binary ethylenimine used to inactivate or attenuate the virus, is not known.

Throughout the 14 days trial, chicks treated with FAdV were healthy, had good appetite, and did not shown any abnormal clinical sign. The body and liver weights of all chicks increased significantly with time, irrespective of treatment dose. Necropsy examination, except for mild fatty liver, did not show significant abnormality.

The FAdV induced antibody response in the chicks. Group B chicks inoculated with10^7 $TCID_{50}$ produced the highest antibody titres among groups on day 7 pi. It can be concluded that inactivated FAdV has potential to be developed as a vaccine.

CONCLUSION

The study showed that inactivated FAdV with virus titre of 10^7 $TCID_{50}$/0.2mL produced the highest antibody titre among treated chicks. There is no evident of side effect in the chicks inoculated with inactivated FAdV, suggesting it is safe to be developed and used as a vaccine.

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DETERMINATION OF BACTERIAL POPULATION AND SOMATIC CELL COUNT IN MILK OF DAIRY BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

Domestic buffalo (*Bubalus bubalis*), commonly found in Malaysia, is classified under two sub-species, river and swamp buffaloes. In Malaysia, buffaloes are reared for their meat and milk. Unlike cattle, information on the characteristics and quality of buffalo milk is lacking. This study determined the bacterial population in milk from normal and subclinically mastitic dairy buffaloes and correlated their presence with the California Mastitis Test (CMT) score. Teat skin swabs and milk from 36 quarters of 9 lactating buffaloes were collected for bacterial isolation and identification. Six quarters were identified as subclinically mastitic udders, while the remaining udders were normal. Seventeen species/strain of gram-positive and 4 species/strain of gram-negative bacteria were isolated from the milk samples. *Staphylococcus aureus* (25.97%) and *Streptococcus intermedius* (15.58%) were isolated from the milk and teat swab samples. *S. aureus* (31.25%) was isolated from the milk of cow with subclinical mastitis. No significant ($p>0.05$) correlation was observed between CMT score and bacteria isolation from the milk samples. The study showed that similar bacteria were present in milk and at the teats of buffalo cows.

Keywords: buffalo, Bubalus bubalis, milk, bacteria, mastitis

INTRODUCTION

The water buffalo (*Bubalus bubalis*), comprising of swamp and river buffaloes, is an Asia-originated bovine species. The river buffaloes, primarily reared for milk, are generally larger and heavier than the swamp buffaloes, have curled short horn, and black in colour. The river buffaloes prefer clean water to wallow.

Raw buffalo milk is common contaminated by microorganisms, including fungi, coliforms, *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Listeria* spp., *Lactobacilli* spp., and *Salmonella* spp, (Han et al, 2007; Mistry et al.,

2015). Bacterial infections causing mastitis is a problem of dairy animals that had resulted in enormous losses to owners. However, buffalo has been traditionally considered to be less susceptible to mastitis than cattle.

Buffalo milk has twice as high caloric value than cow's milk, with high mineral contents. Despite buffaloes being used for milk production in Malaysia, there is still inadequate information on the quality of buffalo milk. This study was conducted to determine the microbiological characteristics of buffalo milk.

MATERIALS AND METHODS

This study was conducted on 9 lactating buffaloes from 2 foster farms of Universiti Putra Malaysia (UPM). Sample selection was random, based on owner consent and selection. Only 25% of lactating buffaloes from the first and all the second farm were used in the study.

Milk and teat swab samples

Aseptic milk samples were collected from teat before milking, using the whole hand squeezing method. Sterilized cotton swabs were used to collect teat swab samples. The cotton swabs were rolled on the outer surface of the udder skin with a swiping movement and placed in sterilized sampling tube. All samples were immediately transported to the Microbiology Laboratory of the Veterinary Laboratory Services Unit, UPM in an insulated ice-box.

Bacteria isolation and identification

Bacteria were cultured on blood agar. The blood agar plates with bacteria culture were incubated at 37°C for 24 h in an aerobic condition. The plates were observed for colony growth. The morphology of colonies was recorded based size, shape, colour, elevation, and edge. Each different colony was picked and sub-cultured on nutrient agar to purify the isolates. Gram-staining and coagulase and oxidase tests were performed on the cultures. The biochemical test was performed to further differentiate the bacteria isolates.

California mastitis test

The California mastitis test (CMT) was used to semi-quantitatively determine the presence of somatic cells in the milk samples according to the method described by Schalm and Noorlander (1957). All CMT scores of 0 and trace were considered negative while CMT scores of 1, 2, and 3 were indicators of sub-clinical mastitis. Positive cows were defined as having at least one quarter with CMT score of 1+.

Statistical analysis

The Kruskal-Wallis test was used to determine the differences in mean CMT scores in the buffalo milk. The Pearson Correlation, Chi-square, and Phi Cramer's V tests were used to determine the correlation/relationship between CMT score and type of bacteria presented in the milk.

RESULTS AND DISCUSSION

The results showed that among the 9 buffaloes, 16.67 % of all quarters were infected, with significantly higher involvement of left forequarters. The prevalence of sub-clinical mastitis in buffaloes at the two farms was 16.67% with highest prevalence in left forequarter (44.44%) followed by left hindquarter and right forequarter (11.11%). None of the right hindquarter was infected.

Seventeen species of gram-positive and 4 species of gram-negative bacteria were isolated from the milk samples. The teat skin swab sample showed 10 species of gram-positive and 1 species of gram-negative bacteria (Table 1).

Based on the Pearson correlation test, there was a weak, positive correlation between CMT score and type of bacteria presented in the milk of buffalo ($r=0.064$, $n=14$, $p=0.709$).

Table 1. Bacteria isolate from milk and teat swab samples

Milk	Teat
Gram-positive bacteria	
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus intermedius	*Staphylococcus intermedius*
Coagulase-negative staphylococci	*Corynebacterium* sp.
Staphylococcus Aureus subsp. *aureus*	*Pantoea agglomerans*
Staphylococcus hyicus	*Staphylococcus hyicus*
Enterococcus faecalis	*Staphylococcus schleiferi* subsp *schleiferi*
Staphylococcus delphini	*Corynebacterium bovis*
Staphylococcus schleiferi subsp. *schleiferi*	*Corynebacterium diphtheriae*
Streptococcus bovis	*Corynebacterium pseudodiphtheriticum*
Streptococcus pyogenes	*Bacillus* sp.
Streptococcus viridans	
Corynebacterium sp.	
Streptococcus pneumoniae	
Streptococcus suis	
Staphylococcus pseudointermedius	
Corynebacterium pseudodiphtheriticum	
Actinomyces sp.	
Gram-negative bacteria	
Brucella sp.	*Acinetobacter baumanni*
Acinetobacter baumanni	
Escherichia coli	
Moraxella bovoculi	

CONCLUSION

The result showed that bacterial growth on the teats is similar to that in raw milk. In this study, *S. aureus* and *S. intermedius* were mostly common bacteria isolated from

the buffaloes. These bacteria are useful predictors of mammary gland infection in buffaloes.

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PREVALENCE OF CONTAGIOUS ECTHYMA DISEASE AMONG SHEEP AND GOATS IN NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

Contagious ecthyma is infectious dermatitis affecting small ruminants primarily sheep and goats and caused by the Orf virus. The infection has been endemic in many countries, including Malaysia. Contagious ecthyma causes huge economic losses to the agricultural sector by reducing animal productivity. This study determined the prevalence of contagious ecthyma among sheep and goats in selected farms in Negeri Sembilan, Malaysia. A total of 182 blood samples from 110 goats and 72 sheep were collected and analysed using the enzyme-linked immunosorbent assay (ELISA) technique to determine serum IgG antibody concentrations. Among the samples, 9 from goats (8.18%) and 3 from sheep (4.17%) were seropositive for contagious ecthyma. The risk factors for the acquisition of contagious ecthyma are age and farm location. Animals aged more than 12 months have longer period of exposure to Orf virus than the young. Farms at closer proximity to other farms are prone to Orf virus infection. Therefore, small ruminant farms should have an Orf viral disease control programme in place to control contagious ecthyma and improve herd immunity.

Keywords: goats, sheep, contagious ecthyma, seroprevalence

INTRODUCTION

Contagious ecthyma, also known as contagious pustular dermatitis or Orf disease, is an extensive infection in small ruminants caused by Orf virus of the genus *Parapoxvirus*. In goats and sheep, the lesions of contagious ecthyma are primarily around the mouth and nares, and sometimes on the hooves (Hajkazemi et al., 2016). These lesions usually start off with papules and pustules and eventually progresses to become scabs before resolving. Orf virus is shed in the crusty scabs from affected animals and can stay infective for at least 4 months. Transmission of the virus occurs when there is a direct contact between susceptible and infected animals or with contaminated fomites. Contagious ecthyma is a zoonotic disease and people that come in close contact with infected goats and sheep are occasionally infected with the disease (Teshale and Alemayehu, 2018).

Orf disease can be an endemic in small ruminants. The disease has been reported in Asia, Africa, and other small ruminant-rearing countries. Orf virus infects sheep and goat in all ages. The morbidity rates of Orf disease in naive sheep or goats are almost 100%; however, the mortality rates are less than 1%. Death of infected animals are usually due to secondary complication of the infection, including anorexia and secondary bacterial infections. Young animals with contagious ecthyma cannot suckle because of the lesions around the muzzles and they eventually die of starvation.

There is no proven treatment for contagious ecthyma. However, a good herd health programme may help to control Orf disease transmission as well as improve herd immunity. Although live Orf virus vaccine can control the disease, Orf virus vaccination is not commonly practiced by the Malaysian small ruminant farmers.

In Selangor, Malaysia, 36.7% of the goats harbour active Orf infections (Jesse et al., 2018). It was also shown that farm location is significantly associated with Orf virus infection.

This study was undertaken to determine the anti-Orf virus IgG antibody titre in small ruminants and the risk factors associated with the prevalence rate of Orf virus infection.

MATERIALS AND METHODS

Sample collection

One hundred and eighty-three goats (n=110) and sheep (n=72) are selected by convenience from 3 farms (A, B, C) in Negeri Sembilan, Malaysia. Approximately 3 mL blood samples were collected from each animal via jugular venipuncture in plain tubes and transported in the Faculty of Veterinary Medicine, Universiti Putra Malaysia in an ice box for analyses. The blood samples are allowed to clot and centrifuged at 300 × *g* for 5 min to collect serum, which was transferred to 1.5 mL microcentrifuge tubes and stored at -20 °C.

Questionnaire

A well-structured questionnaire was given to farmers and farm workers. The questionnaire was designed to determine the association between risk factors and seroprevalence of Orf virus infection in the farm animals. The risk factors considered were farm management and farm biosecurity practices, and age, sex, breed and body condition scores (BCS) of the animals.

ELISA

The IgG ELISA test kit from (SunLong Biotech Co., LTD, China) was used to determine the serum antibody titre to Orf virus. The average value of positive control for the test kit was >1.00, indicating that it is effective. The critical (cut-off) value was obtained from the following equation:

$$\text{Critical value} = \text{Average value of 2 negative controls} + 0.15$$

From the absorbance readings, samples with values lesser than the critical value are taken as negative and those more than the critical value are positive for Orf IgG.

Data analysis

All data collected was analysed using chi-square test (IBM SPSS Statistical software) with statistical significance set at $p<0.05$.

RESULTS AND DISCUSSION

The result revealed that 12 of 182 (6.59%) serum samples were positive for Orf IgG antibodies. Farm A showed 1.69% seroprevalence (1/59) towards Orf virus infection, while in Farms B and C it was 8.16% (4/49) and 9.46% (7/74), respectively.

In goats, the seroprevalence of Orf IgG antibodies was 9 out of 110 (8.18%) while in sheep, it was 3 out of 72 (4.17%). There was no significant association between species and Orf virus infection ($p>0.05$). Goats were generally more susceptible to and develop more severe cases of Orf than sheep (Gao et al., 2016).

The Orf disease seroprevalence was 6.38% (3/47) and 6.67% (9/135) female goats and sheep. There was no significant difference ($p>0.05$) in seroprevalence for Orf virus between male and female animals.

The study showed that young animals was seronegative for Orf virus. However, adult animals showed 8.76% seroprevalence, suggesting age is a risk factor in the development of seroprevalence to Orf disease in goats and sheep (Bala et al., 2019).

The BCS or management system of animals did not determine Orf virus seroprevalence in the animals. There was no association ($p>0.05$) between BCS or management system and Orf virus infection in the goats and sheep.

The farm included in this study that are located close to other farms showed a seroprevalence of 8.94% (11/123), while the farm isolated from other farms showed a seroprevalence of 1%. The results suggest farms in close proximity are at risk of acquiring Orf virus infections.

CONCLUSION

This study showed that 6.59% (12/182) serum samples obtained from Negeri Sembilan were positive for anti-Orf virus IgG antibodies. Age is a significant determinant ($p<0.05$) of Orf virus infection with animals <12 months old more susceptible to Orf virus infection than those aged ≥12 months. Orf virus in young animals may be associated with the herd management practices such as tail-docking and ear-tagging. The location of farms plays a role in the transmission of the disease from farm to farm. Hence, livestock farms should have good biosecurity programmes to prevent spread of Orf virus.

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RETROSPECTIVE STUDY ON CANINE HEART DISEASE IN UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Canine cardiac diseases were extensively reported worldwide but only meagre information is available in Malaysia on the disease. A 5-year retrospective study (2014-2018) on canine cardiac disease cases was conducted in University Veterinary Hospital, University Putra Malaysia (UVH-UPM). This study determined the prevalence, risk factors, and survivability of dogs diagnosed with cardiac disease. Information of canine patient referred UVH-UPM including case number, breed, age and sex were recorded and those diagnosed or suspected with cardiac disease were reviewed. Owners with dog with cardiac disease were contacted to determine the current health status of their dogs and their compliance to therapy. The study showed that the prevalence of heart disease in the dogs was 6.4% (n=479/7529). The senior (>7 years old) were at higher risk ($p<0.05$) than small breed dogs of being affected by the disease. The majority of the heart disease diagnosed were valvular diseases (69.4%) followed by heartworm disease (16.1%), myocardial disease (11.3%), others (2.0%). Congenital heart diseases were of 1.2% prevalence. Dogs diagnosed with degenerative mitral valve disease (DMVD) stage C had relatively longer survivability with medical management. Awareness of cardiac disease among dog owners and clinicians will allow for early detection of heart disease to improve quality of life of affected dogs.

Keywords: canine heart disease, prevalence, risk factors, survivability

INTRODUCTION

Cardiac diseases in dogs are commonly reported and the diagnosis often left dog owners devastated (Parker et al., 2006). The most common canine cardiac disease are valvular and myocardial diseases, pericardial effusion, and cardiac neoplasia without pericardial effusion. Dirofilariosis, endocarditis, hypertrophic cardiomyopathy, and infectious pericarditis were rarely diagnosed (Baumgartner and Glaus, 2004a). It was reported that dilated cardiomyopathy was the second most common cardiac disease in dogs causing considerable morbidity and mortality (Dutton and López-Alvarez, 2018).

Dog diagnosed with cardiac disease were often presented with nocturnal coughing, exercise intolerance, anorexia, ascites, dullness and depression, cachexia, and hepatojugular pulsation (Sarita et al., 2009). Most dogs examined and diagnosed with heart disease were purebred including Cavalier King Charles Spaniel, Dachshund, Doberman Pinscher, Great Dane, Poodles, Collies, Pomeranians, Golden Retriever, Rottweilers, Boxers, German Shepherd, English Bulldog and Miniature Schnauzers. Congenital heart defects were commonly identified in dogs <3 years old and frequently in dogs <6 months of age. Acquired cardiac disease were often identified in middle age and older dogs (Kittleson and Kienle, 1998).

The prevalence of canine cardiac disease in Malaysia is not known. Information on the various types of cardiac disease, the risk factors, the common presenting signs and the survivability rate of dogs diagnosed with heart disease would be useful to clinician to provide advice and management of dogs diagnosed with heart disease. Thus, this study determined the prevalence, risk factors, and survivability of dogs diagnosed with cardiac disease.

MATERIALS AND METHODS

The retrospective study on canine heart disease was conducted in University Veterinary Hospital, University Putra Malaysia (UVH-UPM). Information of dog patients presented to UVH over a period of 5 years, 2014 to 2018, retrieved from Case Log Book were case number, name, breed, age, and sex of patients.

Patient signalment of all the dog patient

The age of dog patients was recorded and categorised according to WALTHAM™ pocket book of responsible pet ownership (Kelly and McCune, 2017). The canine heart disease patients were further categorised into 3 age groups; puppy (<12months old), adult (1 to 7years old), and senior (>7 years old). The breeds of dogs were recorded and further classified into small, medium, and large breed dogs. The sex of the dogs was recorded as either male or female.

Inclusion criteria of dog patients diagnosed with heart disease

The patient medical records of dogs with heart disease and those of suspected and/or has possible cardiac-related condition were retrieved. The inclusion criteria of dog patients diagnosed with heart disease were as follows: retrievable patient medical records, complete patient signalment, complete information on clinical signs and physical examination findings associated to heart diseases, and with a definitive diagnosis of the heart disease. Incomplete information was obtained from owner via telephone communication.

Clinical signs, physical examination and diagnostic imaging

Clinical signs of dog patients diagnosed with heart disease such as coughing, exercise intolerance, tachypnoea, dyspnoea, syncope, ascites, lethargy observed by owners at home and by veterinarians during consultation and/or hospitalisation were recorded.

The canine cardiac patients were also divided into different groups based on clinical signs. Only dogs diagnosed with valvular related heart disease were staged using the ACVIM consensus (Atkins et al., 2007).

Treatment and survival day

Owners of canine heart patients were contacted via telephone to determine the current status of the patients and their compliance to treatments. The date of diagnosis made was assigned as Day 0 and the date of death of canine patient was the last date after diagnosis was made, regardless whether the patient received treatment or not. The cut-off date for survivavility of dog patients was taken as 31 August 2019. Only dogs that did not survived or were euthanised due to chronic stage of the cardiac disease and current dog patients that were still alive were included in the survivability analysis. Dog patients that went missing, no longer owned, did not survived because of other disease, and of noncontactable dog owners were excluded.

Statistical analysis

All statistical analyses were performed using SPSS (IBM, USA). Associated risk factors for canine heart disease were subjected to Chi-square test while the odd ratio was used to determine association among factors. Statistically significant was taken at $p<0.05$. Kaplan Meier Estimator test was used to determine survivability of canine valvular patients in the presence and absence of treatment.

RESULTS AND DISCUSSION

The overall prevalence of heart diseases in dogs presented in UVH-UPM from 2014-2018 was 6.4% (n=479/7529), where 6.30% were diagnosed to have acquired and 0.06% congenital heart disease. Since an earlier study reported a prevalence of heart disease in dogs is 10.0 to 15.0% (Hoque et al., 2019), it is suspected that the prevalence of canine heart disease in this study could have beeb under estimated. The lower prevalence could likely be due to irretrievable patient medical records or missing case files. Besides, some dog patients suspected with cardiac disease remained undiagnosed due to lack of cooperation from owners. Therefore, it remains unclear as to whether the dog patients had heart disease or instead had other diseases with similar clinical signs.

Breed size and age group were identified as significant ($p<0.001$) risk factors in canine heart disease. Small breed dogs were at higher risk ($p<0.001$) compared to medium and large breed dogs. An earlier report showed that the small breeds are more predisposed to chronic degenerative valvular disease (CDVD) than large breed dogs (Atkins et al., 2007). For example, approximately 90.0% of Cavalier King Charles Spaniels were reported to develop myxomatous mitral valve disease (MMVD) by 10 years of age (Borgarelli and Haggatrom, 2010).

Most studies reported that male dogs had a higher risk of heart disease compared to females (Baumgartner and Glaus, 2004b; Oliveira et al., 2011); however, this was not observed in our study. Bitches become calmer when they reach their sexual

maturity; as a result they have lower risks for heart disease. Hormones also play a role in contributing to the higher frequency of cardiomyopathy in males than females (Himalini et al., 2017). Neutering also reduce the risk of heart disease in male dogs.

Senior dogs have higher risks than puppies or adult dogs for acquiring heart disease (Himalini, 2017). In fact, the majority of the dogs diagnosed with heart diseases were more than 6 years, followed by dogs aged 2 to 6 years and least commonly in young dogs <2 years of age. Aging contributes to changes of the structure and function of cardiac pacemaker, collagen accumulation on the atrioventricular nodes and bundle of His (Schmidlin et al., 1992), resulting in the higher frequency of heart diseases in senior dogs.

Long-term treatment regime, depending on the chronicity of the heart disease, may affect the survivability of the patients. Dogs with moderate to severe congestive heart failure caused by MMVD had a longer survival period with medical management (Borgarelli and Haggstrom, 2010). This finding is similarly shown in our study, where dog patients with stage C valvular disease survived longer with treatment than those without treatment. However, it was not the same for patients with stage D valvular disease. It is most likely that the stage D valvular disease dogs were at their terminal stage of heart disease and treatment did not improve survivability.

CONCLUSIONS

The prevalence of canine heart disease in UVH-UPM from 2014 to 2018 was 6.4% (497/7529). Small breed and senior dogs were predisposed to canine heart disease. Thus, it is important for the dog owners to refer their pets for annual health screening. Early intervention with treatment and compliance to treatments would increase survivability of dogs with heart disease.

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IDENTIFICATION OF ECTOPARASITES IN CLIMBING PERCH (*Anabas testudineus*) CULTURED IN EARTHEN POND AND FIBREGLASS TANK

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ABSTRACT

Climbing perch (*Anabas testudineus*) from the family of Anabantidae and order Perciformes have been reported in various countries across Asia including Malaysia. The fish that lives in brackish or freshwater habitats is an obligate air-breather and has superior ability at surviving under adverse condition. The aim of this study was for screen for and identify ectoparasites in climbing perch cultured in earthen ponds and fibreglass tanks. The study also examined the fish for pathological lesions caused by the parasites. Thirty fish were sampled from earthen ponds and fibreglass tanks. Skin scraping were obtained from the fish and wet smear of gills with accessory organs made. The histology of gills showed degeneration and hyperplasia of gills epithelium with infiltration of inflammatory cells. Ectoparasites found in skin scrapings and gills were identified. Five parasites were discovered, namely *Dactylogyrus* sp. and cysts of *Centrocestus formosanus* from the gills while *Ichthyobodo necator*, *Vorticella sp.* and *Trichodina sp.* comes from skin. However, the fish did not show any clinical sign associated with these parasites. Therefore, it can be concluded that fish from different habitat habours different types of ectoparasites, which is associated with water quality.

Keyword : climbing perch, ectoparasite, earthen pond, fibreglass tank, water quality

INTRODUCTION

The climbing perch, *Anabas testudineus* of family Anabantidae and order Perciformes are widely available in various countries including Laos, Vietnam, Bangladesh, India, Indonesia, and Malaysia. There are more than 30 known species of the climbing perch found in brackish and fresh water. Studies in India (Mandal et al., 2016) and Thailand (Luangphai et al, 2004) showed that climb perch are often infested with ectoparasites and endoparasites.

The aim of this study was to determine presence of parasite in climbing perch (*Anabas testudineus*) cultured in earthen pond and fibreglass tank.

MATERIALS AND METHODS

Fish sample

A total of 60 climbing perch were obtained from earthen ponds using pole and hook and fibreglass tanks using a fish net. The fish was brought to the Aquatic Animal Health Laboratory, Faculty of Veterinary Medicine, University Putra Malaysia to be processed. The water quality of the pond and tank were determined using YSI 556 MPS Multi Probe System (YSI Environmental, USA) and HACH Test Kit Model FF-2 (Hach, USA).

Examination of specimens

External lesions of each fish were recorded before decapitation and pithing. Skin scrapings were done at the cranial to caudal part of fish and smears of the mucous in saline solution were made on glass slides and examined microscopically. The gills and accessory organs were removed and placed in petri dishes containing normal saline. The gill filaments were dissected from the gill arch and placed on the glass slide with normal saline and examined microscopically for parasites.

Identification of parasite

The parasites were identified based on the image taken using a digital microscope (with Toupcam camera) and digital phone camera. The image was then used for the identification of parasite based on pictorial guide and description by Kabata (1985) and Scholz and Salgado-Maldonado (2000).

Histopathological analysis of gills

Gills that contained ecoparasites were fixed in 10% formalin solution before processing using standard histological techniques. The slides were stained with hematoxylin-eosin and examine microscopically.

Data analysis

The prevalence of the ectoparasites in the fish was calculated using the following formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish samples}} \times 100$$

The binomial test was used to determine the significance of presence of ectoparasites in the organs at α=0.05.

RESULTS AND DISCUSSION

Sixteen of 30 (53%) climbing perches cultured in fiberglass tank were infested with at least one type of ectoparasite. Among the 16 parasite-infested fish, only one had two types of parasites, the *Vorticella* sp. and *Trichodina* sp. and *Vorticella* sp. The cyst of the digenean trematode, *Centrocestus formosanus*, were found on the gills of 10 of 20 infested fish. *Trichodina* sp. were also found on the gills of two fish.

Twenty of 30 (67%) fish were infested with at least one ectoparasite. Two fish had more than one parasite. The *Dactylogyrus* sp. was present in the gills (gill fluke). The protozoa, *Vorticella* sp., were found on the skin of 5 fish and one gill fluke found in the accessory organ.

Table 1 shows the water pH of earthen pond and fibreglass tank in this study.The earthen pond showed an acidic pH while the fibreglass tank alkaline; both values were out of the reference range. The temperature of the pond and tank reflects the time of sampling, which was near noon. Dissolved oxygen, nitrite, and nitrate were lower than the normal ranges while the ammonia level for fibreglass tank exceeded the normal range.

Table 1: Water quality analysis in earthen pond and fibreglass tank

Parameter	Earthen pond	Fibreglass tank	Reference range
pH	4.74	9.35	6.5-9.0 (Philminaq, 2007)
Temperature (°C)	26.9	28.4	-
Dissolved oxygen (mg/L)	1.96	0.4	3.0-7.0 (Philminaq, 2007)
Nitrite (mg/L)	0	0	0.4 (Philminaq, 2007)
Nitrate (mg/L)	0	0	7.0 (Philminaq, 2007)
Ammonia (mg/L)	0	8.0	<0.05 (Lawson, 1995)

In this study, five types of ectoparasites were found and they comprised of protozoas, monogeneans, and digenean trematodes. There were differences in parasites obtained from the earthen pond and fibreglass tank. This difference is most probably associated with water quality, host specificity, metabolic process, and immunity of the fish (Mandal et al., 2016).

Climbing perch collected from the earthen pond were identified to have only two genus of ectoparasites, *Vorticella* sp. and *Dactylogyrus* sp. *Vorticella* sp. In earthen pond, most of the fish sample were infested with *Dactylogyrus* sp. on the gills. This parasite was identified based on their morphology, that is, they have two pairs of eyes, four head lobes, and opisthaptor with 14 marginal hooks. One fish had gill flukes on its accessory organ. It is postulated that the flukes had migrated from the gills to the organ. Histological analysis revealed severe hyperplasia with degeneration of the epithelium and infiltartion of inflammatory cells

The ectoparasites were identified on fish sampled from the fibreglass tank were *Ichthyobodo necator, Vorticella sp., Trichodina sp.* and *C. formosanus*. The study showed that only two infected fish showed gross and microscopic lesions. The development of these lesions is probably due to impaired immune system.

Trichodina sp. which come from the family of Trichidinidae survives well in shallow and stagnant ponds. They are not strictly host-specific. Trichodinids produce almost similar pathological lesions as *Vorticella* sp. However, in this study, fish infected with *Trichodina* sp. did not show pathological lesions. This could be a testament to the capability of the Climbing Perch to survive under adverse conditions.

The cercariae but not the adult form of *C. formosanus* was detected in the Climbing Perch. The cysts are confirmed to be of *C. Formosanus* based on location, shape, size, and characteristics their excretory bladder which is X-shaped and dark in colour. However, only a few cysts were found in the fish. Since, Climbing Perch is an obligate air-breather, that is they do not rely entirely on its gills for respiration, fish infected C. formosanus on their gills did show respiratory difficulties. However, their gills still showed hyperplasia of the epithelium caused by the cysts.

The pH for earthen pond is acidic while fiberglass tank alkaline. The acidic properties of water in earthen pond reflects the acidity of the soil. Climbing Perch can still survive in extreme acid or alkaline conditions. From our study, the prevalence of parasites was higher in acidic water. On the other hand, the Digeneans seemed to survive in the fiberglass tank water with alkaline pH.

CONCLUSION

In conclusion, the presence of pathological lesions are dependent on the ectoparasites pathogenicity and the nature of the fish itself. The climbing perch are hardy and have high immunity towards parasites. The parasites species identified in fish tanks and ponds is dependent on the pH of the water. Earthen ponds with acidic properties supports the survival of monogeneans and protozoa while fibreglass tanks with alkaline water were infested with protozoa and digenean species. In conclusion, the prevalence of ectoparasites in acidic water was lower than in alkaline water.

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UNDERSTANDING LEVEL OF AWARENESS AND PERCEPTION ON DAIRY CATTLE WELFARE AMONG DAIRY FARMERS IN KENINGAU, SABAH, MALAYSIA

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ABSTRACT

Animal welfare is a public concern and the dairy industry stakeholders including farmers have prioritised welfare as a top management issue in dairy farms. Hence, the objective of this study was to assess the knowledge and perception of farmers on dairy cattle welfare (DCW) in Keningau, Sabah, Malaysia. A questionnaire was developed, validated and distributed by hand to 30 farmers. Demographic information and details of their farm were collected. The farmers 57% (17/30) appeared to have satisfactory level of awareness and good understanding on the criteria and indicators of DCW. The farmers also had good perception on management practices and cows' behaviours with 63.3% (19/30) did not approve to inappropriate management practices affecting cows' behaviour. The main contributing factors of DCW, as perceived by farmers were facilities, worker/employer issues, management practices, well-being, and production. Farmers with high milk-producing cows, with higher education and holding managerial positions in the farms had better understand of DCW. As a conclusion, dairy farmers in Keningau, Sabah were generally aware and understood DCW. However, they still need guidance from veterinarians to strength their knowledge on DCW.

Keywords: dairy cattle welfare (DCW), dairy farmers, knowledge, perception,

INTRODUCTION

Most dairy farmers appreciate the idea good comfort and welfare of their animals (Villettaz Robichaud et al., 2019). In fact, in North America and Europe, welfare of animals is a priority of the dairy industry. Taking care of welfare of cattle will ensure optimum production of milk (von Keyserlingk et al., 2009). Good practices on calving, nutritional management, animal housing management on dairy farms will also improve productivity (Bergman et al., 2014).

Consumers are now more concerned about animal welfare (Martelli, 2007). These animal welfare concerns include the need for natural and humane treatment of

animals and preventing rampant use of antibiotics as prophylactic treatments (Clark et al., 2016).

In Malaysia, we are still far from what developed countries practice on farm animal welfare. However, to overcome this relative lack of sensitivity among the Malaysian public, the new Animal Welfare Act (2015) was officially implemented with the aim of fostering responsible animal ownership. Currently, it is not known whether Malaysians are aware or understand how modern farming practices may affect welfare and productivity of farm animals. Thus, this study was conducted to determine the awareness and perception of dairy farmers and producers on dairy cattle welfare.

MATERIALS AND METHODS

This study was carried out in Keningau, Sabah, located 95.2 km from Kota Kinabalu, Sabah, Malaysia. All dairy cattle farms recruited in the study were located within 5 km radius of the milk collecting center (MCC) of *Stesen Pembiakan Ternakan* (SPT) Sebrang, Keningau. Approval for the study was obtained from the Human Ethics Committee of University Putra Malaysia (Ref: JKEUPM-2019-180).

Questionnaire

A cross-sectional questionnaire study was conducted, using the MCC as the meeting place. The study was conducted over 5 days, from 4th to 8th August, 2019.

Instrument development

Instrument used in this study was based on a questionnaire developed with reference to the Terrestrial Animal Health Code (World Organisation for Animal Health, 2011) and the Animal Welfare Enactment 2015. A pilot study was conducted on May 2019 on 8 respondents during a seminar held at the *Institut Pengurus Veterinar*, Cheras, Kuala Lumpur, Malaysia. The questionnaire was also translated to the Malay language and administered according to the respondents' preference.

Questionnaire comprised of 5 sections. Section 1: respondent demographic information (gender, country of origin, education). Section 2: farm details (location, management system, herd size, cattle breeds, milk production). Milk production was categorised as high or low. Section 1 and 2 were the independent variables of this study and associated with the information obtained in subsequent sections. Section 3: knowledge and perception on animal welfare. Section 4: attitude of farmers towards dairy cattle welfare based on the Likert scale of 1 (strongly disagree) to 5 (strongly agree) with internal consistency tested at Cronbach Alpha of 0.636. Section 5: ranking of own farm based on point system of 1 (flawed) to 10 (perfect). This section was used to assess their opinion on the major contributing factors towards the welfare of their cattle.

Statistical analysis
Data collected from the questionnaires were filled into Google Forms to assist in accurate and efficient data tabulation. The data was later transformed into Excel sheet. Some data were keyed in manually into Microsoft Excel 2016. IBM SPSS Statistics® Version 25 was used to perform statistical analysis to determine the association between variables (Chi-square) and bivariate correlation (Pearson's correlation) between them. A significant level of $p<0.05$ was assumed in both tests.

RESULTS

Dairy cattle welfare

Criteria
Respondents were asked about the criteria of dairy cattle welfare. Criteria chosen the most were morbidity rate (80%, 24/30), followed by changes in body weight, body condition and milk yield (73.3%, 22/30), and handling response (66.7%, 20/30). However, mortality and culling rate (36.7%,11/30), and complications from common procedures (50%, 15/30) were the least considered criteria, followed by physical appearance (53.3%, 16/30), behavior (56.7, 17/30) and reproductive efficiency (60%, 18/30). Sixteen of the 30 respondents (53.3%) were considered knowledgeable based on the criteria of DCW. They obtained a mean score of 4.77 out of 8 marks (SD=2.47).

Indicator
Poor indicators of DCW most recognised by the farmers were injury and reduction in feed intake (each 93.3%, 28/30), and sudden change in body condition (90%, 27/30). The least recognised indicators were isolation of cattle (43.3%, 13/30), conduct of veterinary procedures in milking parlors (53.3%, 16/30) and reduced lying time (56.7%, 17/30).

Overall knowledge of criteria and indicator
The farmers of Keningau, Sabah obtained a mean score of 12.1±3.47 of a total of 18 marks. Their total score was significantly associated with their level of education, work positions in their farm, and farm herd size. However, the score was not associated with experience on farm and the frequency of veterinarian visits to the farms. There was a significant positive correlation between the overall score and the farm performance in milk production (r=0.657, p=0.001). Farmers assessed using indicators of DCW appeared to be significantly more knowledgeable than when assessed using the criteria of DCW ($p<0.05$).

Attitude
In general, the majority of the respondents (60%, 18/30) disagreed with the statements on inappropriate practices and cattle behaviour. Thirty percent (9/30) showed neutral response while only 10% (3/30) agreed to the statements. Most

respondents agreed with the "Fat cows is a sign of good farm practice" and "Reluctance and kicking behavior by the cow during milking is normal". The farmers strongly disagree with the statement "Milk the cow as long as it produces milk".

Contributing factor

The median score on welfare of the animals was 8.00±1.83 out of 10 points. For most farmers, the main contributor to DCW was farm facilities (33.3%, 10/30), followed by workforce (16.7%, 5/30), and management practices and well-being of animals and production (each 10%, 3/30).

A small number of farmers (20%, 6/30) were grouped into miscellaneous since they either failed to state reason or answered unrelated reasons.

DISCUSSION

Awareness of dairy cattle welfare

The most popular criteria of DCW chosen by farmers were "Morbidity rate" and "Changes in body weight, body condition and milk yield". The least understood criterion was "mortality and culling rate". The second least understood criterion was "complications from common procedures". Based on their responses, the farmers showed an average of understanding of the factors affecting DCW. Farmers appeared to understand DCW better when assessed using indicators instead of criteria of DCW.

Generally, farmers with higher education had better knowledge on DCW. This was also shown to be true by others (Nizamuddin and Rahman, 2019; Clark et al., 2016). Farmers with professional positions on the farm also showed greater concern towards farm animal welfare.

Farmers from better performing farms and large herd sizes had good knowledge and understanding of DCW. The study also showed farms managed by these farmers had high milk yields.

Veterinarians can educate to improve the level of knowledge of and motivate farmers (Ritter et al., 2015). In fact, cooperation and open communication between farmers and veterinarians can improve farm performance (Sumner et al., 2018) Unfortunately, our study showed that farmer experience did not suggest that they would be more knowledgeable on animal welfare. This is presumed to be due to lack of knowledge transfer between veterinarians and farmers.

General attitude and perception of dairy cattle welfare contributors

Generally, the farmers in Keningau appeared to show appropriate attitude and perception of DCW. They generally considered that farm facility and workforce as the two most important contributors to DCW.

Body weight, body condition, and milk yield

Similar to a previous study (Algers et al., 2009), The majority of the farmers recognised that the reduction of milk production can be an indicator of poor DCW.

They agreed that sudden changes in body condition indicated a poor welfare since that is usually due to diseases. This may not be generally true because animals with high body condition score may be predisposed to lameness (Ristevski et al., 2017), reduced dry matter intake, and periparturient metabolic disorder (Roche et al. 2009).

Handling response

Most farmers may not have an appropriate understanding on how animal handling response while milking can be an indicator of poor DCW. Kicking, lifting and stepping during milking may be associated with mastitis rather a show of discomfort due to pain at handling. They also failed to recognise that carrying out veterinary procedures such as injections while in milking parlour could have negative impacts on DCW, because cattle become reluctant to enter the milking parlor at subsequent milking sessions (Broucek et al., 2017).

Interestingly, plastic pipes which is a tool widely used to herd cattle by local farms were not favoured by the farmers in this study. The rejection of this method of herding cattle is in agreement with a guideline developed by the Farm Animal Welfare Education Center (FAWEC).

Cattle behaviour

The farmers could recognise simple indicators commonly seen in poor DCW. In fact, the majority of them understood that reduction in feed intake can be an indicator of poor animal welfare (Polsky and von Keyserlingk, 2017).

Licking pen structures was identified as an indicator of poor DCW by the farmers. Excessive licking and grooming can be a sign of mineral deficiency as cattle with salt deficiency often show signs of craving or abnormal appetite for salt. They tend to lick various objects such as rocks, wood, soil and even sweat of other animals in an event of salt deficiency. These behaviors are more prominent in calves. Calves without supplementary mineral would spend more time grooming, licking pen structures and ear sucking (Berger, 2006).

Appropriate amount of grooming by cattle is a favorable behaviour. Mutual and self-grooming are associated with hygiene and comfort among cattle (Algers et al., 2009) while reinforcing and stabilising social relationships (Napolitano et al., 2009). However, in this study, the farmers failed to recognise that dairy cattle are herd animals that need the company and interactions of the same species. Farmers also failed to recognise the importance of the lying time duration for cattle. Reduction in lying time can predispose cattle to ailment such as lameness (Ristevski et al., 2017; Sadiq et al., 2017).

Physical appearance

The majority of farmers agreed that hydration status of dairy cattle is an indicator of DCW. Almost all agreed that injuries are indicators of poor DCW (Costa et al., 2013). Injuries of the limbs especially at the hock region are significantly correlated with lameness that can causes discomfort to cattle (Webster, 2005; Sadiq et al., 2017).

The farmers also recognised that the presence of excessive faeces is an indicator of poor DCW, although a few believed that faeces on the body of cattle is normal.

CONCLUSION

This is the first study in the region on the level of knowledge and attitude on dairy cattle welfare among dairy cattle farmers in Keningau, Sabah, Malaysia. The farmers in the region had a satisfactory level of awareness on DCW. They had average understanding of basic criteria and indicators of DCW. Farmers with higher education, handling farms with large herd size, and with high milk yield tended to have higher level of knowledge on DCW.

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EFFECT OF NESTING SITE SELECTION ON FUNGAL INFECTION RATE AND HATCHING SUCCESS OF GREEN TURTLES

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ABSTRACT

Green turtle, *Chelonia mydas*, is an endangered marine chelonian. The beaches of Chagar Hutang on Redang Island and Turtle Bay on Lang Tengah Island beach are large nesting site for green turtles in Malaysia. However, the fungal colonisation of the nests is a major embryonic mortality factor for turtles. The study was conducted at Chagar Hutang and Turtle Bay beaches to determine the association between site selection, distance to sea and to vegetation, and the effect of distance of turtle nests to vegetation on fungal infection rate and turtle egg hatching success. The majority of the turtle nests were <1 m to vegetation, suggesting turtles prefer to nesting near vegetation. Nesting near dune vegetation had facilitated egg chamber construction as the roots system stabilises beach sub-stratum. Vegetation zone provides shades allowing for cooler nest temperature similar to pivotal temperature, and less temperature fluctuation during incubation. Most nests were positioned at 20 to 30 m from the sea. Nests too close to tide lines were prone to wave wash while those too far inland may cause hatchling misorientation and higher predation risk. There was a significant association ($p<0.001$) between distance of nest to vegetation and fungal infection rate at Chagar Hutang but not at Turtle Bay beach ($p>0.05$). The higher fungal density in nests at Chagar Hutang beach is proposed to be due to the presence of two streams on rainy days, resulting in higher relative humidity. The root systems at the vegetation zone catch water creating an environment that favours fungal growth.

Keywords: turtle, Chagar Hutang, Turtle Bay, nest site selection, distance to sea, distance to vegetation, fungal infection rate, hatching

INTRODUCTION

Nest site selection is important for sea turtles for survival. Nest success is heavily dependent on the suitability of nest site selected by female turtles. Beach vegetation is one of the important factors of nest site selection for sea turtles. The vegetation consists of herbaceous and shrub species that tolerate extreme conditions with mobile system from sand movement and wide fluctuations in the sand temperature, soil

salinity, and wind velocity. Dunes with vegetation are the main sites selected by turtles to ensure nesting success (Zavaleta-Lizárraga and Morales-Mávil, 2013).

Fungal disease is a threat to the survival of wildlife species, including turtles (Fisher et al., 2012). The presence of fungus in nests contributes to turtle egg failure (Limpus et al., 1983). This was evident by the isolation of fungi from soil at nesting sites, and exterior and/or interior of unhatched eggs and embryonic tissue of the sea turtle eggs (Phillott et al. 2001). Among factors that contributes to turtle nest fungal infection is beach vegetation. The presence of vegetation increases relative humidity and decomposed organic material in sand.

This study determined effect of location on fungal infection of turtle nests.

MATERIALS AND METHODS

The study was conducted at beaches on the Chagar Hutang, Redang Island and Turtle Bay, Lang Tengah Island, Malaysia. These beaches are private and free of human activity such as site-seers, egg poaching, turtle capture, or tourism. Chagar Hutang is the biggest primary green turtle nesting site in Malaysia while Turtle bay is a major relocation site of turtle nests from Lang Sari beach.

Nesting site selection

The beach is patrolled every night to track of nesting turtles. The turtles were allowed to dig sand and lay eggs without being disturbed. All nests were marked by a wooden stick with coded tag at the front edge of the clutch. Once turtles had returned to sea after laying eggs, the distance of nest from high water mark and to nearest vegetation are measured.

Sand temperature

Temperature data loggers placed at open beach and shaded areas near vegetation at Chagar Hutang's nesting beach to be as controls and for measuring the ambient temperature of sand at the two nesting sites. Each control data logger was buried 40 cm beneath the sand surface, and the sand temperature of the hatcheries recorded every 3 h for 4 days.

Hatching success and fungal infection rate

Nest checks are done every 3 days after 45 days of oviposition. Nests are excavated by hand after the emergence of hatchlings. Hatching success is determined as the ratio of the number of live hatchlings (that had emerged or were still in the nest when excavated) to the total number eggs in the clutch using the following equation:

$$\text{Hatching success (\%)} = \frac{\text{Empty shells}}{\text{Empty shells + unhatched eggs}} \times 100$$

Fungus infected eggs are identified as unhatched eggs with black discolouration. The fungal infection rate was calculated as the ratio of fungus infected eggs to clutch size using following equation:

$$\text{Fungal infection rate (\%)} = \frac{\text{Fungal infected eggs}}{\text{Empty shells + unhatched eggs}} \times 100$$

Statistical analysis

Statistical analyses were conducted using SPSS Independent t-test and correlation model to evaluate the relationship between nest-to-vegetation distance and hatching success. All data are reported as mean ± SD.

RESULTS AND DISCUSSION

Nesting site selection

Figure 1 shows the nest distribution on Chagar Hutang, Redang Island with respect to distance to sea. Most nests were located at 20 to 30 m distant from the sea. The average of turtle nest from the water edge was 24.51±6.74 m. Nests located close to the ocean had higher possibility of being inundated with sea water and egg loss from beach erosion. Nests located farther inland had a greater likelihood of being desiccated, produce misoriented hatchling, and exposure to predators (Wood and Bjorndal, 2000).

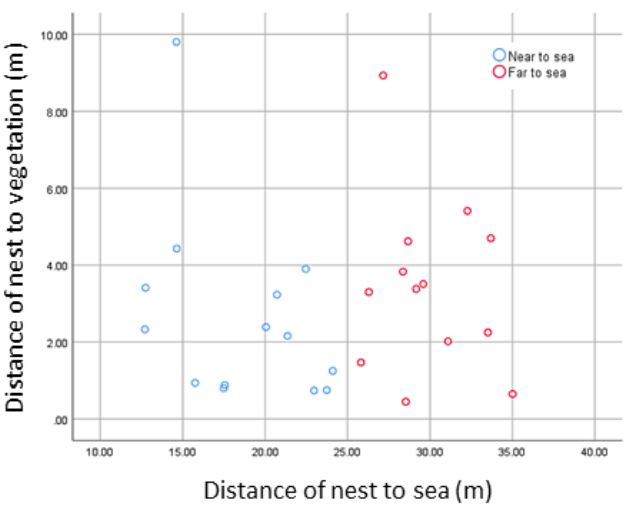

Figure 1. Nest distribution at Chagar Hutang, Redang Island with respect to distance to sea, using 50th percentiles.

The mean distance of turtle nests to vegetation was 2.89±2.30 m. Most nests were close to vegetation at a distance of <1 m (Figure 2), suggesting that turtles prefer

vegetated sites. Nest near dune vegetation are shady and cooler than those at the open beach area. The shade provided by vegetation is pivotal to the maintenance cool nest environmental temperature and for the production equal number male and female offsprings.

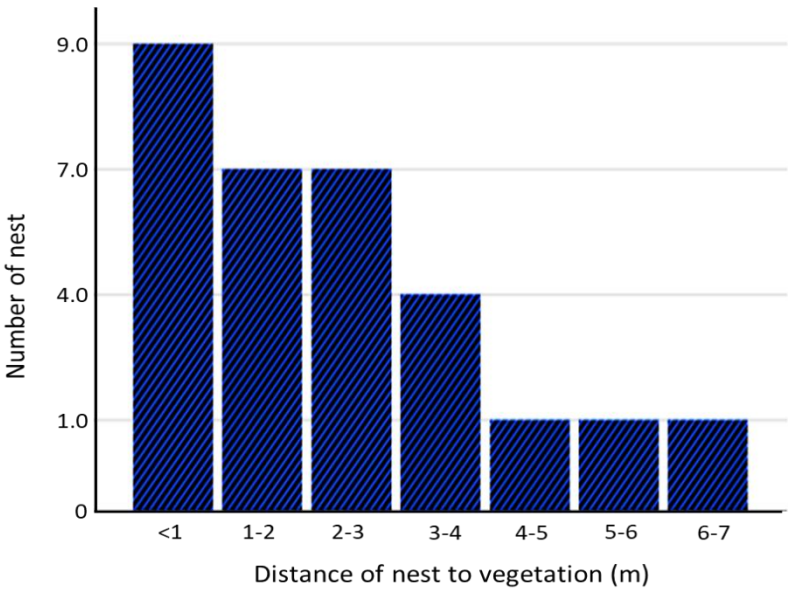

Figure 2. Nest distribution at Chagar Hutang beach with respect to distance from vegetation.

Hatching success and fungal infection rate

Total number of nests was 44 at Chagar Hutang and 25 at Turtle Bay beach. The mean incubation period was 52.57 ± 2.56 days at Chagar Hutang and 57.27 ± 3.60 days at Turtle Bay beach. The difference in incubation period may due to incubation temperature differences between the 2 beaches. The nest at Chagar Hutang were both in shaded and open sand areas, while at Turtle Bay most of the nests were relocated nests to shaded area near vegetation. At Chagar Hutang beach, the temperature 40 cm below the surface of open sand was 30.29 ± 0.64 °C while in the shaded area it was 27.75±0.88°C. At Turtle Bay beach, all of the nests were in shaded area with sand temperature of 28.36 ± 0.54°C.

The hatching success in Chagar Hutang averaged 77.82 ± 19.68%, which was lower than that produced at Turtle bay at 90.27 ± 7.51%. Fungal infection rate in Chagar Hutang averaged 14.38 ± 15.53%, which was much higher than at Turtle Bay at 1.79 ± 3.99%. It seems that the hatching success is negatively correlated with distance of nest to vegetation. The fact that all turtle nests at Turtle Bay beach were located near vegetation, accounts for the higher hatching success of turtle eggs at this beach than at Chargar Hutang.

The sand fungal density at Chagar Hutang was higher than at Turtle Bay beach. Chagar Hutang has two streams flowing from dune vegetation into the sea. On rainy

days, the sand water table level at Chagar Hutang rises and increasing relative humidity at the beach, especially near vegetation zone, where the root system catch water. The damp condition of the sand promotes the growth of fungus at the nests and affecting the hatching success of the eggs.

CONCLUSION

Green turtles have preferences for the location of their nest with respect to the distance to the sea water line and vegetation. More green turtle nests are within 20 to 30 m from sea while < 1 m away from vegetation. Nest close to vegetation at Chagar Hutang beach had higher fungal infection rate which had adversely affected the hatching rate of turtle eggs.

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RISK FACTORS OF OBESITY IN NEUTERED CATS PRESENTED TO UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Overweightness and/or obesity are among reasons discouraging cat owners from neutering their cats. The current study aimed to retrospectively determine the risk factors for obesity in neutered cats at University Veterinary Hospital (UVH), Universiti Putra Malaysia for years 2016 to 2018. A total of 80 clinically healthy cats aged ≥6 months, with complete follow-up history and within 6-12 months post-neutering were admitted for ailments, adjudged not to adversely affect body weight, were included in this study. Information on patient history, including breed, age, gender, household information, management, nutrition, date of neutering, complaints upon follow-up and other information concerning owner-pet relationship were obtained. The pre- to post-neutering weight changes of each cat were recorded. The study showed that gender and age exert a significant effect on pre- to post-neutering weight changes among cats. Cats neutered after 9 months old did not become overweight, whereas those neutered between 5 to 9 months old, especially among females, were 1.65 times (95% CI 1.02 to 2.67) more likely to become overweight. In fact, female cats neutered at an older age were 3.28 times (95 % CI 1.93 to 5.56) more likely than males to become overweight. There is a statistically significant association ($p<0.05$) between cats managed indoor and overweightness and/or obesity. Other factors such as breed, size of cat household, and type of feeds did not contribute to post-neuter weight changes ($p>0.05$). In conclusion, age at neutering, gender of the cat, and whether they were kept indoors or outdoors were significant determinants ($p<0.05$) of overweightness and/or obesity in neutered cats.

Keywords: neutering, cat, overweight, obesity gender, age

INTRODUCTION

Obesity is the most common nutritional disorder among companion animals. It is one of the main animal welfare concerns and a medical issue for dogs and cats. In companion animals, obesity have reached pandemic proportions with 30 to 40% of dogs and cats being overweight to obese. In cats, the underlying cause do obesity is

imbalance between energy intake and energy expenditure, causing the excess energy to be stored as fat. Dietary practices and habits such as feeding of snacks, feeding frequency, and type of food are risk factors for energy imbalance (Fettman et al., 1998; Hoelmkjaer and Bjornvad, 2014). Other risk factor for obesity in cats include genetics, gender, age, indoor living, inactivity, and multi-cat household (Scarlett et al., 1994). Underlying diseases or disorders, for example orthopedic disorders impeding cat activity, may also contribute to feline obesity (Bjornvad et al., 2014).

Neutering is the most common surgical procedure performed on cats. Owners are generally encouraged to neuter their pets when they are 5 months to one year of age. Neutering at early an age of 7 weeks old will protect cats from serious health problems later in life. However, neutering together with increase in food intake, decreased activity and imbalanced energy are risk factors for feline obesity.

This study was undertaken retrospectively to determine the risk factors associated with obesity in neutered cats.

MATERIALS AND METHODS

A retrospective study was done by reviewing medical records of cats admitted for neutering at the University Veterinary Hospital (UVH), Universiti Putra Malaysia UPM from years 2016 to 2018. Clinical healthy cats (n=80) aged ≥6 months with complete follow-up history for the first time within 6 to12 months post-neutering, and readmitted for ailments, adjudged not to adversely affect the body weight, were included in this study. A small number of cats had two or more follow-ups after the first visit. Information on the patient including history, breed, age, gender, household information, management, nutrition, date of neutering and complaints upon follow-up, and owner-pet relationship were obtained. The pre- to post-neutering weights and weight difference for each cat were recorded.

Statistical analysis

The risk analysis method in SPSS software was used to determine the association between risk factors and obesity in neutered cats. All parameters were subjected to descriptive statistical analyses. Effects of age group, gender, breed, household cat number, indoor or outdoor housing, and nutrition on their post-neutering weight gain were analysed using one-way analysis (IBM-SPSS Inc, USA). Significant differences among means were determined at $\alpha=0.05$.

RESULTS AND DISCUSSION

Among the cats, 57.4% (46/80) presented for neutering were of ages between 5 to 9 months, 42.6% (34/80) aged > 9 months old, 43.6% (35/80) were male and 53.8% (43/80) were females. The results showed that gender, neutering age, and indoor management of cats exert a significant effect ($p<0.05$) on post-neutering weight gain in cats. Based on risk analysis cats, especially females, neutered after 9 months old

are did not become overweight or obese, whereas those neutered between ages 5 to 9 were 1.65 times (95% CI 1.019 to 2.67) more likely to become overweight or obese. In fact, female cats neutered at older ages were 3.28 times (95 % CI 1.93 to 5.56) more likely to become overweight compared to males. There is a significant association between cats managed indoor and overweightness and/or obesity ($p \leq 0.05$). Other factors including breed, size of cat household, and type of feeds did not contribute to post-neutering weight gain ($p > 0.05$).

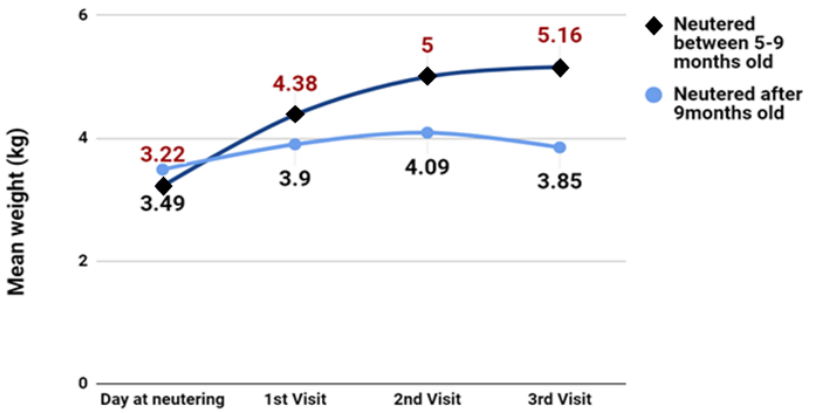

Figure 1. Post-neutering body weights in cats. Cats neutered between ages of 5 to 9 months old showed significantly greater ($p<0.05$) post-neutering weight gain than those neutered after 9 months old.

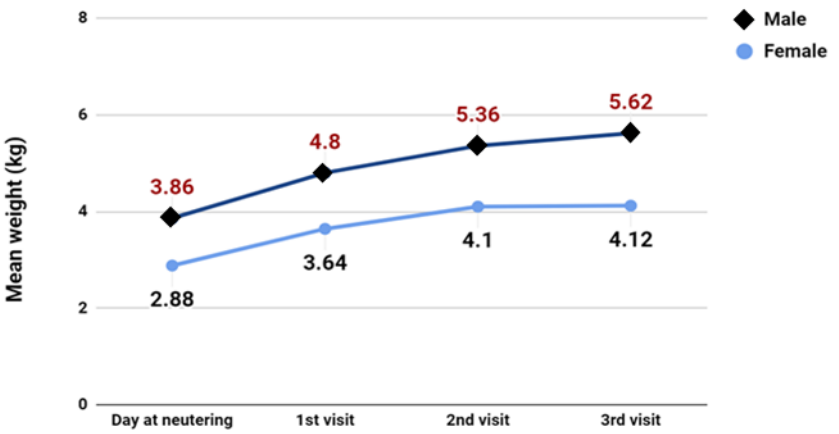

Figure 2. Post-neutering body weights in male and female cats. Female cats neutered at older ages showed significantly greater ($p<0.05$) post-neutering body weight gain at first follow-up visit.

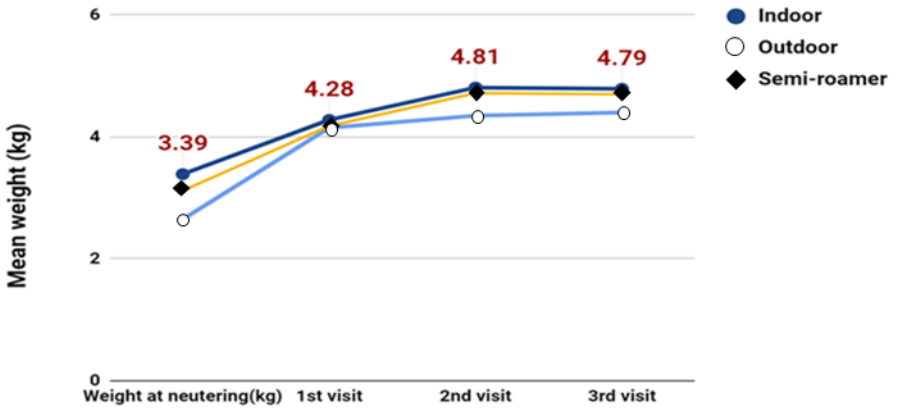

Figure 3. Body weights of indoor, outdoor, and semi-roamer post-neutered cats. Cats managed indoor show the greatest ($p<0.05$) post-neutering body weight gain.

CONCLUSION

Neutering contributes to overweightness and/or obesity in cats. Among risk factors to post-neutering obesity in cats, age at neutering, gender, and management of cats were the most significant. These risk factors are complicated by increase in food intake, low energy expenditure, and hormonal influences. Therefore, owners should be educated on how to manage neutered cats to protect them from overweightness and obesity.

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EVALUATION OF SUBCHONDRAL BONE MICROMORPHOLOGICAL CHANGES IN CHEMICAL- AND SURGICAL-INDUCED OSTEOARTHRITIS IN KNEE JOINTS

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ABSTRACT

Osteoarthritis is a complex disease that affects the synovial joint. Currently, there is no definitive diagnosis workup to diagnose early osteoarthritis. The objective of this study was to determine the micromorphological changes in subchondral bone by determining bone-to-tissue volume (BV/TV), bone surface density (BS/TV), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) in induced osteoarthritis. Thirty-five adult New Zealand white rabbits were equally divided into one control and 6 experimental groups comprising; monosodium iodoacetate (MIA)-induced and surgery-induced osteoarthritis (OA), each with week 4, 8, and 12 groups. Right knee joints from euthanised rabbits were harvested and evaluated using micro-computed tomographic for the changes in the subchondral bone. The results showed significantly lower ($p<0.05$) BV/TV in bone joints of treatment than control rabbits at weeks 8 and 12. This study showed that bone remodeling has occurred in treatment rabbits and the development of AO differed between MIA- and surgery-induced AO in rabbits.

Keywords: osteoarthritis, anterior cruciate ligament, monosodium iodoacetate, micro-computed tomography, rabbits

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis with active anabolic and catabolic processes and it involves all joint tissues (Neogi, 2012). Meniscus, articular cartilage, subchondral bone, and synovial membrane are the four components of the synovial joint involved in this pathology of osteoarthritis (Kuyinu et al., 2016). The pathological changes in OA joints include degradation of articular cartilage, thickening of subchondral bone, formation of osteophytes, variable degrees of synovium inflammation, degeneration of ligaments and menisci, and joint capsule hypertrophy (Loeser et al., 2012; Kuyinu et al., 2016).

Osteoarthritis can be divided into primary, idiopathic and secondary osteoarthritis based on the etiology (Kuyinu et al., 2016) including trauma, hereditary or bone

metabolism disorders. Various techniques have been are used to induce OA in animal models, including chemical-induction with papain, collagenase, sodium monoiodoacetate (MIA) or surgical induction by transecting the anterior cruciate ligament (ACL), meniscectomy and meniscal destabilisation, medial collateral ligament and posterior cruciate ligament and genetic modifications (Kim et al., 2018). In rabbits, the most common technique used to induce OA in rabbit is by rupturing of the anterior cruciate ligament (Gregory et al., 2012).

Imaging modalities typically used to examine osteoarthritis in humans include radiography, magnetic resonance imaging (MRI), micro-computed tomography (micro-CT), and ultrasound. The current gold standard for diagnosing osteoarthritis in a clinical setting is conventional radiography (Okano et al., 2019).

This study was conducted to determine the micromorphological changes in subchondral bone in induced osteoarthritis in rabbits.

MATERIALS AND METHODS

Thirty-five rabbits used in this study was divided into 7 equal groups; control, MIA week 4, 8, and 12 and anterior cruciate ligament (ACLT) week 4, 8, and 12.

Osteoarthritis was induced with 25 mg/mL MIA (Sigma-Aldrich, USA) in saline solution. Five milligram per joint MIA was injected intra-articularly into the right tibiofemoral joint on day 1 and 7.5 mg/joint MIA on day 4. Induction of osteoarthritis was done under general anaesthesia using Zoletil® (Virbac, Australia) induced with 1.5 mg/kg body weight via intramuscular route. Induction of ACLT injury was done under general anaesthesia via intramuscular route with 3mg/kg body weight Zoletil® (Virbac, Australia) and maintained with gas isoflurane. The anterior cruciate ligament in the right stifle joint was transected to induce osteoarthritis. Control rabbits were non-treated.

Sample preparation

Five treatment rabbits each of the MIA and ACLT groups were euthanised either at week 4, 8, or 12 using 200 mg/kg body weight pentobarbital sodium and knee joints harvested. The joints were immediately fixed in 10% buffered formalin (Sigma-Aldrich, USA). After 24 hours, the muscle and tissue surrounding the femur and tibia were carefully removed without damaging the cartilage surface. The proximal femur and distal tibia were placed in 10% buffered formalin for micro-CT evaluation. The joints of the control rabbits were similar treated at the end of the experiment.

Micro-computed thermography

The distal of femur and proximal of tibial were scanned using Skyscan 1076 micro-CT scanner (Skyscan, Belgium). Scanning parameters were set at 70kV and 110µA, with a pixel size of 18µm. The X-ray projections were set at 0.9 degrees angular step with a scanning angular range of 360°. Reconstructions of the image slices were performed with Skyscan NRecon software (Skyscan, Belgium).

With the use DataViewer software (Skyscan, Belgium), two-dimensional images were evaluated qualitatively in both dorsal and sagittal planes to determine morphological alteration. The lining of the joints and presence of osteophytes were noted and described.

Micro-CT images were interrupted quantitatively by using the Skyscan CT-Analyser Software (Skyscan, Luxembourg, Belgium). Volume of interest (VOI), consisting of a stack of regions of interest (ROI) was selected from the distal femoral and proximal radius subchondral bone at the epiphyseal with a semiautomatic contouring method. The subchondral bone micro-architecture parameters, the ratio of bone volume over tissue volume (BV/TV), bone surface density (BS/TV), trabecular thickness (Tb.Th), and size of marrow cavities described by trabecular spacing (Tb.Sp), were determined.

Statistical analysis

Statistical analysis was performed using IBM SPSS 16.0 (IBM, USA) statistical software package. Statistical significance was determined using two-way MANOVA followed by Tukey HSD's post hoc test. Differences between the groups were considered significant at $p<0.05$. The data analysis was separated into data from bone micro-architecture of femur and tibia. Data from both micro-architectures were compared to look for a similar pattern in conjunction with changes in early onset OA.

RESULTS AND DISCUSSION

Qualitative bone micro-architecture assessment

The values for the bone micro-architecture parameters for the MIA and ACLT treatment groups are shown in Table 1 and 2. Figure 1 illustrates the progression of OA after the induction of OA.

Bone micro-architecture

Based on changes in bone parameters, MIA appeared slightly better at inducing AO in the femur and tibia of rabbits than surgery. The BV/TV decreased significantly ($p<0.05$) at weeks 8 and 12 from control values, both for the MIA and ACLT groups. For other parameters, although there were decreasing trends, did not show significant difference ($p>0.05$) with time.

Qualitative morphological findings

The bone joints of rabbits induced to develop OA with MIA did no show significant change. However, there were irregularities on the joint surface with the presence of slight sclerosis and osteophyte formation. These changes indicate early OA of the joints.

The development of OA in the ACLT were as clear as in the MIA rabbits. However, there was more obvious thickening of subchondral bone plate in the ACLT than the MIA rabbits.

Table 1. Microarchitecture parameters of the subchondral bone of right stifle joint of femur and tibia of rabbits with monosodium iodoacetate-induced osteoarthritis

Treatment Group	BV/TV	BS/TV	Tb.Th	Tb.Sp
Femur				
Control	35.68^{a}±2.89	6.43±0.29	0.18±0.01	0.50±0.05
Week 4	31.57^{ab}±6.03	6.10±0.79	0.17±0.01	0.54±0.12
Week 8	30.89^{b}±2.55	5.67±0.55	0.18±0.01	0.59±0.09
Week 12	27.12^{b}±4.39	5.32±0.91	0.18±0.01	0.65±0.18
Tibia				
Control	34.90^{a}±5.04	6.94±0.43	0.18±0.01	0.53±0.05
Week 4	30.88^{a}±6.56	6.58±1.11	0.17±0.01	0.54±0.11
Week 8	30.18^{b}±1.91	6.24±0.27	0.18±0.01	0.60±0.07
Week 12	26.82^{b}±3.50	5.92±1.01	0.17±0.01	0.61±0.14

Values are mean±standard deviation. BV/TV=bone to tissue volume; Means with within column with different superscripts are significantly different ($p<0.05$). BS/TV=bone surface density; Tb.Th=trabecular thickness; Tb.Sp=trabecular separation.

Table 2. Microarchitecture parameters of the subchondral bone of right stifle joint of femur and tibia of rabbits with surgically-induced osteoarthritis

Treatment group	BV/TV	BS/TV	Tb.Th	Tb.Sp
Femur				
Control	35.68^{a}±2.89	6.43±0.29	0.18±0.01	0.50±0.05
Week 4	35.31^{ab}±3.16	5.98±0.37	0.19±0.01	0.54±0.07
Week 8	32.80^{bc}±5.43	6.38±0.81	0.17±0.01	0.50±0.09
Week 12	35.24^{ad}±2.22	6.56±0.86	0.18±0.01	0.50±0.10
Tibia				
Control	34.90^{a}±5.04	6.94±0.43	0.18±0.01	0.53±0.05
Week 4	33.94^{a}±3.39	6.67±0.60	0.18±0.01	0.53±0.08
Week 8	32.24^{b}±5.35	6.79±0.65	0.17±0.01	0.54±0.10
Week 12	32.83^{ac}±1.97	6.83±0.61	0.17±0.01	0.51±0.04

Values are mean±standard deviation. BV/TV=bone to tissue volume; Means with within column with different superscripts are significantly different ($p<0.05$). BS/TV=bone surface density; Tb.Th=trabecular thickness; Tb.Sp=trabecular separation.

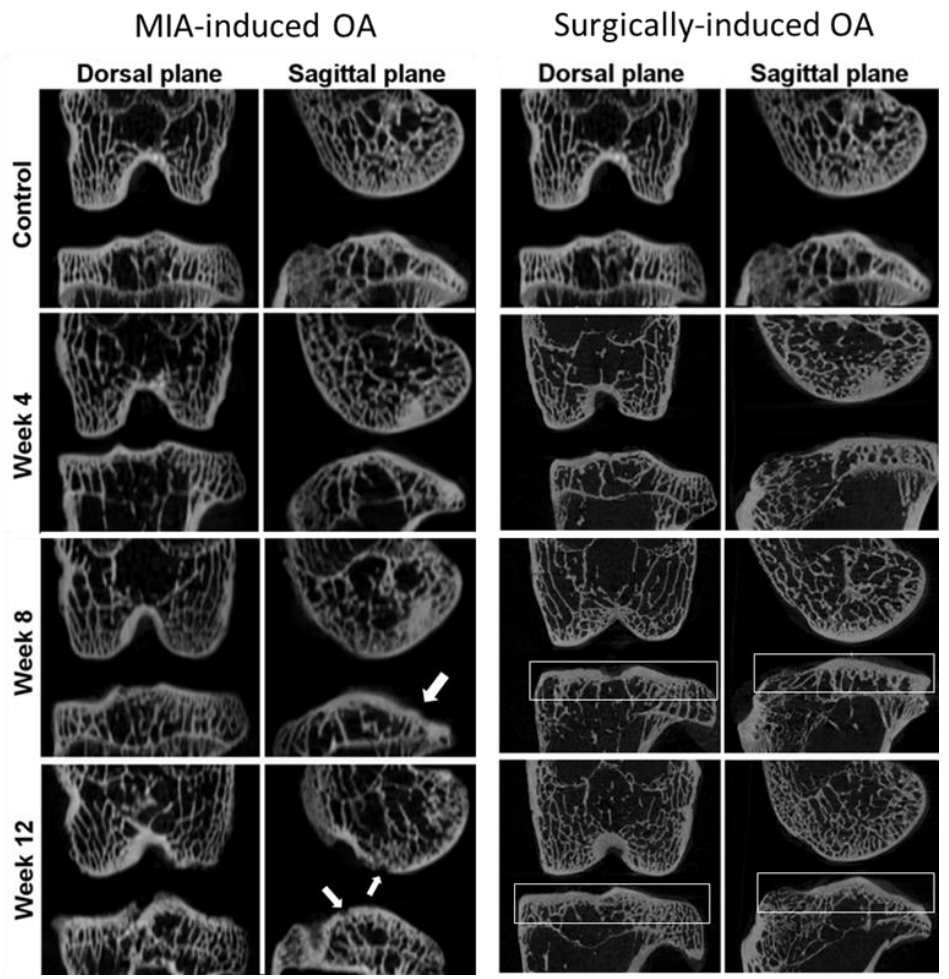

Figure 1. Micro-computed tomography of bone joint induced to develop osteoarthritis (OA) with monosodium iodoacetate (MIA) and surgically (ACLT). MIA-induced OA showed irregularities with slight sclerosis (thin arrows) and osteophyte formation (thick arrow). The ACLT joints showed thickening of subchondral bone plate (white box).

The occurrence of bone resorption indicates early OA, characterised by reduction in trabecular thickness, bone volume and mineral, and increase of trabecular separation. Late OA is characterised by bone formation; the subchondral bone plates thickens but cancellous bone remains osteopenic (Burr and Gallant, 2012). The amount of bone increases but mineral density decreases in late OA and this will cause less tissue stiffness and increasing structural stiffness. The bone becomes under-mineralised at late OA, probably due to defect in osteoblast function.

The MIA- and surgery-induced OA in rabbits obeyed the general pathogenesis of AO development. However, MIA-induced AO mimics early while surgery-induced

OA mimics late OA, suggesting that the mechanisms of OA their developments are different. In research, for example studies on therapeutic interventions, MIA-induced OA is most often used because of the convenience of the method.

CONCLUSION

The mechanisms in the development of MIA- and surgery-induced OA, although obeying the general pathogenesis of the disease, differs slightly. Both methods of AO induction resulted in bone remodelling. It appears AO from surgical induction developed faster than than from MIA-induction. The most significant change in bone morphology is decrease in BV/TV. Osteoarthritis induced by MIA mimicked early OA while that induced surgically mimicked late OA.

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STRESS INTENSITIES OF CATS IN THE CLINICAL ENVIRONMENT

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ABSTRACT

Visits to the veterinary clinic is thought to be a highly stressful event for cats. In fact, stress can affect physiologic parameters which are vital information in the clinical assessment of a patient. Prolonged stress can have adverse effects on immunity, general health and behaviour. Therefore, the objectives of this study were to determine the intensity of stress, based on behavioural signs in cats in a clinical environment and the contributing factors. The study population consisted of 35 cats presented to the University Veterinary Hospital (UVH), Universiti Putra Malaysia for veterinary attention. Stress were monitored remotely using the behavioural score; 1 (relaxed) to 6 (terrified). Patient data, signalment, total time at the consultation room, and distance travelled from home to UVH, were recorded and analysed. The results showed that transportation distance is a significant determinant of stress levels in a clinical environment. Age, gender, breed, specific handling events, duration of consultation, presenting complaints and procedures performed had no effect on the stress level of cats ($p>0.05$). Cats showed the lowest stress level when first taken out of the cage and the highest at physical examination. The stress level of cats when returned to their cage was moderate. It can be concluded that cats were stressed in a clinical environment, especially during physical examination.

Keywords: cat, stress, clinical environment

INTRODUCTION

Veterinary clinic visits are stressful for cats. In fact, physiologic parameters such as blood pressure, rectal temperature, heart rate, and respiratory rate, which are vital for the clinical assessment of a patient, can be affected by stress in a clinical environment (Quimby et al., 2014). Prolonged stress can have adverse effects on the behaviour, general health, and immunity of animals (Blecha, 2000).

How owners perceive stress that affects their pets is a major determinant that either encourage or discourage them from seeking veterinary consultation at clinics. Generally, fewer cat than dog owners bring their pets for regular veterinary visits. The main reason is that they feel the whole process of veterinary visits is stressful

for them and their cats (Lloyd, 2017). Cats has been shown to hide when they see a cat carrier; causing them to physically resist and act aggressively. They also vocalise loudly during transportation to the clinic. These patients display signs of fear and stress while in the clinic waiting area, particularly in the presence of unfamiliar animals, especially dogs. Cats are also known to show physical signs of tension during the examination, and they act remotely and unfriendly for several days after each episode.

It is important to minimise stress levels of patients so that they show normal behaviour to allow for effective handling and monitoring and minimise stress. Controlling and minimising feline stress will also ensure the safety of clinic staff and clients from the uncontrolled behaviour of stressed cats (Trevorrow, 2013), while encouraging owners to revisit the clinics. Therefore, the current study was conducted to determine the level of and contributing factors to stress in cats in a clinical environment.

MATERIALS AND METHODS

The current study was conducted on 35 feline patients presented for outpatient veterinary medical care at the University Veterinary Hospital (UVH), Universiti Putra Malaysia. Stress was monitored remotely using the behavioural score published by Kessler and Turner (1997) and Nibblet et al., (2015). There was no physical contact between the researcher and the cat subjects during the behavioural scoring sessions. Cats were scored as according the range of 1(Relax) to 6 (Terrified). Behavioural scores were conducted at fixed handling events, that is, when taking cat from cage, physical examination, consultation, and at the return of cats to their cages. Patient data collected including signalment, time at consultation, complaints, and procedure(s) performed, and transportation period from home to UVH.

Statistical analysis

The data was analysed using two-way ANOVA and Kruskal Wallis test (SPSS Version 22.0, IBM SPSS Inc, USA) for non-parametric datasets. All statistical analysis was performed at 95 % confidence level.

RESULTS

The results showed that transportation distance is a significant determinant of stress levels in clinical environments ($p<0.05$), even after resting in the UVH reception area. Age, gender, breed, specific handling events, duration of consultation, presenting complaints, and procedures performed had no effect on the stress level of cats ($p>0.05$). Cats showed the highest stress level at the onset of the physical examination, and the least when they were first taken out of the cage. The level of stress level of cats when being returned to the cage was in between these two events.

Thus, it can be concluded that cats were stressed in a clinical environment, especially during physical examination.

Table 1: Behaviour scores for cats subject to veterinary procedures, with respect to transportation distance, at University Veterinary Hospital, Universiti Putra Malaysia

Transportation distance	Behaviour Score		
	When taken from age	During Consultation	When returned to cage
Near	2.667[a] ±0.230	2.889 ±0.230	2.778 ±0.230
Average	2.750[a] ±0.199	3.417 ±0.199	3.167 ±0.199
Far	3.143[b] ±0.184	3.429 ±0.184	3.357 ±0.184

Values are mean ± standard deviation.
[a,b]Mean with different superscripts within column are significantly different ($p<0.05$).

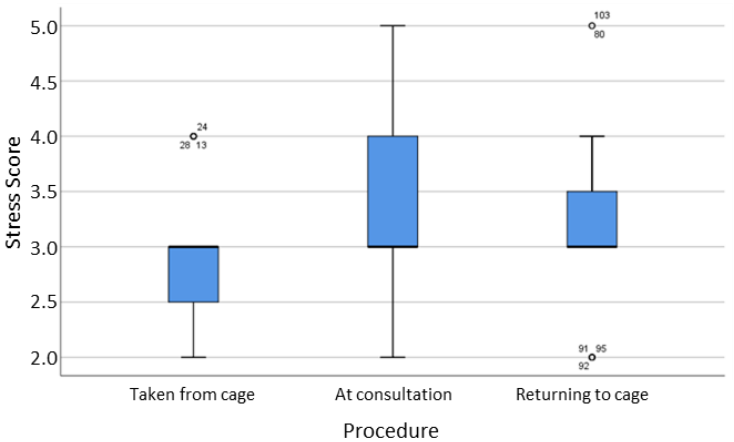

Figure 1: Stress level in cat subject to clinic procedures at the Universiti Veterinary Hospital, Universiti Putra Malaysia.

DISCUSSION

This study assessed the intensity of stress and determined the potential factors contributing to stress in a clinical environment. Distance of transportation was an important determinant of stress level in cats. Most cats are rarely transported and when they are transported, often to unpleasant places such as veterinary clinics. Hence, they usually find transportation frightening and stressful. Transport stress may be reduced by introducing familiar smells, pheromones, food supplements, enjoyable distractions, and baskets or carriers as hiding places, and minimisation of

movement during the journey. In fact, cats should be subjected to transportation training to reduce stress during the car rides (Pratsch et al., 2018).

Veterinarian should make visits of patients to veterinary clinics a pleasant experience. The first visit to the clinic is potentially the most important experience, because it could set the stage to how the animal will react in future visits.

The duration of the cat staying in the consultation room did not appear to significantly affect their stress level. Some cats may spend longer waiting while others may need extended physical examinations. Hence, the stress level may vary according to the procedure or process they have to undergo.

Cats showed the highest stress level at the onset of the physical examination, and less when they were first taken out of the cage and lowest when returned to their cages. Handling during examination can affect their stress level. This further underlines the importance of gentle and appropriate handling of cats. Veterinary clinics should be aware of the needs of the patients during routine care. Thus, staff of veterinary clinics must be well-trained in the art of handling animals during consultation and treatments to minimise stress in patients and avoid incidents. Among means of reducing stress in cats is to gently place hands close to the cat to allow them to sniff and rub. Towels can be used when needed, to allow the cat to hide in during the handling and examination. The surfaces where cats are placed should of a material that allows them to grip for security. When cats appear fearful or anxious during an examination, the handle should step away and allow the cat to settle down again before continuing with the procedure.

CONCLUSION

Cats are indeed stressed in clinical environments, especially during physical examinations. Transportation is a significant contributing factor to stress in cats while in veterinary clinics.

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ASSOCIATION BETWEEN LAMENESS AND REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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ABSTRACT

Lameness in dairy cattle is known to cause substantial economic losses to the farmers and the dairy industry through decreased milk production, low reproductive performance and weight loss. This study was conducted to determine the association between lameness and selected reproductive parameters of dairy cows from a private farm in Johore, Malaysia. A total of 67 milking cows were assessed for lameness using the locomotion scoring system of LS 1 to 5. Cows with LS2 to 5 were further examined for hoof disease identification. The reproductive performance traits were also recorded, which included calving interval, calving to conception interval, number of services per pregnancy, and pregnancy status. A self-filled questionnaire on management system and hoof care programme was also given to the farm manager. The study revealed that the prevalence of lameness (LS>1) in the herd was 15% (10/67). The hoof diseases identified were sole ulcer (9%; 6/67), white line disease (4.5%; 3/67), and interdigital dermatitis (1.5%; 1/67). The calving interval, calving to conception interval, and number of services per pregnancy was 428±141 days, 140±31 days, and 2.8±1.1, respectively. The pregnancy rate for LS1 cows was 9% (5/57), while those with LS ≥2 were not pregnant. There was no significant association ($p=0.053$) between lameness and number of services per pregnancy. In conclusion, although the prevalence of lameness was high in the farm, it was not significantly associated ($p>0.05$) with reproductive performance. Therefore, further studies using larger sample sizes are required to conclude on the association between lameness and the reproductive performance of dairy cattle.

Keywords: dairy cows, lameness, locomotion scoring, reproductive performance, Malaysia

INTRODUCTION

Dairy production is a fast-growing sector in Malaysia. The industry is however, affected by production-limiting factors, particularly mastitis and lameness in cows. Lameness is among important causes of economic losses in the dairy industry (Ózsári, 2017). By definition, lameness is a clinical manifestation of pain from

impaired mobility and abnormal gait and posture, which are mostly related to the locomotion disorders (Van Nuffel et al., 2015). Affected cows also experience lower feed conversion ratio and weight gain, which then cause reduction in milk yield and body condition score, thereby affecting reproductive performance.

Currently, there is no data in Malaysia on lameness and reproductive performance in dairy cows. Hence, this study was conducted to determine the association between lameness and reproductive performance in dairy cattle.

MATERIALS AND METHODS

Lameness assessment

This study involved 67 milking cows from a private dairy farm in Desaru, Johore, Malaysia. All cows were assessed for lameness as they leave the milking parlour. Lameness based on locomotion score (LS) was assessed using the method developed by Sprecher et al. (1997); 1-normal, 2-mildly lame, 3-moderately lame, 4-lame, and 5-severely lame.

Identification of hoof disease

Cows with LS of 2 to 5 were isolated and examined for claw lesions. The claws were washed with water to remove manure and the hoof trimmed using an electronic grinder. The paws were palpated to determine affected area. Claw lesions were identified using the ICAR Claw Health Atlas (ICAR, 2015) as reference.

Data collection

Reproductive performance data collected from the farm included calving and calving to conception intervals, number of services per pregnancy, and pregnancy status. A questionnaire on the management system and hoof care programme was also given to the farm manager to respond.

Data analysis

The data were analysed using the SPSS version 26. The distribution and prevalence of lameness in the herd as well as distribution of claw lesions in lame cows were determined using the frequency table. Descriptive statistics were used for the reproductive performance trait data. Cross-tabulation was used to quantitatively determine the relationship between LS and pregnancy status. The association between lameness and number of services per pregnancy was determined using the Mann Whitney test.

RESULTS AND DISCUSSION

Farm demography

The study was conducted in a large-scale private farm with herd size of 600 cows, comprising of 216 milking cows grouped into high, medium, and low producer

groups. The high producer group (n=80) were cows that produced >25 litres of milk/day. From the high producer group, 67 cows were selected for this study. The farm mainly practices artificial insemination using conventional and sexing semen. Heat detection and artificial insemination and hoof trimming were the therapeutic and preventive measures done by trained farm workers. The milking parlours on the farm are equipped with foot baths.

Lameness

The prevalence of lameness in cows of this study is approximately 15% (Table 1), which is lower than that report in an earlier study (19%) in 8 dairy farms in Selangor Malaysia (Sadiq et al., 2017).

Table 1: Distribution of lameness in the herd

Locomotion score	Frequency (N)	Percentage (%)
1	57	85.1
2	4	6
3	5	7.5
4	1	1.6
Total	67	100.0

Hoof disease

Our study showed the frequency of cases of non-infectious hoof diseases in dairy cows were higher (Table 2) than that reported in a previous study (Sadiq et al., 2017). Among causes of hoof diseases in cows is poor dairy systems. In dairy farms with confined areas with cows walking on hard, solid, and abrasive surfaces like concrete, tend develop claw horn overgrowth and predispose then to claw disorders such as sole ulcers and white line disease (Bergsten et al., 2015). Most confinement systems expose cows to slurry manure and moisture that could cause digital and interdigital hoof diseases.

Table 2. Distribution of hoof disease in the herd

Hoof disease	Frequency	%
Interdigital dermatitis	1	1.5
Sole ulcer	6	9.0
White line	3	4.5

Reproductive performance

The recommended calving interval, calving-to-conception interval and number of services per pregnancy in cattle is 365 and 100 days, and 1.6 to 1.8, respectively (Olechnowicz and Jaśkowski, 2011). The calving interval (428±141 days), calving-to-conception interval (140±31 days), and services per pregnancy (28±1.1) in cows

in this study were higher than the recommended values. Among the 57 cows of the LS1 category, only 5 were pregnant, while none of the inseminated cows from LS category 2 to 5 became pregnant. This indicates that the cow reproductive performance in the farm was poor, which was attributed dietary protein deficiency (Fahar et al., 2018).

The cows in this study showed poor overall pregnancy rate (Table 3). Only a few LS 1 cows (7.5%) were pregnant. The average conception rate in lame cows tends to be lower than in non-lame cows (Olechnowicz and Jaśkowski, 2011). Cows diagnosed with clinical lameness during the first 70 days in milk were 25% less likely to become pregnant. Lameness that occurred within the first 60 days postpartum was also associated with reduced fertility.

Table 3. Frequency distribution of pregnancy status in the herd by locomotion score

Lameness (Locomotion score)	Pregnancy status		Total
	Not pregnant [Number (%)]	Pregnant [Number (%)]	
1	52 (77.6)	5 (7.4)	57 (85.0)
2	4 (6.0)	0 (0)	4 (6.0)
3	5 (7.5)	0 (0)	5 (7.5)
4	1 (1.5)	0 (0)	1 (1.5)
Total	62	5	67

% - Values of total number of cows (n=67)

The mean service per pregnancy was 2.81 in non-lame cows and 1.80 in lame cows. One study suggested that cows suffering from lameness, particularly with interdigital phlegmon required more than 3 services per pregnancy (Mellado et al., 2018). Our study showed that there was no association between services per pregnancy and calving and calving-to-conception intervals in cows.

CONCLUSION

One private dairy cattle farm in Johore, Malaysia had lameness problem and poor overall reproductive performance. None of the LS 2 to 4 cows in the farm were pregnant. Based on the number of services per pregnancy as indicator, the study showed no significant association between lameness and reproductive performance in dairy cattle of this farm. Therefore, further studies using larger sample sizes on more farms are needed.

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PAIN ASSESSMENT IN BULLS SUBJECTED TO ELECTROEJACULATION

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ABSTRACT

Electroejaculation (EE) is one of the methods used to collect semen in animals. This procedure is performed by applying electrical stimulation to the pelvic nerves and the surrounding tissue through the rectum. Nonetheless, this procedure is believed to be painful and stressful to the animals, thus raising animal welfare concerns. The purpose of this study was to assess pain perceived by the bulls during EE stimulation using the electroencephalogram (EEG) as the indicator. Four mature adult bulls aged 4 to 7 years were subjected to the EE stimulation. The EEG was recorded before stimulation (baseline), during, and 20 min after EE stimulation. Physical signs of discomfort were recorded. The results showed significantly higher ($p<0.05$) in EEG parameters during EE stimulation than values at baseline and 20 min post-EE stimulation. The bulls showed signs of discomfort during EE stimulation, but not at 20 min after the procedure. Based on the EEG and signs of discomfort during EE stimulation, the EE method for semen collection is painful to the bulls.

Keywords: pain, bull, electroejaculation, electroencephalogram

INTRODUCTION

Semen collection plays a pivotal role in livestock production in artificial insemination, breeding soundness evaluation, semen banking, and diagnosis of diseases. Semen can be collected by using several methods including with the use of artificial vagina (AV), internal artificial vagina (IAV) (Barth et al., 2004), electroejaculation (EE) (Hill et al., 1956), transrectal massage (RM) (Palmer, 2005; Sarsaifi et al., 2013), and transrectal ultrasound-guided massage (TUGM) (Santiago-Moreno et al., 2013). Since AV and IAV methods almost mimic the natural ejaculation process, they produce the highest concentration of semen. Electroejaculation is effective at inducing semen emission and this method requires minimal facilities, does not require dummy or training, and not physically demanding. The electroejaculation method involves the electrical stimulation of the

pelvic nerves and the surrounding tissues, performed by inserting a probe in the rectum and applying short, low-voltage pulses of electric current. Semen emission is a sympathetic response to the stimulation of the lumbar sympathetic nerves, causing smooth muscle contraction that leads to semen emission into the pelvic urethra from the ampullae and vas deferens. The sacral parasympathetic nerves that form pelvic and pudendal nerves also control erection and ejaculation. These nerves are located at the level of pelvic urethra and body of prostate gland. Although EE is technically convenient and effective, it is considered to be painful and stressful to the bull.

Importation of semen collected by the EE method is now banned by some European Union countries, because it is believed to be inhumane (Palmer, 2005). Although several studies had determined better alternatived to the EE method in semen collection, the assessment of pain using encephalogram (EEG) has not been done in the EE method. The EEG spectrum changes is a tool to in the evaluation of nociceptive responses in animals.

This study was conducted to determine the degree of EE-associated pain response using EEG. The study also determined the correlation between EEG data and behavioural signs of discomfort in bulls.

MATERIALS AND METHODS

Four adult beef bulls aged 4 to 7 years from Ladang 16, University Agriculture Park (TPU), Universiti Putra Malaysia were used in the study. The experimental design in this study was reviewed and approved by the Institutional Animal Care and Use Committee (UPM/IACUC/AUP-U009/2019) of Universiti Putra Malaysia. Two hydrogel conductive self-adhesive sterile disposable pads were placed on the skin over the zygomatic process of the frontal bone for inverting electrode (-ve) and the mastoid process of the skull for non-inverting electrode(+ve). Faeces was evacuated from the rectum before the procedure.

Electroejaculation

The rectal probe was inserted into the rectum and controlled by an operator while another controlled the electroejaculator. The machine was set on a semi-automatic mode where the electrical stimulation was increased gradually. Each stimulus lasted 4 s, paused for 3 to 4 s before applying the following stimulus. The stimulation was for a duration of 10 min, irrespective of ejaculation response. The voltage was increased gradually depending on the bulls' reaction to a max of 10V only.

Electroencephalogram

The total impedance of the circuitry was maintained at <5k ohms. The electroencephalogram was recorded at a sampling rate of 1 kHz and raw EEG was resampled with low pass filter of 200 Hz into delta (0.1 to 4 Hz), theta (4.1 to 8 Hz), alpha (8.1 to 12 Hz), and beta (12.1 to 20 Hz) frequency (Kaka et al., 2015). At the completion of the experiment, the EEG data was analysed using Chart 5.5.5 recording software. The median frequency (MF) and the total EG power (Ptot) were

calculated for consecutive non-overlapping 1-s epochs. The EEG data was taken from 20-s epochs block each time. Data during EE stimulus was taken from 10-s blocks during back arching until 10-s blocks at beginning of ejaculation for statistical analyses. Data contaminated with movement artefacts such as large spikes, over-scale and under-scale were excluded from the analyses.

Recording discomfort signs
Signs of discomfort signs shown by the bulls, which included muscle spasm at the thigh and abdominal regions, irregular hindlimbs movements includes kicking and moving back and forth, arched back, recumbency, and vocalisation were recorded by the same operator throughout the experiment.

Data recording
Signs of discomfort and EEG records were taken before, during EE and 20 min post-EE procedure. The EEG records from stable data was recorded for 1 min before and after EE while the EEG records during EE stimulation, was taken throughout the 10-min procedure. The conduct the electroencephalogram and monitoring of discomfort sign scores were made by different operators.

Statistical analysis
Data obtained for EEG records were statistical analysed using the SAS software package, version 9.1. The data sets were compared across time using the one-way ANOVA. Significant differences among means were determined using the post-hoc Tukey's multiple comparison test. Discomfort signs were compared across time using Kruskal-Wallis test and followed by Dunn's multiple comparison test. (SPSS 25.0 software). The correlation between EEG records and the discomfort signs were analysed using Spearman's correlation test.

RESULTS AND DISCUSSION

All EEG data show apparent increments during the EE stimulation (Table 1). The mean EEG alpha, beta, delta and theta wave values show significant differences ($p<0.05$) between pre-stimulation, stimulation, and post-stimulation periods. There was also significant difference in total power mean values between before and after EE stimulation. The mean median frequency (MF) was higher during EE stimulation than at pre- and post-stimulation periods. The MF and total power changes are associated with noxious stimulation, indicating that the bulls experienced pain during EE stimulation.

Behavioural discomfort signs
All bulls vocalised before EE stimulation (Figure 1). However, at EE stimulation only 29.14% vocalised. The proportion of bulls at recumbency increased from 12.6 to 56.69% from before to at EE stimulation (Figure 2). A significant number of bulls showed muscle spasm during EE stimulation (Figure 3). Among these, 29.13%

showed moderate while 70.87% showed intense muscle spasm. The majority (69.29%) of bulls showed intense movement with kicking of the hindlimbs while the others (30.71%) mainly showed back and forth movement (Figure 4). These behavioural signs of discomfort during EE stimulation suggests the animals were in pain with EE stimulation. All bulls showed arched back response, irrespective of whether they subjected to EE stimulation or not (Figure 5).

Table 1. The electroencephalogram data in bulls with electrical stimulation using the electroejaculator

Parameter	Baseline	At stimulation	Post-stimulation
Alpha	1.72±1.31	2.68±1.75	1.96±1.46
Beta	3.44±2.64	6.07±4.77	3.88±2.76
Delta	3.28±1.87	5.15±3.19	3.48±1.91
Theta	1.30±1.76	1.90±1.24	1.56±0.99
Total Power	11.94±5.57	20.35±9.70	12.01±5.95
Median Frequency	21.60±7.85	28.79±10.08	21.36±5.88

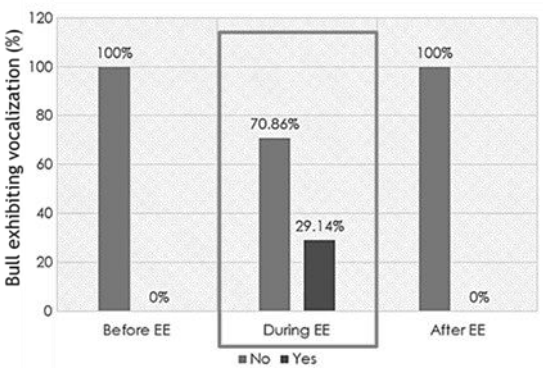

Figure 1. Bulls exhibiting vocalisation with electroejaculator stimulation

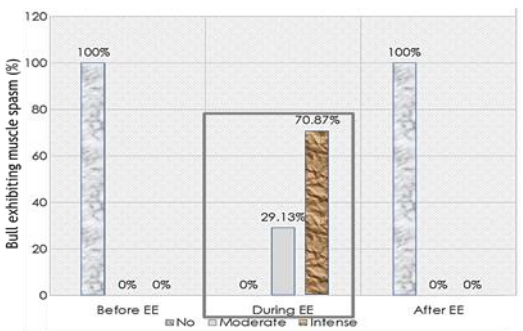

Figure 2. Bulls exhibiting recumbency with electroejaculator stimulation

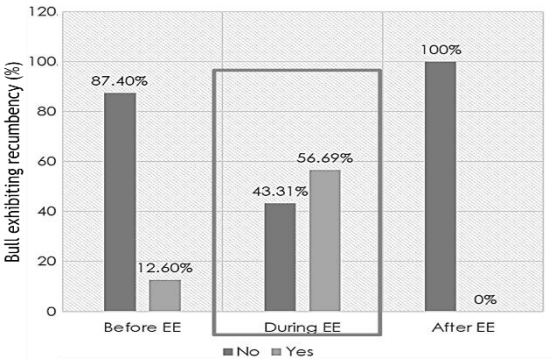

Figure 3. Bulls exhibiting muscle spasm with electroejaculator stimulation

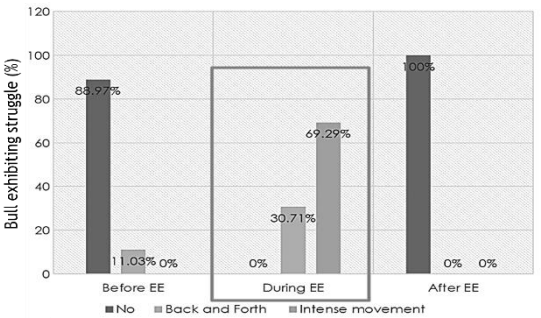

Figure 4. Bulls struggled with electroejaculator stimulation

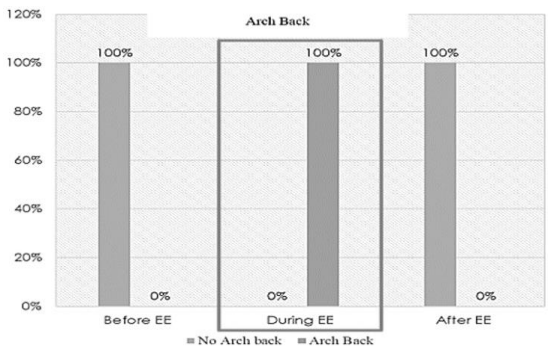

Figure 5. Bulls exhibiting arched back with electroejaculator stimulation

Table 2 shows the correlation between EEG and signs of discomfort expressed by the bulls due to EE stimulation. All mean MF values and signs of discomfort showed strong positive correlations between each other. This correlation strongly supports reports that EE causes pain and stress in bulls (Mosure et al., 1998: Whitlock et al., 2012).

Table 2. Correlation between the median frequency values of EEG and the behavioural signs of discomfort

	Recumbency	Vocalisation	Arched back	Struggle	Muscle spasm
Median frequency	0.97	1.00	1.00	0.99	1.00

CONCLUSION

In conclusion, the EEG showed that semen collection by EE stimulation caused pain to bulls. There is also a positive correlation between EEG readings and the behavioural signs of discomfort in bulls subjected to EE stimulation, which indicates that EE stimulation is painful to animals.

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MUSIC AS AN AUDITORY STIMULUS TO MITIGATE TRANSPORTATION STRESS IN CATS

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ABSTRACT

Transportation stress is unavoidable when cats are in transit to the veterinary clinic for medical examination and treatment. Stress response cats can confound disease diagnosis. Therefore, any non-invasive methods to mitigate stress would contribute significantly to the welfare, and well-being of cats being transported. The study determined the effect of music as auditory stimulus to mitigate transportation stress in cats. A total of 11 cats were enrolled in this study, each serving as its own control. The cats were transported for 30 min with (Tm) and without music (Tnm). The behavioural scores and electroencephalogram (EEG) readings of the cats were taken at the home environment of the cat (T0) and at post-transportation. Stress evaluation was based on quantitative EEG and a verified Cat Stress Score. The study showed that transportation caused a significant increase in stress on cats. Music showed measurable impact in the mitigation of stress response in cats. In fact, music-treated cats during transportation behave as if they were in home environment. In conclusion, calming music during transportation will significantly ($p<0.05$) reduce the intensity of stress in cats.

Keywords: transportation, stress, music, EEG, cat

INTRODUCTION

Stress is a set of physiological and behavioural alteration generated by discomfort or threatening stimuli (Amat et al., 2015). Animals raised under stressful environment will exhibit adrenal hyperactivity, lymphatic atrophy, and peptic ulcers, which are linked to the response of hypothalamic-pituitary-adrenal axis. Prolonged or intense stress may induce negative impact on immunity, general health, and behaviour of animals (Moberg and Mench, 2000).

Music, especially low intensity, lower frequency classical music is known for its soothing effect on emotion, mind, and body. The feline species is sensitive to auditory stimulus that has the same tempo and frequency as their communication bandwidths. According to Snowdon et al. (2015), cats have their own species-specific music genre. Tempo that resonates with purring and the suckling sound

during nursing, and high pitch music are most preferred choices of auditory stimulus for cats. Therefore, music at the right tempo and frequency could positively affect feline physiology.

Transportation stress has been the main concern of owners when visiting veterinary clinics (Volk et al., 2011). How the effect of music relieves feline stress during transportation is still not clear. Thus, this study determined the effect of music on EEG changes in cats.

MATERIALS AND METHODS

The experiment was carried out on 11 cats comprising of 5 males and 6 females. aged 5 months to 3 years. Each cat was transported for 30 min with and without music. The temperature of the car has been fixed at 21.5 °C and the position of the cat carrier was in the middle of backseat. Data was collected from cats before transportation (T_0) as baseline and at post-transportation without (T_{nm}) or with (T_m) music. The stress detection system used was the Cat Stress Score (CSS) (Kessler and Turner, 1997) and electroencephalogram (EEG) recordings. The electroencephalograph leads, using surface gel electrodes, were placed on right ramus and prefrontal areas between the ears of the cat. Cat behaviour evaluation was also based on CSS.

The EEG was recorded using a computer installed with Chart5.0 recording software and connected to the Powerlab 4/20 data recording system (AD Instruments Pty Ltd, Australia). The sampling rate was 1 kHz and raw EEG was resampled with low pass filter of 200 Hz into delta (0.1-4 Hz), theta (4.1-8 Hz), frequency (8.1- 12 Hz), and beta frequency (12.1 – 20 Hz) as described by Zulkifli et al., (2014). The datasets were compared among cats at baseline and post-transportation with music and without music.

Statistical analysis

The data were analysed using IBM SPSS version 25.0 software, (IBM-SPSS Inc, USA). EEG recordings were analysed using the GLM procedure to determine the effect of music, and experimental time points on cat stress scores, and EEG parameters. Differences between groups were tested with Tukey's studentised range test at 95% confidence level.

RESULTS

The mean stress score for cats at T_0, T_{nm} and T_m, was 3.64, 4.82, and 2.91, respectively. The results showed that transportation with music was less stressful to cats than without music.

There were significant differences ($p<0.05$) in EEG readings between cats at T_0, T_{nm}, and T_m. The α, β, and δ waves and total power (Ptot) values were highest

in cats at T_{nm} (Table 1). Generally, the EEG readings approached normal T_0 values when cats were transported with music played.

Table 1: Electroencephalogram of cats transported with and without music

EEG waves	Electroencephalogram		
	T_0	T_{nm}	T_m
α	1.10^{a}±0.03	1.50^{b}±0.09	0.97^{a}±0.04
β	1.23^{a}±0.04	1.21^{a}±0.07	0.96^{b}±0.03
δ	8.68^{a}±0.35	17.48^{b}±0.56	7.15^{c}±0.28
θ	2.03^{a}±0.06	3.27^{b}±0.17	1.86^{a}±0.08
Ptot	10.85^{a}±0.38	20.72^{b}±0.62	9.05^{c}±0.32

Values are mean±standard error.
^{a,b,c}Means, across rows, with different subscripts differ significantly at $p<0.05$. T_0=Home environment (baseline), T_{nm}=After 30 min transportation without music, T_m=After 30 transportation with music, EEG=electroencephalogram, Ptot=total power.

DISCUSSION

The study showed that calming music plays an important role in soothing the nerve of cats under unpredictable and unfamiliar environments. The present study showed that there was a significant increase in mean cat stress score post-transportation. The majority of cats appeared very tense or tense and stiff during transportation without music. Based on the CSS, the cats may be initially stressed, but they became less tensed and relaxed when music was played during transportation. The cat stress score after transportation with music were similar to that at baseline, indicating that they were comfortable in an environment with music. These findings were confirmed by the EEG readings. The typical stressed cats will show brain activities dominated by β wave. β wave is the conscious brain wave that increases in activity in the presence of stress and threat (Zulkifli et al., 2014). β wave is also associated with events that require focused attention and increased alertness, such as under stress and threat (Jena, 2015). The effect of transportation stress in cats was also evident by the changes in EEG, where the β wave increased significantly after transportation. Music appeared to decrease β wave frequency and amplitude in the cats after transportation. Thus, music served as an enrichment for cats during the transportation, which distract them from the discomfort of the unfamiliar environments, unpleasant, and stressful car rides.

CONCLUSION

Transportation is a significant stressor to cats. However, transportation stress can be reduced significantly by exposing the cats to soothing music. Music is safe and

economical to be use in stress management of cats while it also enhances animal welfare and allow for safe handling of cat patients during examination at veterinary clinics.

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ABUNDANCE OF GIBBONS (*HYLOBATES SPP*) AND THEIR TEMPORAL ACTIVITY STATES IN PERIPHERAL VIRGIN FOREST SURROUNDING A DEER BREEDING CENTRE IN LENGGONG, PERAK, MALAYSIA

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ABSTRACT

Gibbons (*Hylobatidae*) are primates that required our utmost attention to ensure their sustainability in tropical rainforests. Gibbons often encroach into human activity areas where the melodious calls can be heard. This study is conducted to estimate the abundance of gibbons and their temporal activity state in forest reserve around a semi-wild breeding deer farm at the periphery of a virgin forest in Perak, Malaysia. An experimental design consisted of preliminary assessment of calling patterns and distribution and capture, mark and recapture was constructed. Auditory sampling was executed using two listening posts set up around the farm to "capture" gibbon calls from different directions during morning, afternoon and evening sessions. Weather data with subjective rate are also recorded to determine association with calling activity. From the calls, the total number of gibbons identified was 20; 14 from Listening Post 1 and 6 from Listen Post 2. Temporal activity showed that the main calling activities were during morning and infrequent during afternoon and evening sessions. The presence of gibbons in the vicinity of the deer farm could be due to the availability of seasonal food patches and habitat disturbance from logging activities in the forest reserve. In conclusion, human encroachment and habitat fragmentation affect the distribution, movement, and composition of gibbon groups in forest reserves.

Keywords: gibbon, auditory sampling, abundance, temporal activity states

INTRODUCTION

Globally, land expansion and timber industries had increased in the past decades, causing losses of tropical rainforest and ultimately invoked significant impacts on the existence of primate species. Habitat fragmentation and loss of dense forest canopy have direct negative impacts on the movement and population of these gibbons (Nijman, 2005).

According to the International Union for Conservation of Nature (IUCN), gibbons are categorised as endangered species. The gibbon or small apes are of the Hylobatidae family, representing the sister lineage of Hominidae, comprise of humans and apes. These arboreal apes comprise of approximately 20 species, making them the largest species group among apes. The gibbons roam in tropical rainforest and are distributed widely in Southeast Asia. Being the only primate that can vocalise, the gibbons demonstrate singing and duetting with their partners in their natural habitat. All gibbon species are known to produce loud, long and patterned morning songs (Geissmann, 2000). This unique behavioral evolution is a way for gibbons to mark their territory. Strict territoriality among gibbon groups has led to monogamous social structure of the species. Hence, these activities influence the composition and spacing of the gibbon groups.

Gibbons are marker species for biodiversity value of the forest. The presence of gibbons in a region indicates that the forest is healthy with great range of biodiversity. Gibbon songs may be absent from their natural habitats and instead their songs may be audible in forests close to village. In this case, it indicates that the gibbons have left their natural habitat and reside in areas close to human settlements (Phiapalath and Saisavanh, 2010).

This study was conducted to estimate the abundance of gibbons and their temporal activity state in forest reserve at the periphery of a virgin forest.

MATERIALS AND METHODS

Site of study

The study was conducted at the Deer Breeding Centre, located in the Bintang Hijau forest reserve, Lenggong, Perak, Malaysia (5°12' 14.51" 100°55' 55.44"). Bintang Hijau forest reserve is a virgin forest located in the northern end of the mountain range, *Banjaran Bintang*. This range runs parallel with, but not connected to the mountain range, *Banjaran Titiwangsa*.

Experimental design

Two listen posts were set up to record the songs of the gibbons. The two posts selected were at highest point near the forest fringes that can equally cover a widespread angle for auditory samplings. A voice recorder was placed at each post to record calls. Morning session samplings were 0530 h and 1000 h. This is the period of highest vocalisation activities among gibbons. The afternoon session was at 1100 h to 1300 h while the evening session at 1800 h and 1900 h. The study was conducted over 5 days from 5th to 9th August 2019. The first day was a preliminary assessment of vocalising pattern and area. The subsequent 4 days was for the capture, mark and recapture of the unique individual or duet sounds.

Data collection

Gibbons calling sounds are sampled by two researchers from different directions. The directions and time of calling are recorded. Gibbons songs were distinguished as either individual calls or group calls consisting of one male, female, and an offspring. Unique individual calls on days 2, 3 and 4 are identified and recorded and "marked". Recapture of "marked" calls were done on days 3 to 5. If there were two distinct calls from the same direction, the sounds were differentiated based on the volume. The sampling ceased when no call was heard for a period of 30 min. Gibbons call after 1000 h were disregarded except in the event of rain in the morning. Unfavorable weather conditions are scored using arbitrary values.

Data analysis

The dataset was subjected to independent T-test to determine significance between two listening posts. Abundance of gibbons was calculated using formula described for the "Capture, Mark, and Recapture" technique of the Schnabel method (Schnabel, 1938).

RESULTS AND DISCUSSIONS

There were significantly ($p<0.10$) abundances of gibbon calls during morning compared to afternoon and evening sessions at Listening Posts 1 and 2. Based on the calculation using the Schnabel method (Schnabel, 1938), the number of gibbons in area covered by listening post 1 was 14 and Listen Post 2 was 6 individuals. No significance ($p>0.10$) difference in number of gibbon's calls were recorded in the afternoon and evening sessions.

The abundance of gibbons in the periphery of the deer farm is thought to be due factors such as availability of niche for the groups and the forceful group movement from the North. This phenomenon is suggested to be due the logging activity that had significantly impacted the movement and distribution of the gibbon groups from their natural habitats to the vicinity of the Deer Breeding Centre in Lenggong, Perak.

Based on the interactive duet songs during the morning session, the gibbons in the forest surrounding the farm were very active. Gibbon activity was not as frequent in the afternoon and evening sessions. There was only one day where calls are heard from one individual during the late sessions.

The results also showed that there was a negative correlation ($r = -.626$) between weather conditions and frequency of calls ($p<0.05$).

CONCLUSIONS

There were abundance of gibbons in the peripheral virgin forest surrounding the Deer Breeding Centre in Lenggong, Perak. The gibbon in this area had established an exclusive territory around the Centre. Their vocalising activity was more frequent in the mornings than afternoon or evening sessions. The presence of gibbons around

the Centre is due to the availability of food patches and degradation of their natural habitat from logging activities.

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ESTIMATING THE COSTS OF REARING DAIRY CATTLE FROM BIRTH UNTIL WEANING USING DETERMINISTIC MODEL

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ABSTRACT

The target of the Malaysian government is to achieve 100% self-sufficiency in fresh milk by 2025. However, among challenges to this arget is high cost of rearing young stock. The objective of this study was to estimate the total cost of rearing a young dairy cattle stock from birth to weaning age. A deterministic partial budget model was built using Microsoft Excel® 2013 with inputs based on information gathered by a questionnaire survey conducted in August 2019 on 13 dairy farms in Sabah, Malaysia. The questionnaire consisted of 23 questions regarding the management of calf and prices from birth to weaning. Using the deterministic model, the costs of feed and labor were estimated by differentiating the feed types, raw milk or calf milk replacer, and adding the costs of water and concentrate. The study showed 75% of farmers immediately and sufficiently fed their calf with colostrum at an average of 5 litres per calf per day. The median weaning age was 90 days and the median total costs of rearing was RM1,060 per calf. At the farm level, the median cost for rearing calf from birth to weaning was RM3,967. The weaning age of dairy calves in this study was higher than the reported normal range of 60 days.

Keywords: birth until weaning age, dairy, rearing costs, young stock

INTRODUCTION

Malaysia's population has grown from 28.6 million in 2010 to 32.4 million people in 2018. This growth in population had influenced demand for food in the country (Dong, 2005; DOSM, 2019). The population growth will influence the ability of the country to achieve the target of fresh milk self-sufficiency level by year 2025. In fact, the milk production had gradually decreased from 2011 to 2017 (DVS, 2018).

Generally, the dairy farming industry is developed and operated based on three main components, land, dairy cattle, and young stock. Young stock management is crucial as these calves are the future replacement heifers. However, currently in Malaysia, farmers keep their heifer calves for a period of 22 to 24 months old while unaware of rearing cost (Gabler et al., 2000; Mohd Nor et al., 2015). Profitable dairy farming could only be achieved with good management practices, including proper

housing, feeding, and good herd management in the farm (Moran, 2009). In Malaysia, dairy farm smallholders have fewer than 30 dairy cows per farm. Data recording is done manually with hand-written records. Some farmers do not practice data recording at all (Jeyabalan, 2010). Thus, the lack of adequate management practices had leads to difficulties in decision-making on farm issues.

Feed contributes the most to the cost of rearing cattle. The cost of feed is between 49 to 57% of the total rearing cost (Heinrichs et al., 2013; Boulton et al., 2015). In feeding pre-weaning calves, the milk used include raw and pasteurised milk or calf milk replacer. The cost of rearing from 3 days old until the weaning age of 58 days comprise only 12.3% of total rearing cost for replacement heifer (Gabler et al., 2000). The pre-weaning period incurs the highest daily cost among rearing periods.

This study estimated the total costs of rearing a young dairy cattle stock from birth until weaning age.

MATERIALS AND METHODS

A survey was conducted on 13 smallholder dairy farms in Keningau, Sabah, Malaysia in July and August 2019. The survey was based on a set of questionnaires that included farmers' background, calf management practices, neonatal care, feed management, and utilities. An individual spreadsheet for each farm was developed in Microsoft Excel ® 2013 whereby all data such as feed and labour costs were recorded and analysed. The deterministic model was built using Microsoft Excel ® 2013. The costs of rearing calf were estimated by the sum of feed and labour costs from birth to weaning. The feed cost data consisted of cost for fresh milk, calf milk replacer (CMR), starter dry feed, and water intake for each calf. The cost of fresh milk was calculated using Equation 1. The farmgate price was RM2.90 for raw milk (Table 1) and RM336 per 25 kg for CMR. The price CMR per litre is based on the preparation ratio practiced by the farmer. Therefore, the price of CMR per litre is determined and multiplied with the daily amount given to each calf [Equation 2]

The assumption made in this study was that the calves were given concentrate at 200 g/calf/day (personal communication). This information was use to generally estimate the cost of concentrate. Thus, the price of concentrate/kg was determined and multiplied with the daily amount given to each calf [Equation 3]

The estimation of water intake per calf was depending on live body weight of calves with an assumption of 0.1001 L water intake kg body weight (Boulton et al. 2015). The rate was then calculated daily and based on weaning age [Equation 4].

The cost of labour for young stock management was calculated from the total worker time to manage the animals. The worker was assumed to spend 2 min/day milk feeding using either milk tub, pail or container and 6 min/day using bottled milk. An additional 6 min/day spent is assumed if the farmer uses calf milk replacer (CMR) to feed the calves. This period included time spent for CMR preparation. The labour wage/min on the management of calves was calculated based on their salaries, which was is obtained by multiplying min spent/day with the labour wage [Equation 5]

Cost of fresh milk (FMC) = freshmilk AMT x freshmilkpP…….Equation 1
Where: Freshmilk AMT= amount of fresh milk given (L/calf/day)
FreshmilkP = Price of fresh milk (RM/L)

Cost of calf milk replacer (CMRC) = CMRAMT x CMRP……..Equation 2
Where: CMRAMT = amount of CMR given (L/calf/day)
CMRP = Price of CMR (RM/L)

Cost of concentrate (ConcC)= ConcEAMT X ConcP……Equation 3
Where: ConcEAMT= Estimated amount of concentrate consumed (kg/calf/day)
ConcP = Price of concentrate (RM/kg)

Cost of water intake (WIC) = (BW X 0.1001 L) X RegT……..…Equation 4
Where: BW= Body weight (kg)
RegT = Regional tariff rate (RM)

Cost of labor (LabC) = LabW X LabTime……..……..Equation 5
Where: LabW= Labor wage (RM/min)
LabTime = Time allocated to feed a calf (min/day)

Table 1. Type of input used in the deterministic model

Item	Value	Source
Biological		
Calf milk replacer (CMR) preparation (min/day)	6	Farm survey
Work time with milk feeding method (min/calf/day)		
a. Milk tub	2	Expert opinion
b. Pail	2	Expert opinion
c. Container	2	Expert opinion
d. Bottle milk	6	Expert opinion
Labour work time spent on concentrate feeding	2	Expert opinion
Economical		
Farm gate price of fresh milk (RM/Litre)	2.90	PPITPS
Calf milk replacer commercial price (RM/kg)	13.44	Farm
Dairy concentrate pallet price (RM/kg)	1.62	KITSB

PPITS – Pusat Perkhidmatan Industri Tenusu, Sabah; KITSBK – Koperasi Industri Tenusu Sabah Bhd

RESULTS AND DISCUSSION

The median weaning age record in the study was 90 days (range: 75 to 105 days) which is longer than the suggested normal range. Based on DVS (2010), calves should be weaned by 60 to 120 days old. However, additional cost is incurred, especially milk feeding cost, if the calves are weaned after 60 days old. In this study, the estimated median total cost of rearing a young stock from birth to weaning was RM1,060 (Table 2). This cost is slightly higher than reported by other studies in the United Kingdom (Boulton et al, 2015) and United State of America (Heinrichs et al., 2013), at RM1,009 and RM909, respectively. However, the comparison will vary with currency rates.

Table 2. Heifer rearing cost from birth to weaning age in 12 farms

Item	N	Cost (RM/calf)		
		Mean ± SD	Range	Median
Birth to weaning	12	1344.50 ± 658.34	606.70 - 2637.50	1060.00
Feed	12	1330.35 ± 662.60	559.80 - 2619.70	1049.40
Labor	10	16.95 ± 11.10	6.30 - 46.90	14.87
	Total	2691.80 ± 1332.04	1172.80 - 5304.10	2124.30

Unfortunately, treatment cost cannot be determined in the study because of poor data recording at the farms. Farmer could not identify sick animals, treatment used or cause of animal deaths on their farms. For that reason, the morbidity and mortality rate could not be estimated. It was reported that the mortality rates of calves between tropical and temperate countries is 15 to 25% and 1 to 8%, respectively (Svensson et al., 2006; Mee, 2008; Moran, 2012; Raboisson et al., 2013). It should be emphasised that each farm should target to reduce the mortality rate of pre-weaning calves to less than 8% (Brand et al., 1996). This is to minimise cost and maximise profit in their calf production. It is estimated that the pre-weaning mortality could lead to an economic loss of RM1,060 per calf.

CONCLUSION

The median total cost for rearing a calf from birth until weaning was estimated at RM 1060. At farm level, with median total number of young stocks at 4 calves, the median total cost spent on calf-rearing was estimated at RM3,967.

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EFFECT OF SHORT-TERM DIETARY SUPPLEMENTATION OF BLACK SOLDIER FLY LARVAE ON COGNITIVE FUNCTION IN MICE

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ABSTRACT

Black Soldier fly (BSF) larvae are being used both for human consumption and animal feed. The BSF larvae is a good source of quality protein and fats. Although BSF larvae are known to have nutritional value, its effect as a feed supplement on brain cognitive function of mammals remains unclear. Therefore, this study determined the effect of short-term dietary supplementation with BSF larvae on the cognitive function of mice. Four weeks old C57BL/6 mice (n=16) were divided into 2 equal groups. The treatment group was fed commercial pellets supplemented with 10% BSF larvae, while the control group fed commercial pellets only. Feed and water were made available at libitum for the mice. After 4 weeks of dietary treatment, all mice were tested for spatial working memory using the Y-maze test. Mice supplemented with BSF larvae showed longer time and higher number of entries in the novel than other arms of the Y-maze ($p<0.05$). The result suggested that short-term dietary supplementation with BSF larvae for 4 weeks could improve brain cognitive performance in mice. In conclusion, alternative protein and amino acid sources from BSF larvae may help to improve neurocognitive function.

Keywords: Black Soldier fly larvae, Y-maze, cognitive function, mice.

RELIABILITY OF AN INFRARED THERMOMETER USED IN A SMALL ANIMAL HOSPITAL

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ABSTRACT

Body temperature measurement animal patients is an integral part of physical examination. In veterinary practice, rectal temperature is the gold standard for the prediction core temperature of patients. However, this method is not without limitations; being uncomfortable for the animal and the value tends to lag behind core temperature. Technology advancements in veterinary medicine are inclined to follow the trends in human medicine. However, there are products intended for human use that was shown to be unsatisfactory for animal use. Infrared thermography (IT) is replacing digital thermography for core body temperature measurement in humans and there are now IT equipment designed for animal use. The current experiment was undertaken to determine the reliability of an IT equipment designed for animal use. The experiment involved 210 paired core body temperature readings from cats and dogs and 100 paired temperature readings each for the environmental and feed. The results showed that the IT was very reliable (r=0.951) to be used for measuring environmental temperature and feed (r=0.824) but not for core body temperature (r=0.611). However, using the built-in correction function and calibration for animal species incorporated in the IT equipment, yielded extremely reliable results. Although IT is rapid to use and do not cause discomfort to animals, it cannot be conveniently and reliably used for all patients. Nevertheless, IT is still a useful tool for patients not cooperative when the rectal temperature method is applied to determine core body temperature.

Keywords: body temperature, thermometer, infrared, reliability

DETERMINATION OF TRIGLYCERIDES STORAGE IN HEPG2 CELLS SUPPLEMENTED WITH EXOGENOUS LIPIDS AND *STEVIA* EXTRACT

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ABSTRACT

The extract of *Stevia* sp. (*Stevia rebaudiana Bertoni*), is a sweet herb that is used as a safe sugar substitute for consumption. The extract is widely used not only as an alternative for sugar but also as a form of cholesterol-reducing compound in the prevention of heart diseases. The aim of this study was to determine the anti-triglyceride effect of a commercial *Stevia* extract by quantifying the amount of neutral lipids internalisation by the HepG2 cells. The amount of lipid was quantified using the lipid extraction method adopted from Bligh and Dyer lipid extraction protocol. The quantity of lipid internalisation by HepG2 cells was observed under immunofluorescent microscopy. HepG2 cells supplemented with exogenous lipid and commercial *Stevia* extracts showed increased amount of stored lipid droplets. The study showed that *Stevia* extract has potential to be developed as an anti-triglyceride and anti-cholesterol agent.

Keywords: HepG2 cells, lipid droplets, immunofluoresence, *Stevia* (*Stevia rebaudiana Bertoni*), triglyceride

A RETROSPECTIVE STUDY OF THE RELATIONSHIP BETWEEN AVERAGE DAILY INCUBATION TEMPERATURE AND GREEN TURTLE HATCHLING SEX RATIO

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ABSTRACT

Marine turtle species lack sex-determining chromosomes and they undergo a thermosensitive period (TSP) for sex determination during the second third of incubation period. During the TSP, cooler temperature will result in higher male while warmer temperature produces high female hatchlings ratio. Therefore, monitoring the beach temperature profile and turtle hatchling sex ratio is crucial to ensure successful conservation measures. This project was undertaken to determine the relationship between beach temperature profile of Chagar Hutang, Redang Island, Malaysia and hatchling sex ratio of Green Turtles (*Chelonia* Mydas). The average daily incubation temperature (ADIT) was recorded using the Thermochron iButton temperature data loggers. Hatchling sex ratio was determined by histological examination of the gonads obtained from dead Green Turtle hatchlings. The ADIT was higher during middle than early season nesting ($p=0.00$). The ADIT of middle third incubation periods for both early and middle nesting season were higher than the recorded pivotal temperature for Peninsular Malaysia at 29.4°C. Therefore, hatchling sex ratios for both periods was speculated to be female-biased. The results in the study is in agreement with the temperature profiling, where the calculated female ratio for early nesting season was 57.1% and for the middle nesting season was 81.8%.

Keywords*:* Green Turtle (*Chelonia Mydas*), hatchling sex ratio, average daily incubation temperature, histology.

CORRELATION BETWEEN BACKFAT THICKNESS AND REPRODUCTIVE PARAMETERS OF SOWS

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ABSTRACT

Maintaining optimal body condition of sows can improve their reproductive performances and maximise productivity of a farm. However, very few studies have been done to determine the influence of sow body condition on their swine reproduction in Malaysia. Thus, this study was undertaken to determine the association between backfat thickness and the reproductive parameters of the sows and with body condition score (BCS) and flank-to-flank measurement. Thirty-six Yorkshire × Landrace crossbred sows were selected from two reproductive phases in a pig farm at Tanjung Sepat, Selangor, Malaysia. Backfat thickness was measured using BSW Sowfit® System ultrasonic probe at P2 position. The results showed that there was an insignificant association ($p>0.05$) between high backfat thickness and low percentage of live piglets born. This may suggest that high backfat level could reduce reproductive performance in sows. A moderate positive correlation ($r=0.30$-0.60) was found between backfat thickness and BCS and flank-to-flank measurement. The study showed that BCS and flank-to-flank measurements are not totally reliable to be used to estimate backfat level in sows. The level of backfat in sows should not exceed the optimal level to ensure sow reproductive efficiency.

Keywords: backfat thickness, reproductive parameters, body condition score, flank-to-flank measurement

EFFECT OF STORAGE CONDITION ON PHYSICAL CHARACTERISTICS AND NUTRITIONAL COMPOSITIONS OF SOY WASTE

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ABSTRACT

The majority of smallholder farms use cost-effective agriculture by-products, including soy waste, to meet nutritional requirement of goats. Storage of feedstuff must be done properly to ensure that the nutritional compositions are not compromised. Therefore, the objective of this study was to determine the effect of storage conditions on physical characteristics and nutritional composition of soy waste. Three farms that either practiced freezer, gunny sack, or blue barrel storage of soy waste was chosen for the study. Three soy waste samples were obtained from each farm at days 0, 3, and 6 post-arrival. Physical characteristics were assessed by evaluating the colour, mouldiness, odour, texture, and pH of the soy waste. Proximate analysis was done to determine dry matter, ash, and crude fat, fibre, and protein contents. The results showed that there were significant differences ($p<0.05$) in physical characteristics of the soy waste samples. The pH dropped from 6 to 4 and the soy waste became discoloured from white to yellow following storage. Soy waste stored in the freezer appeared to maintain nutritional composition while showing the highest moisture content. It can be concluded that storage conditions effect the physical characteristics and nutritional compositions of soy waste used for feed.

Keywords: soy waste, storage conditions, physical characteristics, nutritional compositions, goat.

INFLUENCE OF STORAGE TIME INTERVAL ON HUMORAL IMMUNE RESPONSE INDUCED BY INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS

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ABSTRACT

Inclusion body hepatitis (IBH) is highly associated with fowl adenovirus (FAdV) infection. The efficacy of a vaccine is determined by serum antibody response. However, length of storage period may influence the efficacy of vaccines. Therefore, the present study determined the impact of different storage period of the inactivated FAdV (UPM1137) on the antibody response in broiler chickens. Forty-four day-old-chicks were divided into five equal groups. On day one of age, Groups A and B were treated with 10^9TCID_{50} UPM1137 vaccine that was stored for 1 and 6 months, respectively, Group E was non-inoculated and served as the control group. Clinical signs were monitored thrice daily. Group E with four chicks were sacrificed by cervical dislocation at day-old age. Four chicks from each Groups A, B, C, D and E were sacrificed on days 7 and 14 post-inoculation (pi). Body weights and blood samples were collected prior to sacrifice and on necropsy, the gross lesions and liver weights were recorded. Liver samples were collected at days 0, 7 and 14 pi and fixed in 10% buffered formalin for histological examination. This study revealed that there were neither abnormal clinical signs nor significant gross and histological lesions in the liver in all chickens throughout the trial. Body weights, liver weights and liver weight to body weight ratios of the broiler chickens also do not show any significant difference ($p>0.05$) among all groups. The ELISA test resulted Group C (5727 ± 1675) induced the highest FAdV antibody titre, followed by Groups A (3700 ± 948), B (3549 ± 698), D (2444 ± 1073) and E (1742 ± 609) at day 7 pi. The FAdV antibody titre dropped gradually in all groups at day 14 pi with Group C still showing high antibody titres (1444 ± 956). The FAdV antibody titre did not differ significantly ($p>0.05$) among groups. In conclusion, the study showed that inactivated FAdV stored up to 18 months can still induce high antibody response. No side effect was observed in chickens inoculated with the UPM1137 vaccine.

Keywords: inactivated fowl adenovirus, storage time intervals, efficacy, commercial broiler chickens, antibody titre.

EFFECT OF DIETARY ENERGY AND PROTEIN SOURCES ON NUTRITIONAL COMPOSITION OF DAIRY BUFFALO MILK

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ABSTRACT

Buffalo milk is a rich source of nutrients and is an excellent alternative dairy product for human consumption. Differences in dietary protein and energy sources may be reflected in the composition of milk. Therefore, this study determined the nutrient compositions of buffalo milk from three buffalo farms in Selangor, Malaysia. The buffaloes were fed diets with different energy and protein ratio. Raw milk samples were randomly collected from the bulk tanks, preserved with 40% formalin, and stored at -20°C prior to analysis. The milk fat, protein, lactose, solid non-fat and total solid composition of the samples were analysed using the Foss Milkoscan FT2. Feed samples were collected and analysed using the Association of Official Agricultural Chemists protocols. The fat and protein content of buffalo feed was higher in Farm A and B, respectively, than in Farm C. The fat, lactose, solid-non-fat ,and total solid of milk were higher in Farm A than Farms B or C. However, the milk protein content was higher in Farm B than the other two farms. In conclusion, the nutritional composition of buffalo milk were influenced by the dietary energy and protein contents.

Keywords: buffalo milk, composition, dietary energy, protein

CHOLESTEROL STORAGE IN HEPG2 CELLS SUPPLEMENTED WITH EXOGENOUS LIPID AND *STEVIA* EXTRACT

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ABSTRACT

Statin has been widely used to treat lipid-related diseases in animals. However, statin can cause many negative side effects including toxicity in animals. The *Stevia* sp. plant extract has been suggested as an alternative to statin for the lowering cholesterol levels in the blood. This study was conducted to investigate the effect of *Stevia* extract on cholesterol storage in the liver hepatocellular carcinoma (HepG2) cells. Cholesterol concentration was extracted using the modified Bligh and Dyer method. Immunofluorescence was conducted to determine formation of lipid droplets and the expression of the low-density lipoprotein receptor (LDL-R). There was an increase in total cholesterol storage and LDL-R expression in the HepG2 cells treated with *Stevia* sp. extract. The study showed that *Stevia* sp. extract has anti-cholesterol property and can potentially be used as an alternative therapeutic compound.

Keywords statin drug, *Stevia* sp. extract, HepG2 cell, cholesterol

QUANTITY AND QUALITY ASSESSMENT OF TWO DNA EXTRACTION KITS USING ANIMAL BLOOD

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ABSTRACT

Isolation of genomic DNA (gDNA) is a crucial step in genomic-related studies. There are commercial kits available for DNA isolation. However cheaper products have been introduced to the market albeit their unknown efficacies. Using cheaper products is cost-effective, however the results can be unsatisfactory. The objective of this study was to compare the yield, purity, and integrity of gDNA products using an expensive (Qiagen) and a low-cost (Analytik-Jena) gDNA extraction kits. Sixteen cattle and goat and 6 archived canine blood samples stored at -20^{o}C for 2 years were used in this study. The results showed that Analytik-Jena produces higher gDNA yield compared to the Qiagen kit. However, Qiagen kit produced higher purity gDNA. The typical yield and A260:A280 ratio suggested by manufacturer was met with the Qiagen (3-6 µg; 1.7-2.0) but not with Analytik-Jena (>30 µg; 1.7-2.0) kit. The DNA products from both kits showed excellent gDNA at gel electrophoresis even with samples stored more than 2 years. In conclusion, the study showed that both DNA extraction kits were efficient at gDNA isolation, although the Qiagen kit produced product of high purity gDNA.

Keywords: DNA extraction, blood, cattle, goat, dog

EFFECT OF A TRAINING MODULE ON ATTITUDE AND KNOWLEDGE OF VETERINARY STAFF AND STUDENTS TOWARD PAIN MANAGEMENT IN CATS

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ABSTRACT

Pain management is important in veterinary practice; unfortunately, it remains inadequate and understudied, especially in cats. Data on the current attitude and knowledge of staff and students are fundamental to the continuous revision of related education program. The study investigated the current attitude and knowledge of veterinary staff and students on pain management in cats, and assessed the effect of a training module. Veterinarians (n=10), non-veterinarian staff (n=23), final- (n=72) and fourth-year (n=50) veterinary medical students completed a duplicate set of questionnaires, i.e. before and after participating in a one-hour training module on feline acute pain. The module was conducted by a referenced veterinarian. Almost all respondents value pain control in their patients and acknowledged the importance of pain assessment. However, only a few respondents agreed they received adequate training and possessed adequate pain assessment skills. Most respondents mistakenly believed that the heart and respiratory rates are indicators of pain in cats, although these changes are often non-specific. Respondents were more likely to identify overt behavioural changes such as vocalisation and aggression compared to subtle signs, such as facial expression and rigid body during pain assessment. There was significant improvement in pain assessment by students after they received training. Respondents that benefited most from the training module, in order of effectiveness, was fourth-year followed by final-year students, non-veterinarians, and veterinarians; suggesting that training on pain management is beneficial to students should be given early in the veterinary curriculum.

Keywords: training module, veterinary staff, veterinary students, pain management, cats (*Felis catus*)

SEROPREVALENCE OF BRUCELLOSIS IN A DEER POPULATION AT UNIVERSITY AGRICULTURE PARK, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Brucellosis, a zoonotic disease of significant public health importance, is responsible for more than 500,000 human cases annually. Humans can acquire brucellosis through consumption of meat, unpasteurised milk of infected animals, and through direct contact with infected animal tissues. Domestic animals and wildlife can be the reservoir for *Brucella* species. Brucellosis cases were recorded in the deer population at University Agriculture Park (TPU), Universiti Putra Malaysia (UPM) in year 2017. All seropositive animals were culled immediately as recommended by the Malaysian Veterinary Protocol, recommending 'test and slaughter'. This present study was conducted to determine the current seroprevalence rate of brucellosis among the deer population at TPU, UPM. Serum samples were obtained from 111 deer of various ages. The Rose Bengal plate test (RBPT) was to determine the presence of antibodies against *Brucella abortus* and *Brucella melitensis* in the serum samples. The result shown that all samples were negative for brucellosis. The study showed that brucellosis was not prevalent in deer of TPU. The 'test and slaughter' programme implemented for brucellosis cases was effective in controlling the disease at TPU, UPM.

Keywords: brucellosis, deer, Rose Bengal plate test

ANTIMICROBIAL RESISTANCE OF MASTITIS PATHOGENS FROM DAIRY CATTLE HERDS IN SELANGOR AND NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

Antimicrobial resistance is a major public health concern. Antimicrobial resistance may naturally occur because microorganisms can adapt to their surrounding environments. The purpose of this study was to determine the microbial populations and the status of antimicrobial resistance of subclinical and clinical mastitis pathogens isolated from milk of cows from Ladang 16, University Agriculture Park (TPU), UPM and from UPM foster farms in Semenyih, Selangor and Lenggeng, Negeri Sembilan, Malaysia. Thirty dairy cattle were sampled and subjected to California mastitis test (CMT). Based on CMT tests, 42 mastitic quarter milk samples were identified and subjected to microbial analysis. The study showed that the total overall prevalence of mastitis was 70% comprising of 13.33% clinical and 56.67% subclinical mastitis. The was no significant differences in prevalence of overall and subclinical mastitis among farms. Fifteen bacterial species were isolate, with *Staphylococcus aureus* being the most prevalent (21.15%), followed by coagulase-negative *Staphylococcus* (19.23%), *Streptococcus viridans* group (15.83%), *Staphylococcus hyicus* and *Bacillus* sp. (7.69%), *Actinomyces sp*. and *Streptococcus agalactiae* (5.76%) and *Staphylococcus intermedius* (3.85%). Seven other isolated bacteria had a total prevalence of 1.92%. Twenty-two representative bacterial isolates from each farm were subjected to antimicrobial susceptibility test. The bacteria were sensitive to amoxicillin/clavulanic acid (90.91%), streptomycin (81.82%), and erythromycin (77.27%). This study reveals that the selected farms in Semenyih and Lenggeng had a high prevalence of mastitis. In general, the bacteria isolated in this study were susceptible to commonly used antibiotics.

Keyword: mastitis, antimicrobial resistance (AMR), pathogen

MILK COMPOSITION OF AUSTRALIAN FRIESIAN-SAHIWAL DAIRY CATTLE OF VARIOUS GENETIC GRADES

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ABSTRACT

Australian Friesian-Sahiwal is a dairy crossbreed from Sahiwal bulls and Holstein-Friesian cows. The Friesian-Sahiwal cow is known for its high milk production while well-adapted to tropical environment. The aim of this study is to compare the composition of milk from between different genetic grades of Friesian-Sahiwal crossbred in a commercial dairy farm in Keningau, Sabah. Four groups of Sahiwal-Friesian crossbred cows, 10 each of F2 (62.5%), F3 (75%), F4 (87.5%), and F% (93.7%) were evaluated. The cows were in same lactation groups during the sampling. Manual milking was done during evening milking hours. The milk collected was evaluated for fat, solid non-fat, protein, total solid, and lactose using the Master Pro Milk Analyzer. The was insignificant correlation ($p>0.05$) between the milk compositions and the genetic grade groups. Thus, it can be concluded that the phenotypic traits of Friesian-Sahiwal cows did not influence milk compositions.

Keywords: crossbred, genetic grades, milk composition, Sahiwal-Friesian

SEROLOGICAL AND MOLECULAR DETECTION OF WEST NILE VIRUS IN CATTLE

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ABSTRACT

West Nile virus (WNV) is a zoonotic RNA virus belonging to the genus *Flavivirus*. The viral transmission is enzootic between mosquitoes and birds. Wild birds act as reservoirs while mosquitoes, primarily *Culex* sp., served as a vector for virus transmission. Indigenous people (*Orang Asli*), bats, macaques and both captive and wild birds in Malaysia were shown to be seropositive for the WNV. However, the information on WNV seroprevalence in ruminants is still lacking. This study determined the serological and molecular prevalence of WNV in cattle. In this study, convenient serum and rectal swab samples were obtained from 30 cattle from University Veterinary Hospital (UVH), University Agriculture Park (TPU), and selected foster farms of Universiti Putra Malaysia. Serum was tested for the presence of WNV anti-protein E using a commercial kit (ID Screen® West Nile Competition). Sera that were positive for WNV antibodies were subjected to bovine Japanese encephalitis IgG ELISA test kit to rule out the cross-reactivity. Meanwhile, swab samples were subjected to molecular detection by one-step RT-PCR targeting the highly conserved gene between capsid and pre-membrane. Out of 30 samples, 17 (56.67%) were positive for WNV antibody. The positive samples came from UVH (3/4), TPU (3/8), and foster farms (11/18). All samples were negative at RT-PCR analysis. In conclusion, the cattle in study had been exposed WNV although they were not seropositive for WNV.

Keywords: West Nile virus, cattle, ELISA, RT-PCR

EFFICACY OF SPRAY *STREPTOCOCCUS AGALACTIAE* VACCINE IN RED HYBRID TILAPIA FINGERLINGS

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ABSTRACT

Streptococcosis is an emerging infectious disease caused by the *Streptococcus* sp. This disease can infect cultured fish and cause heavy economic losses to the aquaculture industry. This study determined the efficacy of spray *Streptococcus agalactiae* vaccine against streptococcosis in Red hybrid tilapia fingerlings. Sixty tilapia fingerlings were randomly divided into two groups; Group A (n=40) and B (n=20). The fish of Group A were sprayed with formalin-killed *S. agalactiae* once daily for three consecutive days, followed by a booster dose 2 weeks after the first vaccination. Group B fish were not vaccinated and served as the control. Both groups were challenged with pathogenic *S. agalactiae* via intraperitoneal injection. The fish were then observed for clinical signs and mortality. Mucus and gut-lavage samples from each group were collected and subjected to indirect ELISA to determine the IgM antibody levels against *S. agalactiae*. The results showed that vaccinated showed significantly higher ($p<0.05$) IgM antibody response than nonvaccinated Red Hybrid tilapia fingerlings. This study showed that spraying formalin-killed *S. agalactiae* vaccine effectively stimulated the immune system of Red hybrid tilapia fingerlings.

Keywords: Red Hybrid tilapia fingerlings, *Streptococcus agalactiae*, spray vaccine, ELISA

OCCURRENCE OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX IN ASIAN ELEPHANTS AT NATIONAL ELEPHANT CONSERVATION CENTRE, KUALA GANDAH, PAHANG, MALAYSIA

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ABSTRACT

Tuberculosis (TB) is a zoonotic disease caused by *Mycobacterium tuberculosis* complex (MTBC), a group of the multi-host pathogen that is moribund at the wildlife-livestock interface. The elephants at the National Elephant Conservation Centre (NECC), Pahang, Malaysia were earlier shown to be 25.93% seropositive for TB. This study was conducted to re-evaluate and determine the occurrence of MTBC in captive Asian elephant at the NECC. Sixty-three trunk washes and 21 fresh whole blood were collected from 21 elephants. Trunk wash samples were examined for acid-fast bacillus by Ziehl-Neelsen staining. The serological test was carried out using the DPP VetTB assay on the serum samples. Trunk washes and whole blood were subjected to PCR analysis using two sets of primers, 16MYC-F and 16MYC-R for detection of genus *Mycobacterium*, and TB1-F and TB1-R for MTBC. Serological antibody and PCR analyses on trunk wash tested negative for MTBC while PCR analysis of the whole blood showed 23.8% (5/21) positivity based on sequence identity with MTBC. In conclusion, the study showed that captive elephants at NECC harbour MTBC.

Keywords: *Mycobacterium tuberculosis* complex, PCR, trunk wash, tuberculosis, elephant

EFFECT OF STORAGE CONDITION ON EGG QUALITY AND NUTRITIONAL COMPOSITION OF COMMERCIAL AND VILLAGE CHICKEN EGGS

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ABSTRACT

Eggs are a cheap source of protein with limited shelf-life depending on storage conditions. Environmental factors such as storage time and temperature might affect the quality of eggs. Therefore, the objective of this study was to determine the effect of storage duration and temperature on physical quality and nutritional composition of eggs from commercial and village chickens. A total of 120 eggs, comprising of 60 each of commercial and village chicken eggs were stored for 0 (control), 5, and 10 days in a refrigerator (4°C) and room temperature (20°C) (n=10/storage time/temperature). The physical qualities measured were weight loss, Haugh unit, yolk index, albumen pH and yolk pH. The dry matter, protein, and fat content of eggs were determined using proximate analysis. The decrease of weight loss of eggs was rapid, while Haugh unit and yolk index gradually from day 0 to 10 at room temperature. Dry matter content decreased gradually with storage duration at room temperature. Based on crude protein and crude fat level the quality of the eggs were maintained at storage. In conclusion, storage duration and temperature play an important role in the preservation of nutritional composition and quality of eggs.

Key words: egg quality, durations, temperature, commercial, village

DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX IN MACAQUES IN SELANGOR, MALAYSIA

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ABSTRACT

Tuberculosis (TB) is an infectious disease in animals and humans. The disease is caused by *Mycobacterium tuberculosis* complex (MTBC). Human development is accompanied by the human-wildlife conflict, for example between humans and non-human primates. Among diseases that can be carried by wild non-human primates include TB. This study determined the presence of MTBC in non-human primates in selected areas in Selangor, Malaysia. Opportunistic sampling was done on fresh carcass of 30 long-tailed macaques (*Macaca fascularis*) from the culling programme of the Department of Wildlife and National Parks, Malaysia. Samples were subjected to gross post-mortem assessment for TB-like lesion. Serum samples were obtained for anti-*M. tuberculosis* antibody using the Ecotest® kit. Polymerase Chain Reaction (PCR) and Ziehl-Neelsen staining was conducted for antigen detection. Two sets of primers were used, 16MYC-F and 16MYC-R for detection of genus *Mycobacterium*, whereas TB1-F and TB1-R for MTBC. TB-like lesions were found in one sample while the Ecotest® kit gave 3.33% antibody detection. PCR analyses were negative for MTBC but positive for 564 bp antigen of the genus *Mycobacterium*. In conclusion, antibody and antigen for *Mycobacterium spp.* was detected in wild macaques, which may come from the environment. However, the study showed that there is potential risk of TB transmission between humans and non-human primates.

Keywords: tuberculosis, *Mycobacterium tuberculosis* complex, detection, human-wildlife conflict, non-human primates

EFFICACY OF KILLED-*AEROMONAS HYDROPHILA* VACCINE IN RED HYBRID TILAPIA

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ABSTRACT

Aeromonas hydrophila is a short, gram-negative bacillus, and a normal flora of freshwater. The bacteria are one of the most opportunistic pathogens associated with the epidemic that can cause massive mortality and heavy losses to the aquaculture industry. The disease caused by *A. hydrophila* is characterised by tissue swelling, dropsy, red sores, necrosis, ulceration, and haemorrhagic septicaemia. Vaccination is the best method to control the infection. This study determined the efficacy of killed-*A. hydrophila* vaccine against *A. hydrophila* infection in Red hybrid tilapia. Group 1 tilapia (n=40) were orally vaccinated with 10^7 CFU/mL (low dose) and Group 2 (n=40) with 10^9 CFU/mL (high dose) feed-based vaccine, twice daily. Group 3 Tilapia (n=40) were nonvaccinated and served as the control. Booster doses were given at 2 weeks post-vaccination. Serum, body mucus, and gut lavage were collected weekly from all fish and to determine antibody concentration the indirect-ELISA At week 4 post-vaccination, vaccinated fish were challenged intraperitoneally with 200 µL 1.5×10^8 CFU/mL of *A. hydrophila*, and the antibody response determined. The results showed that vaccinated and unvaccinated fish showed significantly ($p<0.05$) higher antibody response that the control. However, no significant different ($p>0.05$) in antibody response was observed between fish vaccinated at low and high doses. In conclusion, fish vaccinated with killed-*A. hydrophilia* vaccine induced a high immune response to *A. hydrophilia*.

Keywords: Red Hybrid tilapia, *Aeromonas hydrophila*, feed-based vaccine, indirect-ELISA

CONSUMER AWARENESS OF ANTIBIOTIC RESIDUES IN CHICKEN MEAT

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ABSTRACT

Antibiotics plays crucial roles in the prevention, control, treatment and growth promotion in poultry in Malaysia. However, use of antibiotics can cause accumulation of residues in the body. The objective of this study was to determine the awareness of consumers on the presence antibiotic residues in chicken meat. One hundred and thirty-five respondents at 3 wet markets at Pasir Gudang, Johore, Malaysia were interviewed. Questions asked were to determined their level of knowledge and awareness on antibiotic use and the presence of residues in chicken meat. Demographic variables such as gender, age group, ethnicity, education level, and working status were recorded. The results showed that 28% of respondents were ambiguous and only 6% were very sure and aware of the presence of antibiotics residues in chicken meat. The awareness level of antibiotics residues in chicken meat was associated with the education level of consumers.

Keywords: antibiotics residues, chicken meat, awareness

ISOLATION AND IDENTIFICATION OF AQUATIC FUNGUS IN NATURAL WATER BODIES IN SELANGOR, MALAYSIA

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ABSTRACT

Fungi play a crucial role in biodiversity especially in the biodegradation of biowaste products. In Malaysia, information on aquatic fungus is still scanty. In other countries, there are more data on marine than freshwater fungi. Therefore, the objectives of the study were to isolate and identify freshwater aquatic fungus species from various aquatic environments. Twelve locations from four natural water bodies in Selangor, Malaysia were investigated for the presence of aquatic fungus. The fungi were isolated using insect wing, green peas, and maize grain in a baiting method. Sporulated baits were cultured in autoclaved pond water (APW) and Sabouraud Dextrose Agar (SDA). The study isolated 92 aseptate hyphae fungi, 57 from SDA and 35 from APW. On the other hand, 62 septate hyphae fungi were isolated, 42 from SDA and 20 from APW. Thus, this study showed that aquatic fungi are prevalent in the water bodies of Selangor, Malaysia. The fungi were mainly saprophytic and can be a potential threat to aquaculture, especially under adverse conditions.

Keywords: aquatic fungi, septate, aseptate, freshwater, natural water bodies

OCCURRENCE OF GASTROINTESTINAL HELMINTHS IN VILLAGE CHICKENS IN SELANGOR, MALAYSIA

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ABSTRACT

Village chicken (*Gallus gallus domesticus*) production is gaining in importance as an industry in Malaysia. High worm burden can cause village chickens to have low body scores. The aim of this study was to determine the prevalence of gastrointestinal parasite in village chicken. Forty-five village chickens were selected from intensive and semi-intensive farms. Their body condition scores of the chicken prior to slaughter were recorded and the intestinal tract processed for worm identification. The prevalence of worms in the chicken was 33.33% (15/45). The total prevalence of worm in semi-intensively raised chicken was 28% (7/25) while those of the intensive-farming was 40% (8/20). The parasites recovered from the intestinal track was cestode, *Raillietina* sp. (8.89%, 4/45), nematodes, *Ascaridia* sp. (11.11%, n=5/45), *Heterakis* sp. (13.33%, n=6/45), *Capillaria* sp. (15.56%, n=7/45), and *Cheilospirura* sp. (4.44%, n=2/45). The body condition score was negatively correlated ($R=-0.46$, $p<0.01$) with worm burden and positive correlation with management system ($R=0.26$, $p<0.05$). This study showed that gastrointestinal helminths are prevalent in village chickens in Selangor.

Keywords: village chicken (*Gallus gallus domesticus*), body condition score, worm burden, nematode, cestode

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF CAPRINE ARTHRITIS ENCEPHALITIS INFECTION

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ABSTRACT

Caprine arthritis encephalitis (CAE) is a significant viral disease of small ruminants that can cause economic losses. Caprine arthritis encephalitis can cause chronic multisystemic inflammation in animals with long incubation period and a persistent lifelong infection. Malaysia first experienced a CAE outbreak in 2010. In 2018 it was shown that the seroprevalence of CAE was 8.8% in goats in Selangor, Malaysia. The study determined the seroprevalence of CAE among goat in farms in Negeri Sembilan, Malaysia and the risk factors associated with the disease. Blood samples were randomly collected from 82 goats. The sera were analysed by using Shanghai Qayee Biotechnology ELISA kit to detect anti-CAE virus (CAEV) antibody. Among the 82 goat samples, 43 (52.44%) were positive for CAEV antibody. Gender of the goats was shown to be an important risk factor for CAEV infection. Higher number of does (58.33%) were seropositive for CAEV than bucks (44.12%). Based on the CAEV seroprevalence, this disease of small ruminants has been increasing in incidence since 2018.

Keywords: caprine arthritis encephalitis, goats, risk factors, seroprevalence

KNOWLEDGE, ATTITUDE AND PRACTICES ON CAT- AND DOG-ASSOCIATED ZOONOTIC DISEASES AMONG ANIMAL RESCUERS IN MALAYSIA

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ABSTRACT

Stray cats and dogs are common sights in Malaysia. Issues pertaining to stray animals are not limited to their welfare, but also on their roles as potential carriers of zoonotic pathogens and the subsequent infection in humans. Animal rescuers are often in close contact with the strays and at a higher risk of acquiring zoonotic infections. Thus, this study was conducted to determine the level of knowledge, attitudes and practices (KAP) of animal rescuers in Malaysia on cat and dog associated zoonoses. Convenience sampling via offline and online forms had recruit 77 respondents for the study. The questionnaire was designed to assess the KAP for cat- and dog-associated zoonotic diseases including rabies, leptospirosis, toxoplasmosis, bartonellosis, pasteurellosis and salmonellosis of the respondents. The mean score for knowledge, attitudes, and practice was 49, 80, and 85% respectively with overall score of 60% used as the cut-off. A positive correlation was identified between rescue experience and KAP score ($r=0.26$, p-value <0.05). Previous education background of rescuers did not seem to influence the KAP score ($r=0.07$, p-value >0.05). Overall, animal rescuers, from their work experience, had moderate to good KAP level on the cat- and dog-associate diseases. Although the score for the knowledge section was low, the score for attitude and practice of the rescuers were satisfactory. Thus, to improve their KAP on animal diseases, the rescuers can be further educated on the compliance to preventive measures and understanding of the risks of zoonoses.

Keywords: knowledge, attitude, practices, cats and dogs, zoonotic diseases, animal rescuers, public health

SEROPREVALENCE AND RISK FACTORS OF SCHMALLENBERG VIRUS INFECTION AMONG SELECTED SMALL RUMINANT FARMS IN NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

Schmallenberg virus (SBV) was recently discovered in Germany. Schmallenber virus infection occurs predominantly in cattle, and to a lesser extent in sheep and goats. In Malaysia, the extent of SBV infection is still unknown. Hence, this study was conducted to determine the seroprevalence and risk factors of SBV infection among small ruminants. Blood samples were randomly collected from three small ruminant farms in Negeri Sembilan, Malaysia. Parameters such as age, breed, gender and reproductive status were recorded and farmers were interviewed to obtain information on the farm and animal management. Sera obtained from centrifuged blood samples were subjected to serology testing using the ID-VET Schmallenberg virus Competition Multi-species ELISA kit. The results revealed that 59 out of 182 samples were positive for SBV infection with a seroprevalence of 32.42%. The significant risk factors for SBV infection are species, reproductive status, management, ownership, and breed. Goats (40%) showed a higher seroprevalence rate for SBV infection than sheep (20.83%). Pregnant animals showed the highest seroprevalence rate (78.95%), followed by mature animals (34.18%), lactating animals (30%), and immature animals (11.36%). Small ruminants raised under semi-intensive (55.10%) showed higher seroprevalence rate than those under extensive (35.14%) and intensive (10.17%) management. Private small ruminant farms (43.09%) showed higher SBV seroprevalence rate than government farms (10.17%). Among goats, Boers (50%) had showed higher seroprevalence rate than local breeds (35.14%). Among sheep, mixed-breed sheep (69.23%) had higher seroprevalence rate than pure-breed White Dorpers (10.17%). This study is a first report on the seroprevalence of SBV infection in small ruminants in Malaysia. It is suggested more extensive studies are conduct in other states in Malaysia using larger sample sizes to determine the true prevalence and endemicity of SBV infection among small ruminants in Malaysia.

Keywords: Schmallenberg virus, small ruminants, seroprevalence, risk factors

MOLECULAR DETECTION OF NOVEL KOBUVIRUS IN DOMESTIC CATS

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ABSTRACT

Kobuvirus belongs to the family Picornaviridae and has been associated with acute gastroenteritis in humans and animals. Recently, feline kobuvirus (FeKoV) was reported in domestic cats in Korea, China, Italy, and the United Kingdom. The FeKoV is suspected to cause diarrhoea and thus far there is no report on its detection in Malaysia. Therefore, the objective of the study was to determine the presence of FeKoV in faecal samples in cats and its association with diarrhoea. In this study, 56 faecal samples were collected from nonvaccinated shelter Domestic Shorthair cats with diarrhoea. The cats were predominantly female (65.1%) and below the age of one year (39.5%). Faecal scoring was performed and signalment recorded. Total RNA was extracted from faecal samples and 43 samples with good RNA quantity and purity were subjected to One-Step reverse transcription polymerase reaction (RT-PCR) analysis targeting the RNA polymerase gene of FeKoV. Melt curves and quantification cycle (Cq) values were evaluated followed by gel electrophoresis and gene sequencing of the product. The sequences were aligned with the reference strains in the GenBank database using MEGA 7 software. Seven samples (16.3%) were positive at RT-PCR and gel electrophoresis (216 bp). Two of the representative samples were confirmed to be FeKoV on gene sequencing and BLAST analysis. Moreover, cats with diarrhoea had 3 times higher odds of being positive for FeKoV (p=0.072, CI; 0.948-10.046). Phylogenetic analysis showed that the two Malaysia FeKoV were 94% closely related to isolates from Korea and China. This is a first report on the identification of partial genetic sequence of FeKoV, with genetic variations, that is circulating among domestic cats in Malaysia. The FeKoV infection is suggested to be associated with diarrhoea in cats.

Keywords: kobuvirus, feline, phylogenetics, diarrhoea

ORAL SALIVARY pH IN CATS WITH GINGIVITIS AND FELINE CHRONIC GINGIVOSTOMATITIS

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ABSTRACT

Common oral inflammatory diseases in cats include gingivitis and feline chronic gingivostomatitis (FCGS). Salivary pH may partake in the development of such diseases. Thus, this study aimed to determine the saliva pH in cats with gingivitis and FCGS. Twenty-one cats comprising of 7 clinically healthy cats, 7 with gingivitis and 7 with FCGS were sampled. The saliva pH of the cats was determined semi-quantitatively using Macherey-Nagel pH indicators. Oral lesions of cat with FCGS were examined clinically and using computed tomography of the skull. The saliva pH range of cats with gingivitis was the same as in normal cats at 8 to 9, while in cats with FCGS, the pH was 7 to 9. Clinical findings in FCGS were ulcerated oral mucosa and tongue, inflamed palatoglossal folds, and bleeding gingiva while computed tomography showed alveolar vertical and horizontal bone loss and tooth resorption. The study showed non-significant difference ($p=0.101$) in saliva pH between healthy cats and those with gingivitis and FCGS.

Keywords: saliva pH, feline chronic gingivostomatitis, gingivitis, cat

DETECTION OF PORCINE PROLIFERATIVE ENTEROPATHY IN SELECTED PIG FARMS IN MALAYSIA

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ABSTRACT

Porcine proliferative enteropathy (PPE) caused by *Lawsonia intracellularis*, is among the most important enteric diseases in pigs that contributes to high economic losses to the industry. As little information is available on the prevalence of PPE in Malaysia, the objective of this study was to determine the seroprevalence and seroprevalence pattern of the PPE in Malaysia. Eight farms were selected from Perak, Selangor, and Malacca, Malaysia and 400 serum samples obtained by convenience sampling. The commercial Svanovir® L. intracellularis/Ileitis-Ab blocking ELISA test kit was used as the ante-mortem serological assay. Data was analysed according to age group, farm size, and region. Seropositivity were recorded in all farms at 29.3%. Seronegativity was observed in 4 to 12 weeks old pigs. The overall seroprevalence was very high in pigs aged 18 to 24 weeks. There was no significance difference ($p>0.05$) in seropositivity among pigs of different farm sizes or from various regions of Malaysia. However, small-scale farms showed higher PPE seropositivity than large-scale farms. Nevertheless, the PPE seroprevalence pattern of Malaysia showed that the control and prevention of PPE in pigs in Malaysia has been effective.

Keywords: *Lawsonia intracellularis*, porcine proliferative enteropathy ELISA

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF *COXIELLA BURNETII* INFECTION AMONG SMALL RUMINANTS IN NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

Q Fever or coxiellosis is a disease caused by *Coxiella burnetii*, a bacterium that infects a wide range of species and is a zoonotic threat. In ruminants, this disease causes abortion, stillbirths, premature deliveries, and weak offsprings. Currently, there is no record on the prevalence of this disease among small ruminants in Malaysia. Thus, this study determined the seroprevalence of Q fever among goats in two small ruminant farms in Negeri Sembilan, Malaysia. This study also assessed the risk factors associated with Q fevers in goats. Blood samples were collected via jugular venipuncture from 91 goats. The sera were subjected to serological testing using the Goat *C. burnetii* (Q Fever) ELISA Kit (Sunlong Biotech Co. Ltd.). The results showed that the seroprevalence of Q fever in goats was 3.30% (3 of 91). The significant risk factor for Q fever was sex, where male goats (8.11%) showed higher seroprevalence than female (0%) goats. In conclusion, the seroprevalence of Q fever among goats in Negeri Sembilan was low. However, detection of a few goats positive for Q fever antibodies suggests that they are at risk of acquiring the disease.

Keywords: *Coxiella burnetii*, goat, Malaysia, Q Fever, risk factors, seroprevalence

SELF-RECOGNITION BY THE BORNEAN ORANGUTAN (*PONGO PYGMAEUS*)

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ABSTRACT

Self-recognition is defined as the capability to distinct oneself as an individual apart from its environment and other individuals. The mirror self-recognition (MSR) is a behavioural technique introduced to determine the self-recognition ability in animals. The main objective of this study was to determine the self-recognition ability in orangutan (*Pongo pygmaeus*). The study was conducted at Sepilok Rehabilitation Centre, Sabah, Malaysia. The subjects comprised of 10 captive orangutans aged 5 to 20 years and 8 semi-wild orangutans age 7 to 12 years. Acrylic mirrors (63.5 × 87.5 cm) were placed outside the cage and around the centre. The animals were then observed through close mirror, open mirror, and mark test. All behaviours were recorded with a hidden video camera. The observation was conducted over 5 days at 1000 to 1200 h each day. All data were analysed using an ethogram procedure. The results showed that the orangutan exhibited positive open mirror test with higher social responses behaviour. In the mark test, the data were inconsistent, especially the frequency of touching the mark on their own body. This inconsistency in observations may be due to their lost in interest towards the mirror over time and the loss of their body mark from grooming. The ability of orangutan to use mirrors in image identification is most likely related to self-awareness and cognitive convergence.

Keywords: orangutan, self-recognition, mirror, Sepilok Rehabilitation Centre

IDENTIFICATION OF WEST NILE VIRUS IN PIGS USING SEROLOGICAL AND MOLECULAR METHODS

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ABSTRACT

West Nile virus (WNV) is an enveloped RNA virus of the genus *Flavivirus*. Birds are known as the amplifier while mosquitoes especially Culex species are vectors for virus transmission. Humans, reptiles, amphibians and other mammals includes horse, pigs, cows while deer are known to be the dead-end host. Seroprevalence studies in Malaysia showed that the indigenous (*Orang Asli*) population have been exposed to WNV. Although the WNV antigen has been detected in horses and bats, it is not known whether pigs in Malaysia are susceptible hosts. Thus, this study determined the presence of WNV antibody and antigen in pigs in Peninsular Malaysia. Thirty archived serum samples from the Virology Laboratory, Veterinary Laboratory Services Unit, Universiti Putra Malaysia, were subjected to competitive ELISA for the detection of IgG antibodies against pr-E of WNV and one-step RT-PCR targeting highly conserved gene between capsid and pre-membrane. The results showed that out of 30 samples 23 (76.67%) were positive for WNV antibody. The positive samples were from 3 weaners, 16 growers and 4 gilts. Seven sows were negative for WNV. RT-PCR failed to detect circulating WNV antigens. There was no association between location of pigs and exposure to WNV. In conclusion, although no circulating WNV antigen was detected in the pigs, they have been exposed to WNV.

Keywords: West Nile virus, swine, ELISA, RT-PCR, Peninsular Malaysia

COMMON BACTERIA ISOLATED FROM SECRETIONS OF BITING INSECTS

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ABSTRACT

Biting insects inject secretions into humans during bites. These secretions may potentially transmit infectious agents including antibiotic-resistant bacteria. This study determined the presence of antibiotic-resistant bacteria in secretions of centipedes, honeybees, and mosquitoes. Centipede venom and dead bees were donated. Female honey bees were selected and micro-dissected to obtain venom sacs and sting. Mosquitoes were trapped using mosquito light trap, pooled, and homogenised. Standard bacterial cultures and biochemical tests were performed on bacteria isolated. Suspected methicillin-resistant *Staphylococcus aureus* (MRSA) colonies were further identified using PCR. The bacteria were tested for antimicrobial resistance towards common human antibiotics using the Kirby-Bauer method. Surprisingly, the centipede venom did not yield any bacterial colony. *Bacillus* sp. and *Serratia* sp. were isolated from bee venom and mosquito homogenates. A yeast species was also isolated form one mosquito homogenate. The highest rate of bacterial resistance was towards cephalexin. Among bacterial isolates, 56% were resistant to at least one antibiotic. None of the bacteria isolates were resistant to gentamicin. In conclusion, secretions of biting insects carry both gram-positive and gram-negative bacteria which may be resistant to commonly used antibiotics.

Keywords: biting insects, venoms, bacteria, antibiotic resistance

SERUM CARDIAC TROPONIN I AS AN INDICATOR OF MYOCARDITIS IN DOGS DIAGNOSED WITH LEPTOSPIROSIS

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ABSTRACT

Myocarditis was recently reported in humans diagnosed with leptospirosis, an infectious and potentially fatal zoonotic disease. Currently, the extent of cardiac involvement in canine leptospirosis is still poorly understood. In clinical setting, cardiac biomarkers specific for myocardial damage could be useful in the detection of myocarditis. This study aimed to quantify and compare the severity of myocarditis in dogs with leptospirosis and to determine the serum cardiac troponin I (cTnI) concentrations in these and healthy dogs. Seven healthy dogs and 11 dogs diagnosed with leptospirosis were identified and the dogs' archived hearts in formalin were retrieved. The mid-section of the left ventricular free wall (LVFW) and papillary muscle (PM) were isolated and subjected to histopathological investigation. The inflammation in endocardium, myocardium, and pericardium of the LVFW and PM was significantly increased ($p<0.05$) in dogs diagnosed with leptospirosis than healthy dogs. Similarly, perivascular inflammation in all sites were frequently observed in the diseased dogs. The serum cTnI concentrations in dogs with leptospirosis were significantly higher ($p<0.05$) than in healthy dogs. The results showed that serum cTnI is a good marker for the detection of myocarditis and based on serum cTnI and histopathological findings, dogs with leptospirosis showed evidence of heart disease.

Keywords: leptospirosis, myocarditis, cardiac troponin I, histopathological investigation, dogs

RABBIT MEAT AS A POTENTIAL SOURCE OF FOODBORNE DISEASES

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ABSTRACT

Rabbit farming is gaining popularity in recent years mainly for production of an alternative protein source with high nutritive values and low fat and cholesterol contents. In Malaysia, there is no information on the rabbit meat as a source of foodborne diseases in humans. Therefore, this study determinef the potential foodborne diseases that could be transmitted by rabbit meat. The meat was obtained from a rabbit farm in Selangor, Malaysia. The microbiological profile was established by culturing 5 samples of fresh rabbit meat obtained immediately post-slaughter and on 20 samples of chilled meat stored at 4°C for 7 days. In the study, *Staphylococcus aureus* (80%), *Staphylococcus intermedius* (40%), and *Enterobacter cloacae* (40%) were isolated from rabbit fresh meat sampled at post-slaughter. *Staphylococcus aureus* (75%), *Staphylococcus intermedius* (65%), and *Enterobacter cloacae* (25%), *Acinetobacter* sp. (25%) and *Pseudomonas aeruginosa (5%)* were isolated from chilled meat. Histopathological examination was conducted on 10 sets of randomly selected abandoned organs collected at post-slaughter. Organs from 2 rabbits suspected to be infected with encephalitozoonosis showed typical lesions of chronic interstitial nephritis and granulomatous meningoencephalitis. In conclusion, except for the noticeably high isolation of *Staphylococcus aureus* in rabbit meat, other isolates, including *Encephalitozoon cuniculi*, are of minor foodborne disease concern. Thus, rabbit meat in Malaysia is safe for human consumption.

Keywords: foodborne diseases, rabbit meat, microbiology, histopathology

DETECTION OF *FELIS CATUS GAMMAHERPESVIRUS 1* IN BLOOD OF DOMESTIC CATS

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ABSTRACT

Felis catus gammaherpesvirus 1 (FcaGHV1), was first discovered in 2014 in North America in domestic cats and subsequently in Australia, Singapore, United Kingdom, Central Europe, Japan, and Brazil. The infection was positively associated with male cats, increasing age, and co-pathogens such as *Retrovirus* and *Haemoplasma*. This study determined and described the prevalence of FcaGHV1 in blood of domestic cats in Malaysia using molecular techniques. Using convenience sampling method, blood samples were obtained from 59 shelter and 71 pet cats. The cats were predominantly Domestic Shorthair (n=110), males (n=65), more than one year of age (n=84), and positive for feline leukemia virus (FeLV) (n=4) and feline immunodeficiency virus (FIV) (n=2). DNA extracted from whole blood was subjected to conventional PCR (cPCR) assay using published specific primers designed to target the glycoprotein B gene of FcaGHV1. Based on cPCR and gel electrophoresis, all cats were negative for FcaGHV1. To determine possible presence of low viraemia, a quantitative real-time PCR (qPCR) assay was performed on 50 randomly selected blood samples from 25 shelter and 25 pet cats. The qPCR showed low positive FcaGHV1 detection in 2 shelter and 1 pet cats with specific peaks that was subsequently confirmed on nucleotide sequencing that showed 96% similarity with the Yamaguchi, Japan isolate. None of the 3 positive cats were positive for FeLV or FIV and all were males >1 year of age. From this study, it can be concluded that the prevalence of FcaGHV1 in cats in Malaysia is low.

Keywords: Felis *catus gammaherpesvirus* 1, qPCR, domestic cat, sequencing

PREVALENCE OF ENTERIC PARASITES IN SHELTER DOGS IN SELANGOR, MALAYSIA

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ABSTRACT

Constraints in resources often compromise animal health and biosecurity in animal shelters, and may result in animals becoming reservoirs for enteric parasites, including those with zoonotic potential. The paucity of regional studies prompted the present investigation on the prevalence and associated risk factors for enteric parasites among shelter dogs. A total of 105 canine faecal samples were collected from 4 animal shelters across Selangor, Malaysia, and screened for potentially zoonotic enteric helminths *via* faecal floatation, and protozoa *via* conventional microscopy and PCR amplification using genus-specific primers. Positive amplicons were sequenced for species identification. *Ancylostoma* was the most prevalent (39.0%) enteric helminth, followed by *Trichuris* (3.8%) and *Toxocara* (1.0%). Molecular detection revealed the prevalence of *Cryptosporidium* (7.6%), *Giardia* (7.6%) and *Blastocystis* (0.95%) in the dogs. The prevalence of *Ancylostoma* and *Cryptosporidium* were significantly high ($p<0.05$) in dogs that were not dewormed in the last 3 months prior to faecal examination. *Cryptosporidium* infection was also significantly ($p<0.05$) high in dogs that were not neutered. These results suggest that regular deworming is an important measure to control enteric helminths among shelter dogs, and to minimise the risk of zoonosis to shelter workers and volunteers.

Keywords: shelter dog, enteric parasites, zoonosis, risk factor

IN VITRO ANTHELMINTHIC ACTIVITY OF INDIAN *ACALYPHA* ROOT EXTRACT TOWARDS L3 STAGE STRONGYLE LARVAE IN SHEEP

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ABSTRACT

Gastrointestinal nematode (GIN) infection is the second most important cause of small ruminant morbidity and mortality in Malaysia. To control GIN infection, commercial anthelminthic drugs (AHD) are used. However, continuous unscrupulous usage of AHD has inevitably resulted in rampant anthelminthic resistance in small ruminant farms in Malaysia. Hence, ethnoveterinary medicine is being explored to discover alternative therapeutic compounds for the treatment GIN infections. This study aimed to evaluate the anthelminthic activity of *Acalypha indica* extract, a common tropical weed, towards L3 stage strongyle larvae in sheep. A total of 300 L3 larvae were assigned to 5 equal groups and each treated in replicates with either 40, 50 or 60 mg/mL Indian *A. indica* root methanolic extract (IARME), ivermectin or distilled water. The average L3 larvae mortality was recorded at 0, 10, and 30 min, 1, 2, 3, 4 and 24 h post-treatment. The results showed that 60 mg/mL IARME produced the highest average L3 mortality with time, reaching 100% mortality after 3 h. Generally, the anti-nematode activity of IARME increased with increase in treatment doses. In conclusion, this study showed that the IARME has anthelminthic properties can potentially be used as an alternative treatment for GIN in sheep.

Keywords: *Acalypha indica*, anthelminthic resistance, gastrointestinal nematode, methanolic extract, sheep

ULNAR TROCHLEAR NOTCH IN LABRADOR RETRIEVERS WITH MEDIAL CORONOID DISEASE

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ABSTRACT

Medial coronoid disease (MCD) is a constituent of canine elbow dysplasia, and the most frequently diagnosed developmental orthopedic disease that causes forelimb lameness in young, large breed dogs. Increased loading of the ulnar trochlear notch due to increase in body weight during growth may affect the development of the trochlear notch. It is believed that the development of ulnar trochlear notch affects the progression of MCD. This study examined the ulnar trochlear notch curvature in healthy Labrador retrievers and those diagnosed with MCD. The micro-computed tomography (CT) images on the growth of the antebrachia and both elbow joints obtained from previous study were analysed on sagittal plane and six parameters were recorded by the same observer: medial coronoid-ulnar length, anconeal-ulnar length, mid-trochlear notch-ulnar length, width of trochlear notch, depth of trochlear notch at anconeal process, and depth of trochlear notch at medial coronoid process. The MCD in these elbows were confirmed through previous CT and histological studies. The results showed that there is significant difference ($p<0.05$) in all parameters between disease and non-disease elbow joints, indicating there are differences among the ulnar trochlear notch curve of the dogs. The disease status, age, and body weight showed significant effects ($p<0.05$) on all parameters.

Keywords: medial coronoid disease, ulnar trochlear notch, Labrador retrievers, computed tomography

INFLUENCE OF SOUND OF PREYS ON BEHAVIOUR OF CAPTIVE MALAYAN TIGERS (*PANTHERA TIGRIS JACKSONI*)

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ABSTRACT

The Malayan Tiger (*Panthera tigris jacksoni*) is an endangered and threatened species under IUCN Red List. Preys are important food resources for the survival of tigers in the wild. Among factors threatening their survival are illegal poaching for wildlife trade, habitat loss, and reduction in number of prey animals. The objective of this study was to determine the influence of sound of prey animals on the behaviour changes in captive Malayan tigers. Five Malayan tigers of both sexes, different ages, origin, and period of captivity were chosen for this study. Camera traps were placed around the cages and out of the site of tigers. The baseline behaviours of tiger, e.g active/aggressive, locomotion and exploratory, were recorded for two h in the morning. Then, the sounds of prey animals, e.g of sambar deer, barking deer and wild boar, were played on the loud speaker for 20 min each morning for 5 consecutive days. The behaviours of the tigers were recorded and analysed using an ethogram. The results showed that the sound of sambar deer had greatest influence on tigers that had been in captivity for 5 years. The results indicate that the tigers are instinctively sensitive and react to the sound of prey animals. The findings from this study can be used in the preparation of tigers to hunt preys when they are eventually released to the wild.

Keywords: sound of prey, behaviour, Malayan tigers

EFFECT OF ADJUVANT ON HUMORAL IMMUNE RESPONSE INDUCED BY INACTIVATED FOWL ADENOVIRUS IN COMMERCIAL BROILER CHICKENS

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ABSTRACT

Fowl adenovirus (FAdV) is an infectious agent, associated with inclusion body hepatitis, that causes high mortality in poultry. Vaccination is the key method in the control and preventiion of FAdV infections. Currently, there is no universal adjuvant that is being used with inactivated FadV virus. Thus, the objective of this study was to determine an ideal adjuvant for an inactivated FAdV (UPM1137) vaccine. Forty one-day-old commercial broiler chicks were divided into five groups. On one day of age, Group A, B, C, and D chicks were inoculated subcutaneously with 0.2 mL 1×10^9 TCID50 inactivated FAdV vaccine prepared in alum, montanide ISA 71R VG, Emulsigen-D as adjuvants, respectively. Group E chickens were not inoculated and served as the control. The chickens were observed twice daily for abnormal clinical signs. Four chicks from Group E were sacrificed at one day of age. At days 7 and 14 post-inoculation (pi), four chicks from each group were sacrificed. The body weight of the chicks measured and blood samples were collected prior to sacrifice. Liver weights and gross lesions were recorded at necropsy. Liver samples were collected for histological examination. FAdV antibody titre were determined using ELISA. None of the chickens showed abnormal clinical sign, gross or histological lesion. The body weight, liver weight and liver weight to body weight ratio of chicken did not differ significantly ($p>0.05$) among groups. Group D (5407±2117) had the highest anti-FAdV antibody titre, followed by Groups B (4421±778), A (3885±1659), C (2913±2051) and E (1742±609) on day 7 pi. On day 14 pi, the antibody titre in Group B (3324±1917) remained highest among groups. However, there was no significant difference ($p>0.05$) in anti-FAdV antibody titer among groups. In conclusion, the study showed that the UPM1137 vacccine with Montanide ISA 71R VG as adjuvant induced a higher and longer humoral immune response among vaccines with adjuvants in this study. All inactived UPM1137 vaccines with adjuvant are safe to be used in chickens.

Keywords: inactivated fowl adenovirus (FAdV), UPM1137, adjuvant, commercial broiler chickens

DETECTION OF POTENTIALLY TRANSMISSIBLE ZOONOTIC BACTERIAL AND FUNGAL DISEASES IN RABBITS

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ABSTRACT

Commercial rabbit farming in Malaysia is gaining popularity due to the nutritional benefits of rabbit meat and lucrative business. Currently, there is little information on bacterial and fungal zoonoses among rabbits in Malaysia. This study investigated the presence of potentially zoonotic bacterial and fungal diseases in rabbits of a commercial farm located in Dengkil, Selangor, Malaysia. Thirty New Zealand White rabbits were randomly selected immediate at post-slaughter. Four rabbits had diarrhoea and all rabbits showed various degrees of crusty lesions on the nose and ear pinna. Nasal swabs were obtained and bacterial cultures performed to determine presence of Pasteurella *multocida* and rectal swabs to detect *Salmonella* spp. and *Escherichia coli*. Hair and scales samples were collected using Mackenzie's brush technique for microscopic examination and fungal culture on Sabouraud Dextrose Agar. Nasal mucosa, skin, and congested intestinal sections were collected and processed for histopathological examination. All rabbits were negative for *P. multocida*, *Salmonella* spp. and dermatophytes. A total of 26.67% (8/30) of the cultures were positive for *E. coli*, which was consistent with the histopathological findings of enteritis. In conclusion, commercial rabbits may pose risk of bacterial infections to farm personnel and the public.

Keywords: commercial rabbit farming, zoonosis, bacteria, fungal

LIPID SIGNALLING PATHWAY GENE EXPRESSION IN HEPG2 CELLS SUPPLEMENTED WITH EXOGENOUS LIPIDS AND STEVIA EXTRACT

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ABSTRACT

Atherosclerosis is caused by dyslipidaemia such as hypercholesterolaemia or hypertriglyceridaemia. Although simvastatin has been used widely to control hypercholesterolaemia in humans, it has side effects. There is current interest in alternative treatments for hypercholesterolaemia in humans and animals. *Stevia rebaudiana* (*Stevia*) was previously shown to have a hypolipidaemic effect. Thus, this study has been designed to determine the effect of a commercial *Stevia* extract and *Stevia*-derived stevioside and rebaudioside A on HepG2 cells. The cells were subjected to RT-PCR analysis and gel electrophoresis to determine expression of genes of the lipid signalling pathway; low-density lipoprotein receptor (LDLr), 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMGCR), scavenger receptor protein class b type 1 (SCAR-B1), acyl-coenzyme A: cholesterol acyltransferase (ACAT2), perilipin 2 (PLIN2), proprotein convertase subtilisin/kexin type 9 (PCSK9). The expression LDLr gene determined with RT-qPCR increased significantly by 10.8-, 14.0-, 19.1-fold in positive control, high dose commercial *Stevia*-, and high dose stevioside-treated HepG2 cells, respectively.

Keywords: dyslipidaemia, HepG2 cells, LDLr, *Stevia*

MOLECULAR DETECTION OF PORCINE CIRCOVIRUS TYPE 3 IN PIGS

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ABSTRACT

Porcine circovirus type 3 (PCV3) belongs to the genus Circovirus of the family *Circoviridae*. Many countries including Malaysia have reported the presence of PCV3 in their swine herd. The PCV3 infection is associated with various clinical syndromes, including porcine dermatitis and nephropathy syndrome, reproductive failure, porcine respiratory disease complex, and cardiac and multisystemic inflammation. The study determined the presence of PCV3 in clinically ill and apparently healthy weaners, growers, and finishers. Using the convenient sampling method, 46 clinically ill animals and 18 healthy animals were selected for the study. Organs collected include inguinal and mesenteric lymph node, lungs, spleen, tonsil, and the kidneys. The tissues were subjected to PCR assay, partial sequencing, and phylogenetic analyses. Specific primers targeting the capsid gene (OFR2) of PCV3 was used in PCR. The results showed that 28.26% (13/46) of the clinically ill field animals were positive for PCV3. All healthy animals were negative for the virus. Nucleotide sequencing showed that the 6 local PCV3 strains obtained were highly homologous with each other. All Malaysian strains can most likely be phylogenetic grouped into 2 clusters and they were evolutionary close to the USA, Spain, and Germany strains. It is speculated that the Malaysian PCV 3 strains originated from these countries through importation of breeder animals. In conclusion, in Malaysia, PCV3 is prevalent in clinically ill animals. All 6 PCV3 strains in this study were identical to the previously reported local PCV3 sequences and they are clustered close to each other in the same clades.

Keywords: porcine circovirus type 3, Malaysia, PCR, sequencing, phylogenetic analyses

SEROPREVALENCE OF H9N2 INFLEUNZA VIRUS IN COMMERCIAL POULTRY FARMS IN PENINSULAR MALAYSIA

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ABSTRACT

The most prevalent avian influenza virus subtype circulating endemically in poultry producing countries including Asia, the Middle East and Northern Africa is H9N2. Outbreaks of H9N2 in poultry cause significant economic losses mainly due to losses in egg production and weight gain. This study determined the seroprevalence of H9N2 in commercial poultry farms in Peninsular Malaysia. A total of 1910 serum samples from 21 breeder, 41 broiler, 10 layer, and 12 blinded farms (n=84) in from Johore (374), Malacca (103), Negeri Sembilan (395), Selangor (898), Pahang (80), Perak (50) and Penang (10) were obtained for the study. Out of 1910 samples, 1310 (69%) from 61 (73%) farms tested positive for H9N2. Out of 61 positive farms, 15 were breeder, 29 broiler, 7 layer, and 10 unknown type farms. The H9N2 seroprevalence varied with state; Johore (65.2%, 15/23 farms), Malacca (50%, 3/6 farms), Negeri Sembilan (84.2%, 16/19 farms), Selangor (74.1%, 20/27 farms), Pahang (80%, 4/5 farms), Perak (100%, 3/3 farms) and Penang (100%, 1/1 farm). Based on age, 434 (69.7%) samples were from poultry aged <6 weeks, 176 (70.4%) 6 to 20 weeks old, 118 (53.2%) 21 to 40 weeks old, 129 (58.9%) 41 to 60 weeks old, 55 (87.3%) 61 to 80 weeks old, and 10 (31.3%) >80 weeks old; 388 samples were from poultry of unknown age. The study showed that the H9N2 seroprevalence in commercial poultry in Peninsular Malaysia is high.

Keywords: H9N2, avian influenza virus, commercial poultry, seroprevalence

USE OF ANTIMICROBIALS IN SMALL ANIMAL PRACTICE AT UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Inappropriate usage of antimicrobials contributes to the development of antibiotic resistance. Indiscriminate use is detrimental to humans and animals, for example, the result of transmission bacteriar with resistance genes. Thus, this study determined the antimicrobial usage in cats and dogs referred to the University Veterinary Hospital, University Putra Malaysia. A retrospective analysis of 1348 cat and dog cases with antimicrobial prescription for the period January to December 2018 was done. The antibiotic prescribed were described according to the category of Veterinary Important Antimicrobials and Veterinary Non-Important Antimicrobials and for confirmed and suspected infections. Eighty-nine percent of cases prescribed antimicrobials under the veterinary important drugs category, whereas 11% were not. Furthermore, only 9.8% of cases with antimicrobial prescriptions were for confirmed infections and 90.2% for suspected infections. There is a positive association between antimicrobial prescription and the confirmation of infections ($p<0.05$). In conclusion, companion animal practitioners must ensure antimicrobial usage is justifiable to reduce the occurrence of antimicrobial resistance.

Keywords: companion animals, antimicrobial resistance, antimicrobial prescription

ASSESSMENT AND ENUMERATION OF T CELLS IN FRESH AND FROZEN WHOLE BLOOD AND PERIPHERAL BLOOD MONONUCLEAR CELL SAMPLES

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ABSTRACT

Cryopreservation of whole blood and peripheral blood mononuclear cells (PBMC) is a technique used for long-term preservation of cell viability. In immunological research, flow cytometric analysis of lymphocyte subsets is often used to determine disease progression. This study was undertaken to determine viability and percentage of T cells in fresh and frozen whole blood and PBMC samples. Blood samples were obtained from 14 cats and PBMCs isolated by first lysing erythrocytes followed by density centrifugation technique. Frozen whole blood samples were kept in storage media in a -80°C freezer for 7 days. All fresh cell samples were assessed for cell viability using trypan blue exclusion technique and T cell percentage by flow cytometric analysis. Fresh whole blood and PBMC samples showed similar percentage cell viability; however, the cell viability of frozen samples decreased significantly upon thawing. The percentage of $CD8^+$ T cells was lower in frozen than fresh whole blood and PBMC samples. The percentage of $CD4^+$ T cells was lower in frozen than fresh PBMC. This observation was not true for fresh and frozen whole blood samples. The study showed that fresh PBMC sample is the best sample to be used for the enumeration of lymphocytes, while frozen PBMC samples is preferred for long-term cell storage. It is proposed that the preservations of feline lymphocyte can be improved by optimising the storage media and thawing protocol.

Keywords: PBMC, whole blood, cryopreservation, cell viability, T cell, flow cytometry

LAMENESS IN SMALL RUMINANTS REFERRED TO THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA FROM JANUARY 2013 TO JULY 2019

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ABSTRACT

Lameness, a clinical manifestation of pain resulting in impaired locomotion and gait abnormalities, has become an animal welfare issue. The condition can cause substantial economic losses to farmers. In Malaysia, information on lameness in small ruminants is scanty. Therefore, this study described the distribution of lameness cases in sheep and goats referred to the University Veterinary Hospital, Universiti Putra Malaysia (UPM). Patient case records for the period January 2013 to July 2018 were extracted from Ruminant Log Book and patient file. The cases were selected based on the following criteria; case with complaint of lameness, physical examination findings of lameness, and final diagnosis of lameness. The total number of small ruminant cases for the period was 2,519, of which 5.6% were lameness cases. Among lameness cases, 12.6% were sheep, 87.3% goats, 64.1% adults, and 64.8% females. The highest number of lameness cases recorded was in March (16.2%), followed by November (14.1%), and May (12%). As expected, because of regular visits by UPM veterinarians, approximately 80% of lameness cases were acquired from UPM foster farms (n=116). The study also showed that the common cause of lameness in small ruminants, in order of frequency was fracture (26.1%), traumatic injury (18.3%), cutaneous myiasis (8.5%) and foot rot (7.7%). Routine herd health programmes with good husbandry practice should help to minimise the incidence of lameness in small ruminant farms.

Keywords: lameness, small ruminant, traumatic injury, fracture, foot rot

MOLECULAR DETECTION OF HEPADNAVIRUS IN LIVER SAMPLES OF DOMESTIC CATS

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ABSTRACT

Domestic cat hepadnavirus (DCH) is classified under the Hepadnaviridae family and genus of *Orthohepadnavirus*. This is a new virus first discovered and characterised in Australia in a cat with lymphoma. Hepadnaviruses are generally hepatotropic; however, the potential pathogenic roles of DCH in the liver is not clear. Therefore, this study determined the presence of DCH in liver tissues of domestic cats in Malaysia and the association between the presence of the virus and hepatitis. The cats were all Domestic Shorthair; 94% were adults and 60% females. Liver tissues were evaluated via routine histopathology as showing mild or severe hepatitis. Fifty liver tissues samples were collected at post-mortem by convenience sampling from domestic shelter-feral cats. DNA was extracted and subjected to PCR using specific primers for Hgap region of the virus genome. Gel electrophoresis was performed to identify the corresponding 230 bp DCH band. The positive control was the whole genome sequence obtained from a sample previously confirmed with DCH infection. Among the liver tissues, 62% had mild and 38% severe hepatitis. The results showed 26% of liver tissues were positive for DCH. The presence of DCH was significantly associated with severe hepatitis ($p=0.007$, OR=6.075, CI= 1.523 – 24.228). In conclusion, this study presents a novel molecular detection of DCH in tissues of feline liver with severe hepatitis. The role and pathogenesis of DCH in feline hepatitis and other hepatic pathologies warrants further investigations.

Keywords: domestic cat hepadnavirus, PCR, hepatitis, liver, histopathology

EFFICACY OF POTASSIUM MONOPERSULPHATE WITH SODIUM DICHLOROISOCYANURATE AS A COLIFORM BACTERICIDAL DISINFECTANT IN PIG FARROWING PENS

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ABSTRACT

The coliform bacteria are among the most important aetiological agents that cause diseases in sow and piglets, affecting their health, welfare, and performance. In sows and piglets, the disease can be controlled by cleaning and disinfecting farrowing pen. The objective of this study was to determine coliform bactericidal effect of a new commercial product that contains potassium monopersulphate and sodium dichloroisocyanurate (PMS-SDIC). Fifteen farrowing pens at a farm in Selangor, Malaysia were selected for this study. Five pens designated as control were not cleaned with disinfectant, 5 pens were cleaned with disinfectant PMP-SDIC, and the final 5 pens cleaned with a traditional disinfectant that contained PMP only as active ingredient. Phosphate buffered saline swabs were taken before and 24 h after application of the disinfectant PMS-SDIC. The swabs sample were sent to a commercial laboratory for coliform count. The study showed no significant difference ($p>0.05$) in the coliform counts between pens cleaned with PMS-SDIC and traditional disinfectant.

Keywords: disinfectant, pig, potassium monopersulphate, sodium dichloroisocyanurate, coliform bacteria.

PREVALENCE OF *ANCYLOSTOMA* SPECIES IN SHELTER CATS IN SELANGOR, MALAYSIA

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ABSTRACT

Ancylostoma spp. is an endemic zoonotic helminth in Malaysia. It can inhabit the intestines of humans and animals. Cats can potentially transmit the infection to humans. The present study determined the prevalence of *Ancylostoma* spp. in cat faeces. Sixty faecal samples were collected from shelter cats in Selangor, Malaysia. Simple floatation technique and microscopic examination were performed to determine the presence of hookworm eggs. DNA extraction and PCR were used to amplify the selected genes of *Ancylostoma ceylanicum* and *Ancylostoma caninum* using species-specific primer. Positive PCR samples were sent for sequencing and blasted to GeneBank for species confirmation. The study showed that the microscopic prevalence *Ancylostoma* spp. was 15% (n=9/60). There was no association between ancylostomiasis in cats and the regional location of shelters (p=0.069, >0.05). Based on molecular detection, 10% (n=6/60) of faecal samples yielded *A. ceylanicum*. All samples were negative for *A. caninum.* In conclusion, the use of combination of microscopic and molecular method allows for the detection of *Ancylostoma* species in faecal samples.

Keywords: *Ancylostoma* spp., shelter cats, Selangor, prevalence

PREVALENCE OF *PLATYNOSOMUM* SP. IN STRAY CATS IN SELANGOR AND KUALA LUMPUR, MALAYSIA

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ABSTRACT

Platynosomum sp. is a hepatic parasite in cats, causing platynosomosis or lizard poisoning. The route of infection by the parasite is mostly through the ingestion of immediate or paratenic hosts, such as terrestrial isopods or lizards. This study determined the prevalence of platynosomosis among stray cats in Selangor and Kuala Lumpur, Malaysia and its correlation with gender of animals. A total of 42 liver and 42 bile samples were collected from 42 euthanised stray cats. Liver samples were examined for adult fluke and bile for ova. The DNA of adult flukes were extracted and subjected to PCR analysis, sequencing, and species identification using GeneBank. The prevalence of platynosomosis among stray cats in this study was 21.43% (CI 10.84 - 37.24). There was no significant association ($p>0.05$) between incidence of platynosomosis and gender of cats. Sequencing showed that 7 of 9 adult flukes were *P. illiciens* (96.96 - 99.89%) and 2 were *P. fastosum* (99.12 - 99.78%). In conclusion, the prevalence of *Platynosomum* sp. in Selangor and Kuala Lumpur is significant.

Keywords: *Platynosomum* sp., prevalence, gender association, stray cats

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR, INTERLEUKINS 12 AND 10 CONCENTRATIONS IN CATTLE BLOOD WITH DEGENERATIVE LEFT SHIFT

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ABSTRACT

The bone marrow leukocyte reserves of cattle are naturally low and thus may not respond adequately to inflammatory diseases and easily manifest as degenerative left shift. In inflammatory diseases cytokines are released to complement the immune response and overcome the effect of invading organisms. The objective of this study was to determine the response of serum granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 12 (IL-12) and interleukin 10 (IL-10) in cattle showing degenerative left shift. Forty-one serum samples were harvested and divided into two groups of cattle with degenerative left shift; anaemic and non-anaemic. The serum GM-CSF, IL-12, and IL-10 concentrations were determined using sandwiched bovine ELISA kits. There were no significant ($p>0.05$) differences in serum IL-12 and IL-10 levels between anaemic and nonanaemic cattle. However, the serum GM-CSF concentration was significantly ($p<0.05$) higher in non-anaemic cattle than anaemic cattle with degenerative left shift. In cattle, GM-CSF may have caused inhibition of proliferation of burst-forming unit-erythroid (BFU-E) resulting in anaemia. In conclusion, the serum GM-CSF level is higher in non-anaemic cattle with degenerative left shift than anaemic cattle with degenerative left shift.

Keywords: haemosiderin-laden macrophages, haemolytic anaemia, cattle, blood protozoa

IDENTIFICATION OF ENDOPARASITES IN CLIMBING PERCH (*ANABAS TESTUDINEUS)* CULTURED IN EARTHEN POND AND FIBREGLASS TANK

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ABSTRACT

Climbing Perch, *Anabas testudineus*, is a freshwater fish species commercially grown in Southeast Asian countries. Endoparasitism indirectly affect production of this fish. The Climbing Perch is also believed to be a carrier or host for some food borne parasites such as helminths. This study determined the prevalence of common endoparasites and the correlation between the total body length and endoparasite burden of climbing perch cultured in earthen pond and fibreglass tank. In this study, among 60 climbing perch collected, 76.6% (46/60) were infested with intestinal parasites. However, none of the fish were positive for blood parasites. The endoparasites were counted and morphology of the endoparasites examined under light microscopy. Three nematodes, *Camallanus* sp. 1, *Camallanus* sp. 2 and *Capillaria* sp., one Acanthocephala, *Pallisentis* sp., and one unidentifiable cestode larvae were identified. The study showed that there was no significant correlation ($p>0.05$) between the total body length and endoparasite burden of Climbing perch cultured in earthen pond ($r=0.046$) or fibreglass tank ($r=0.026$).

Keywords: Climbing Perch, *Camallanus* sp., *Pallisentis* sp., *Capillaria* sp.

IDENTIFICATION OF PARASITES IN RATS AT *PASAR BORONG*, SELANGOR, MALAYSIA

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ABSTRACT

Rattus rattus and *Rattus norvegicus* are the two most common rats and they are well adapted to the environment. Rats are reservoirs or vectors of diseases and may facilitate the transmission of parasite infections to humans and animals. This study identified ectoparasites, blood parasites, intestinal protozoa and intestinal helminths in rats caught at *Pasar Borong*, a wholesale wet market in Selangor, Malaysia. A total of 32 rats were caught; 71.9% (23/32) *R. rattus* and 28.1% (9/32) *R. norvegicus*. The rats were sacrified under diethyl ether. The parasites detected using peri-anal tape test, simple floatation, direct examination of intestine, and faecal smear for detection of intestinal parasites, hair pluck, skin scraping and full-body combing for ectoparasites, thin and thick blood smear, microhaematocrit centrifugation, and buffy coat technique. The ectoparasites identified were mites (*Laelaps* sp. and *Ornithonyssus* sp.) and fleas (*Xenopsylla cheopis*). Intestinal parasites identified were cestodes (*Hymenolepis nana (H. nana)*), nematodes (*Nippostrongylus brasiliensis*, *Strongyloides* sp., *Trichuris* sp., *Capillaria* sp. and *Syphacia* sp.) and intestinal protozoa (*Coccidia* and *Giardia* sp.). The only blood protozoan identified was *Trypanosoma lewisi*. The study showed that rats caught at *Pasar Borong* harbour several zoonotic parasite species that can infect humans.

Keywords: rats, ectoparasite, blood parasite, intestinal parasite

ACARICIDAL ACTIVITIES OF ESSENTIAL OIL ON BROWN DOG TICK (*HIPICEPHALUS SANGUINEUS*)

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ABSTRACT

The brown dog tick, *Rhipicephalus sanguineus*, is a vector for several diseases that could be transmitted to humans and animals. The current method of eradiacation of *R. sanguineus* is with the use acaricides. However, because frequent use, ticks have become resistant to the drugs. Essential oil has gained popularity as an alternative tick repellent for dogs. This study investigated the potential of essential oil (Union B.I.O solutions natura) in the killing of dog ticks. The essential oil contained 0.5% *E. globulus* leaf oil, 0.4% geraniol, and 0.5% *C. winterianus*. The ticks are divided into 3 treatment groups. Ticks in Group A were fully immersed in essential oil, Group B ticks were placed on folded filter paper impregnated with 0.4 mL essential oil, and Group C ticks were nontreated. The ticks were incubated at 27°C for 14 days. The result showed 100% nymph and adult mortality in the treatment groups. However, the essential oil failed to inhibit oviposition and there was no significant difference ($p>0.05$) in the weight of eggs. In conclusion, the essential oil can be used as an alternative acaricide for non-engorged ticks.

Keywords: *Rhipicephalus sanguineus*, essential oil, immersion test, packet test

PREVALENCE OF *TOXOCARA* SPP. IN SHELTER CATS IN SELANGOR, MALAYSIA

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ABSTRACT

Toxocariasis is a zoonotic disease caused by *Toxocara* spp. that is commonly found in the small intestines of dogs and cats. *Toxocara* spp. are ascarid nematodes of the order Ascardida, family Toxocaridae and they include *Toxocara canis*, *Toxocara cati*, *Toxocara malaysiensis*, and *Toxascaris leonine*. The objectives of the study were to determine the prevalence of *Toxocara* spp. in faecal samples of cats and the best method for the detection the parasite. Sixty faecal samples were collected from shelter cats in Selangor, Malaysia. The samples were subjected to simple floatation test, DNA extraction, and PCR analysis targeting the internal transcribed spacer of *Toxocara* sp. Microscopic examination yielded *Toxocara* spp. in 3.33% (2/60) of shelter cats. However, identification using the molecular method proved negative for *Toxocara* sp. The best method for the identification of *Toxocara* spp. in faecal samples of cats is the combination of microscopic method and DNA extraction from eggs or adult *Toxocora* spp. This is a first report on the prevalence of *Toxocara* spp. in faecal samples of shelter cats in Selangor determined by the combination microscopic and molecular methods.

Keywords: toxocariasis, *Toxocara* spp., prevalence, microscopic and molecular methods

IN VITRO ANTHELMINTHIC ACTIVITY OF INDIAN BORAGE (*PLECTRANTHUS AMBOINICUS*) EXTRACT TOWARDS L3 STAGE STRONGYLES IN SMALL RUMINANTS

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ABSTRACT

Parasitic gastroenteritis (PGE) is an important cause of mortality and morbidity in small ruminants in Malaysia. The control of PGE relies greatly on the use of anthelmintics. However, the uncontrolled usage of anthelmintics had led to development of drug resistance, especially towards the benzimidazoles. Therefore, there is need for ethnoveterinary options in the treatment of parasitic infections in animals. This study assessed the anthelminthic activity of Indian borage extract (IBE), towards L3 strongyle larvae in sheep. Three hundred L3 larvae were equally divided into 5 groups; each group was treated with either 10, 30 or 50 mg/mL IBE, ivermectin (positive control) or distilled water (negative control). The percent mortality of L3 larvae was recorded at 0, 10, and 30 min, 1, 2, 4, and 24 h post-treatment. The results showed that IBE caused L3 larvae mortality in a dose-dependent manner. The highest rate of L3 larvae mortality was recorded after treatment with 50 mg/mL IBE. Time-wise, 97% mortality was recorded within 4 h post-IBE treatment. In the positive control group, ivermectin produced 100% L3 larvae mortality. The study showed that IBE exhibited anthelmintic properties and can potentially to be used as an alternative therapeutic compound for the control of PGE in sheep.

Keywords: *Plectranthus amboinicus*, ethnoveterinary, anthelminthic, nematode, small ruminants

FELINE HAEMOTROPHIC MYCOPLASMOSIS IN SHELTER CATS

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ABSTRACT

Feline haemotrophic mycoplasmosis is a global infectious disease, known to induce haemolytic anaemia in cats. The three species known to cause feline haemotrophic mycoplasmosis are *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum*, and *Candidatus Mycoplasma turicensis*. The objective of the study was to determine the molecular prevalence, species diversity, and blood parameter changes associated with feline haemotrophic mycoplasmosis in shelter cats. A total of 84 feline blood samples were collected from 4 shelters; A (n=13), B (n=26), C (n=29), D (n=16) in Johore, Selangor, and Penang, Malaysia. The blood samples were subjected to species-specific PCR amplification, complete blood count (CBC) and plasma biochemical analysis. Positive amplicons were sequenced for species identification. *M. haemofelis* was the only species detected with an overall prevalence of 8.3% (7/84). The highest prevalence at 15.4% each was in Shelters A (2/13) and B (4/26) followed by Shelter C at 3.4% (1/29). Shelter D was negative for *M. haemofelis*. There was no significant difference ($p>0.05$) in CBC or values of plasma biochemical parameters between infected and uninfected cats. The low parasitaemia in cats in the shelters suggests they have instituted antimicrobial treatments to their cats. The overall molecular prevalence of feline haemotrophic mycoplasmosis in shelter cats in Peninsular Malaysia was relatively low in comparison to that reported by other countries.

Keywords: shelter cats, feline haemotrophic mycoplasmosis, prevalence, blood parameters.

PROBIOTIC POTENTIAL OF *PAENIBACILLUS PABULI* AGAINST *STREPTOCOCCUS INIAE* INFECTION IN RED HYBRID TILAPIA

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ABSTRACT

Streptococcus iniae is a common pathogen that causes disease in both freshwater and marine fish. *Paenibacillus pabuli* strain D12 has probiotic potential towards *Vibrio alginolyticus*. In this study, the probiotic potential of *P. pabuli* strain D12 against *Streptococcus iniae* infection in Red Hybrid tilapia and growth rate of fish supplemented with *P. pabuli* strain D12 were determined. Fifty Red Hybrid tilapia were divided into 5 equal groups; control, challenged with *S. iniae,* fed *P. pabuli* strain D12 for 1, and 3 days, and challenged with *S. iniae.* The length of fish was measured before instituting the probiotic and every week post-challenge. The fish were observed for clinical signs and mortality. At week 1 post-challenge, 3 fish from each group were sacrificed and the brain, eyes, and kidney obtained for bacterial isolation. Fish gut was collected for histopathology. The results showed that the mortality of the Red Hybrid tilapia that did not receive probiotic supplementation was slightly higher than those that received. Clinical signs of *S. iniae* infection were only observed in fish fed probiotic for 1 day and in challenged fish. During the 4-week treatment period, the growth rate of fish was significantly lower ($p<0.05$) in fish challenge with *S. iniae* than control fish. However, there were no significant difference ($p>0.05$) in growth among other groups of fish. It is clear that the probiotics managed to prevent *S. iniae* from causing excessive damage to the fish. The growth this fish was not affected by the probiotic supplementation. In conclusion, mortality of fish appeared to be higher in fish that were not fed than those fed probiotic.

Keywords: *Streptococcus iniae*, *Paenibacillus pabuli* strain D12, growth, mortality

MOLECULAR DETECTION OF SCHMALLENBERG VIRUS IN SMALL RUMINANTS

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ABSTRACT

The Schmallenberg virus (SBV) was first discovered in Germany in 2011. Since then, the vector-borne virus has spread to various parts of the world, causing abortions, stillbirths, and congenital malformations in ruminants. This study determined the prevalence of SBV in ruminants. Eighty-seven ruminant serum samples were collected from selected farms in Terengganu and Negeri Sembilan, Malaysia for RT-PCR analysis. The primers and positive control used were designed and optimised to target the L-segment of SBV. RNA was extracted from serum samples and its concentration measured before subjecting to one-step RT-PCR for antigen detection. Gel electrophoresis was done on the RT-PCR product. All serum samples were negative for SBV antigen. However, the lack of demonstration of SBV antigen does not mean the virus is not present, because several samples showed seroconversion for SBV. The results indicate that the animals may, some point, been exposed to the virus. The lack of seroconversion could be due low viraemia. It is recommended future studies should be conducted on acutely infected animals or use external placental fluid and umbilical cord of infected offspring for molecular detection of SBV.

Keywords: Schmallenberg virus, molecular detection, RT-PCR

MICROBIOLOGICAL PROFILE OF RESPIRATORY AND INTESTINAL TRACTS OF *RUSA TIMORENSIS*

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ABSTRACT

Rusa deer (*Rusa timorensis*) farming is a rising industry primarily for the production of high-quality venison and velvet antler. Like all animals, deer have normal microflora. This study was conducted to determine the microflora of the respiratory and intestinal tracts of Rusa deer and antibiotic susceptibility of the isolated pathogenic bacteria. Nasal swab and faecal samples were collected from 30 Rusa deer at the University Agriculture Park (TPU), Universiti Putra Malaysia. All samples were subjected to isolation and identification of bacteria species. Pathogenic bacteria obtained from the samples were selected for antibiotic susceptibility test. Six gram positive-bacteria and 3 gram-negative bacteria isolated from the respiratory tract were coagulase-negative *Staphylococcus*, *Bacillus* sp., *Staphylococcus spp.*, *Streptococcus viridans, Corynebacterium* spp., *Pseudomonas aeruginosa, Enterobacter cloacae* and *Pantoea agglomerans.* In contrast, 3 gram-positive and 4 gram-negative bacteria isolated from the intestinal tract were *Bacillus* sp., *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterobacter cloacae, Corynebacterium* spp. and coagulase-negative *Staphylococcus*. The antibiotic susceptibility test revealed that the representative pathogenic bacteria were resistant to penicillin G (57.7%), followed by amoxicillin (46.2%), erythromycin (34.6%), sulfamethoxazole/trimethoprim and oxytetracycline (7.7%) but susceptible to enrofloxacin (96.2%). The study showed that the normal microflora of the respiratory and intestinal tracts of Rusa deer comprise of opportunistic pathogenic bacteria that had developed antimicrobial resistance.

Keywords: *Rusa timorensis*, normal microflora, respiratory, intestinal, antibiotic susceptibility test, antimicrobial resistance

ASSOCIATION BETWEEN ANTIBODY TITRE, PRESENCE OF *BARTONELLA HENSELAE* ANTIGEN AND CLINICOPATHOLOGICAL FINDINGS IN FELINE BARTONELLOSIS

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ABSTRACT

Cats, as reservoir hosts for *Bartonella* sp., expose their owner to the risk of contracting bartonellosis. Although molecular screening among client-owned cats has been done in Malaysia, the serological status of these cats remains unknown. This study was conducted to determine the association between antigenemia, antibody titre, and clinical findings in cats with bartonellosis. Blood and serum samples from 50 client-owned cats were used to determine the presence of *Bartonella* sp. antigen, serological status and complete blood count. Molecular detection was performed using PCR targeting 16S – 23S rRNA intergenic transcribed spacer (ITS) region and presence of IgG antibody against *Bartonella henselae* was determined using indirect immunofluorescence assay. Based on PCR analysis, only one cat was positive of *Bartonella* sp.; however, 44% (22/50) cats were seropositive for the infection. The PCR-positive sample sent for DNA sequencing confirmed *B. henselae*. The only *Bartonella* PCR-positive cat in the study had flea infestation and gingivostomatitis, had seroconverted, and showed eosinophilia. The study suggests that *Bartonella* sp. seroprevalence was much higher than molecular prevalence among client-owned cats. The results suggest that cats with bartonellosis can be asymptomatic. This phenomenon is of significant public health issues because asymptomatic disease-carrier cats may transmit the infection to humans.

Keywords: *Bartonella*, zoonotic, PCR, immunofluorescence assay, prevalence

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