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# Preface

This year fifty-five graduating students conducted their final year projects. Student projects incur purchases of consumables which are often costly; thus the final student projects are more often than not sponsored by the research projects of the academic staff members. Subsequently, the student projects become a means for the lecturers to acquire additional data for their research. Occasionally the data from the research are publishable and/or patentable and cannot be included in the proceedings. This is particularly true for this group of researches where two (2) of the projects have managed to obtain information that is deemed patentable and cannot be published. As a result, this year the number of articles/abstracts published in the proceedings totals 53 – two less than the number of the graduating class.

Last year, the editors made a plea to the lecturers to publish full-length articles instead of mere abstracts. There has been an improvement this year as the number of full-length articles increased from 14 in 2010 to 20 for the current proceedings. Although the editors understand that lecturers need to publish in cited journals and journals with impact factor in order to meet their KPIs, we hope this is done judiciously and not at the expense of the proceedings. Like it or not, the proceedings still remains a source of reference for future students to conduct their final year projects. Thus the onus is on us to make certain that the proceedings is as good and relevant as possible.

The editors would like to take this opportunity to express their gratitude to students and their supervisors for once again making the proceedings a reality.

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# Antipyretic Effect of Crude Methanolic Extract of *Mitragyna speciosa* in Mice

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## Abstract

*Mitragyna speciosa* is a species of tropical indigenous plant that can be found mainly in Southeast Asia. This study aims to ascertain the existence of antipyretic properties of the crude methanolic extract of *Mitragynaspeciosa*, and determine its effective dose against Brewer's yeast-induced pyrexia in mice. Thirty BALB/c mice were randomly divided into three treatment groups and two control groups. Pyrexia was induced by subcutaneous injection of 30% Brewer's yeast. Rectal temperature was recorded before and 18 h after induction of pyrexia every 30 min for 5 h. All groups treated with the crude methanolic extracts of *Mitragynaspeciosa* (50 mg/kg, 100 mg/kg, 200 mg/kg) were observed to produce significantreduction of rectal temperature as compared to the negative control group at different times. Ketoprofen at the dosage of 1 mg/kg caused significant ($p<0.001$) inhibition of fever from 0.5 to 5.0 h after treatment. In conclusion, the crude methanolic extract of *Mitragyna speciosa* possessed dose-dependent antipyretic properties in mice. The antipyretic effective dose of the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg.

**Keyword**: *Mitragyna speciosa*, mice, pyrexia

## Introduction

*Mitragyna speciosa* (commonly known as "kratom", "ketom", "ketum", or "biak-biak") is a tree which is part of the family Rubiaceae. Genus mitragyna can usually be found in swamp and valleys in tropical and subtropical Asia such as Thailand, Laos, Cambodia, Malaysia (Burkill, 1935) and in the East and West Africa and India (Harvala and Hinou, 1988). In Malaysia, this species can be widely found in the northern half of the Peninsula (Burkill, 1935) and Selangor (Houghton and Said, 1986).

Effects of *Mitragyna speciosa* are known to be dose-dependent, where at high doses the subjects usually exhibited opioid-like effects, while at lower doses, it tends to result in stimulant-like effects. Its usage to treat pain and opium withdrawal syndrome was described as early as the nineteenth century (Shellard, 1989).

However, there is no scientific data on the antipyretic effect of the crude extract of *Mitragyna speciosa*. Therefore, the objectives of this study were to determine the antipyretic effect of crude methanolic extract of *Mitragyna speciosa* in mice and the effective dose of crude methanolic extract of *Mitragyna speciosa* against Brewer's yeast-induced pyrexia in mice.

## Materials and Methods

### *Preparation of the crude methanolic extract of Mitragyna speciosa and ketoprofen*

Ketoprofen 20 mg tablet was crushed using mortar and pastel. The ketoprofen powder or the crude methanolic extract of *Mitragyna speciosa* was then dissolved in 20% Tween 80 using a magnetic stirrer until a homogenous solution was achieved. This homogenous solution was then mixed with 0.9% NaCl, and stirred using a magnetic stirrer until a homogenous, foamy solution was produced.

### *Animals*

Thirty BALB/c female mice weighing 20 to 25 g were used. The mice were acclimatized for at least one week and housed eight per cage under standard 12-h light: 12-h dark cycle. Food and water were available *ad libitum*. Mice were allowed to acclimatize to the laboratory environment 24 h prior to experimentation.

### *Antipyretic activity*

The antipyretic activity was evaluated in mice according to the method described by Makonnen *et al*. (2003). A thermister probe was inserted about 1 cm into the rectum of the mice, and basal rectal temperature was recorded by a digital thermometer. Pyrexia was induced by injection of 30% (w/v) suspension of Brewer's yeast in 0.9% NaCl subcutaneously at the dosage of 10 mL/kg. The rectal temperature was recorded 18 h after the induction of pyrexia. Only mice which showed increase in the rectal temperature of > 0.5°C were subjected to the experiments.

### *Dosage test*

The crude methanolic extract of *Mitragyna speciosa*, ketoprofen (positive control), and 20% Tween 80 in 0.9% NaCl (negative control) were administered intraperitoneally. For each dosing, six mice were used. The crude methanolic extract of *Mitragyna speciosa* was administered at the dosage of 50 mg/kg, 100

mg/kg, and 200 mg/kg. Ketoprofen was administered at the dosage of 1 mg/kg. As for the negative control group, the vehicle (20% Tween 80 in 0.9% NaCl) was administered at the dosage of 10 mL/kg. Eighteen hours after the induction of pyrexia, and immediately after dosing crude extract, the rectal temperature was measured every 30 min for 5 h.

### *Analysis of data*

The mean change in the rectal temperature over the 5 h period was calculated for each mouse and expressed as percentage of reduction. All the data from the experiments were analysed using SPSS version 16.0. The data were analyzed using General Linear Model repeated measure ANOVA. The results were expressed as mean ± S.E.M. and the statistical significance ($P<0.05$, $P<0.01$, $P<0.001$) between the treatment group to the negative control group were analyzed using one-way ANOVA followed by Tukey's test at each point of time.

## Results and Discussion

In the negative control group, the subcutaneous injection of 30% Brewer's yeast has increased the body temperature to 38°C which was maintained for 5 h, and the highest temperature was recorded at 1.0 h (38.67°C). All treated groups showed significantly lower rectal temperature as compared to the negative control group. From Figure 1, ketoprofen (1 mg/kg)-treated group showed significant reduction in rectal temperature from 0.5 to 1.0 h, and from 3.5 to 5.0 h. Ketoprofen was observed to produce the highest percentage of inhibition of fever at 5.0 h (36.65°C).

As shown in Table 1, mice treated with the methanolic extract of *Mitragyna speciosa*at the dose of 50 mg/kg had significant ($P<0.05$) reduction in rectal temperature from 2.5 h until 5.0 h. At the dosage of 100 mg/kg, *Mitragyna speciosa* seems to produce significant ($P<0.05$) reduction in temperature at 0.5 h and, from 2.0 to 5.0 h. The group of mice treated with the crude methanolic extract of *Mitragyna speciosa* at the dosage of 200 mg/kg resulted in significant reduction ($P<0.001$) in rectal temperature from 0.5 to 5.0 h. The greatest percentage of reduction of temperature can be seen in the group treated at the dosage of 200 mg/kg, at 2.0 h. However, administration of methanolic extracts of *Mitragyna speciosa*at a dose of 200 mg/kg caused hypothermia in mice from 1.0 to 3.5 h.

The dose-dependent effect of the methanolic extract of *Mitragyna speciosa* also revealed findings similar to that reported by Babu *et al*. (2008), where opiate or morphine-like effects seem to predominate when high dose of *Mitragyna speciosa* extract were administered. In this study, administration of 200 mg/kg of the crude methanolic extract induced hypothermia in mice for 2.5 h. This is possibly due to the presence of other compounds in the crude extract, which may act synergistically to produce more potent hypothermic effect than that of a single compound.

Thus, it was shown that the effective antipyretic dose for the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg. The results also showed that ketoprofen, known to inhibit the cyclo-oxygenase, causes significant inhibition of pyrexia. It is assumed that the mode of action of antipyretic activities of *Mitragyna speciosa* methanolic extract might involve a mechanism possibly mediated via inhibition of cyclo-oxygenase activity. However, more studies need to be carried out to determine the actual mechanism of action of the antipyretic properties of *Mitragyna speciosa*.

## Conclusion

This study revealed that the crude methanolic extract of *Mitragyna speciosa* possesses a dose-dependent antipyretic effect, where the highest dose of the crude extract resulted in opioid-like effect.The antipyretic effective dose of the crude methanolic extract of *Mitragyna speciosa* was 100 mg/kg.

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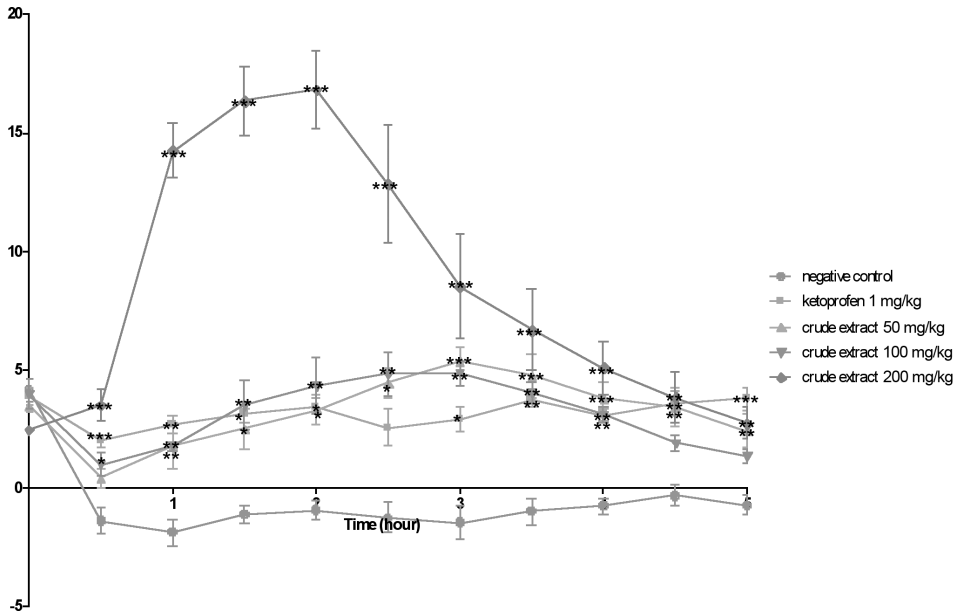


**Figure 1.** Percentage reduction of fever in Brewer's yeast-induced pyrexia in mice treated with the crude methanolic extract of *Mitragyna speciosa*. *n*= 6, data= mean ± S.E.M., all values marked with asterisk are statistically significant * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to negative control.

**Table 1.** Effect of crude methanolic extract of *Mitragyna speciosa* on yeast-induced pyrexia in mice

| Treatment | Dosage (mg/kg) | Rectal Temperature (°C) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Before pyrexia induction | 18h after pyrexia induction | Time after treatment with Mitragyna speciosa crude extract (h) | | | | | | | |
| | | | | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3 .0 | 3.5 | 4.0 | 4.5 | 5.0 |
| Vehicle | | 36.38 ± 0.145 | 37.97 ± 0.109 | 38.48 ± 0.164 | 38.67 ± 0.199 | 38.38 ± 0.168 | 38.32 ± 0.176 | 38.43 ± 0.276 | 38.52 ± 0.313 | 38.33 ± 0.235 | 38.25 ± 0.118 | 38.08 ± 0.199 | 38.23 ± 0.141 |
| Ketoprofen | 1 | 36.62 ± 0.048 | 38.10 ± 0.132 | 37.33 ± 0.092*** | 37.07 ± 0.067* | 36.90 ± 0.115 | 36.78 ± 0.111 | 37.12 ± 0.189 | 36.98 ± 0.091 | 36.68 ± 0.079* | 36.92 ± 0.060** | 36.73 ± 0.112** | 36.65 ± 0.163*** |
| *M. speciosa* | 50 | 36.82 ± 0.054 | 38.15 ± 0.159 | 37.98 ± 0.105 | 37.45 ± 0.281 | 37.18 ± 0.280 | 36.88 ± 0.220 | 36.45 ± 0.219* | 36.10 ± 0.207** | 36.33 ± 0.304** | 36.70 ± 0.256** | 36.83 ± 0.260** | 37.22 ± 0.180** |
| *M. speciosa* | 100 | 36.48 ± 0.172 | 38.00 ± 0.103 | 37.62 ± 0.135** | 37.32 ± 0.070 | 36.67 ± 0.384 | 36.35 ± 0.447* | 36.17 ± 0.340* | 36.15 ± 0.198** | 36.47 ± 0.169** | 36.82 ± 0.095** | 37.27 ± 0.056 | 37.48 ± 0.075* |
| *M. speciosa* | 200 | 37.06 ± 0.081 | 38.00 ± 0.055 | 36.66 ± 0.238*** | 32.58 ± 0.418*** | 31.78 ± 0.530*** | 31.60 ± 0.585*** | 33.12 ± 0.936*** | 34.76 ± 0.829*** | 35.46 ± 0.661*** | 36.06 ± 0.432*** | 36.54 ± 0.421** | 36.96 ± 0.268** |

Values are Mean±S.E.M. of the rectal temperature of BALB/c mice treated with crude methanolic extract of Mitragyna speciosa administered intraperitoneally in Brewer's yeast-induced pyrexia. n= 6, all values marked with asterisk are statistically significant * P<0.05, ** P<0.01, *** P<0.001 compared to negative control.

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# Microbiological Quality of Raw Goat's Milk

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## Abstract

A study was conducted to determine the microbiological status of raw goat's milk from a few sources in Selangor and to detect milk-borne pathogens; especially *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., and *Brucella melitensis*. Forty samples from nine different sources in Selangor were collected. The study found that the samples had a mean Total Plate Count (TPC) of $5.2 \pm 1.36 \times 10^5$ cfu/mL. The levels of coliform of all the samples were high with the mean of $1.5 \pm 4.17 \times 10^6$ cfu/mL. *Staphylococcus* aureus were detected in 14 of 40 samples of raw goat's milk (35%). *Salmonella* spp., *Campylobacter* spp. and *Brucella melitensis* were not isolated from any of the samples.

**Keywords**: Microbiological quality, TPC, CPC, raw goat's milk, pathogens

## Introduction

Milk is an essential food for newborn and is rich in proteins, carbohydrates, fats, minerals and vitamins. Milk spoils easily and the reasons for milk spoilage are numerous which includes infected milking animals, unhygienic milking processes and improper milk storage methods. Microorganism not only can cause spoilage of milk but may also cause milk-borne infections to humans. Milk is a good medium for the growth of many microorganisms, including pathogens (Bishop and White, 1986; Sorhaug and Stepaniak, 1997).

More than 90% of all reported cases of dairy-related illness are of bacterial origin. At least 21 milk-borne or potentially milk borne diseases has been identified (Bean *et al*., 1996). In the past 20 years, illness from dairy consumption have been predominantly associated with *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* O157:H7, which can be present in milk obtained from apparently healthy looking animals, typically as a consequence of contamination that occurs during and after milking. The present study examines the microbiological quality of raw goat's milk and attempts to detect a few important milk-borne pathogens.

## Materials and Methods

### *Sample and data collection*

Raw goat's milk was purchased from nine different sources in Selangor (either farms or retail shops). Forty samples of raw goat's milk were obtained between 29th November and 22th December 2010 for the purpose of this study. From the goat farm, the samples were collected from individual goats and stored in 30 mL bottles. The sample collection for 3 farms was performed by the author of this study and for the other 3 farms was performed by the workers in the farm. All samples were placed in a styrofoam box filled with ice and transported to the Veterinary Public Health Laboratory in UPM, Serdang Selangor.

### *Bacteriological Analysis*

#### *Total Plate Count*

Plate Count Agar (PCA), (OXOID, Basingstoke, U.K.) was used to determine the Total plate count of the raw goat's milk. One mL of milk was pipetted aseptically and transferred into universal bottles containing 9 mL 0.1% Buffered Peptone Water (BPW). Serial dilutions were carried out and 0.1 mL was spread onto the PCA. The plates were incubated at 30ºC for 48 h. At the end of incubation period, plates containing colonies between 30 and 300 were selected for colony count.

#### *Coliform Plate Count*

Violet Red Bile Agar (VRBA), (OXOID, Basingstoke, U.K.) was used to detect the presence of coliform in the raw goat's milk. Serial dilution was carried and 1 mL was pipetted into the plate and mixed well with the VRBA using pour plate method. Then a thin layer of VRBA was poured onto the solidified agar. The plates were incubated at 37ºC for 48 h. At the end of the incubation periods, coliform appear as typical red colonies. At the end of incubation period, plates containing colonies between 30 and 300 were selected for colony count (Harrigan, 1998).

#### *Staphylococcus aureus*

The presence of *S. aureus* was determined by surface plating the samples on Mannitol Salt Agar (MSA) (OXOID, Basingstoke, U.K). One loopful of milk samples were cultured onto the MSA. Then the plates were incubated at 37ºC for 24 h. The presence of positive isolates is indicated by the presence of yellow colonies and the agar turning colour to yellow.

#### *Salmonella spp.*

One mL of milk samples was pipetted into 10 mL of BPW, and then incubated at 37ºC for 24 h. Then, one mL of sample was transferred into 10 mL of Rapapport – Vassiliadis (RV) (OXOID) and was incubated at 42ºC for 24 h. After the incubation periods, one loopful of enrichment was streaked onto the Chromogenic Agar (CA)

(OXOID) and XLT4 Agar (OXOID). The plates were incubated at 37ºC for 24 h. On the CA plates, purplish colonies indicates *Salmonella* spp. while on XLT4 agar plates, black colonies is indicative of *Salmonella* spp. Biochemical test were perform to confirm the presence of *Salmonella* using Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), and urease. To confirm for *Salmonella*, agglutination test using polyvalent O and H antiserum was carried out.

### *Campylobacter spp.*

One mL of milk samples was pipetted into 9 mL of enrichment media that was prepared according to the manufacturer's instruction with *Brucella* broth (Becton Dickinson, Germany) and supplemented with 5% lysed horse blood, 1 vial of growth supplement (SR023) and 1 vial of CCDA selective supplement (SR155). The samples were then incubated at 42ºC for 48 h. Then, one loopful of the enrichment media was streaked onto Campy Cefoperaxone Deoxycholate Agar (CCDA) (OXOID, U.K) and incubated at 42ºC under microaerobic atmosphere for 48 h. Then, motility test were carried out on the small, greyish, translucent colonies and observed under microscope. *Campylobacter* appeared as motile, small, curved rod organism (seagull-shaped). Those samples with positive motility test were subcultured on Columbia Blood Agar (CBA) and incubated further. Then, another motility test was done before further confirmation test including oxidase test, catalase test, hippurate hydrolysis and indoxyl acetate hydrolysis tests were performed.

### *Brucella melitensis*

One mL of milk samples were pipetted into a micro centrifuge tube. Then, the tubes were centrifuged at 2000 x g for 15 min. Then, the milk cream was separated from the sediment part. The milk cream and the milk sediments were transferred and streaked onto *Brucella* Agar (BA) (OXOID). The plates were incubated at 37ºC for at least 2 to 3 d to allow the organism to grow. Small, honey colour, translucents colonies were subcultured onto BA and were incubated at 37ºC for another 2 to 3 d. Then Modified Acid Fast staining was done to check for *B. melitesis* which should appear as red and small coccobacilli.

## Results

### *Bacterial counts*

Fifteen representative samples out of 40 samples of raw goat's milk were examined for TPC and coliform count. The TPC number ranges from <1 x 10 to 5.2 x $10^6$ cfu/mL. Coliform was found in all samples examined. All samples show a moderate count of coliform ranging from $10^3$ to $10^6$ cfu/mL.

### *Isolation of pathogens*

Of the 40 samples tested, 14 (35%) were positive for *S. aureus*. *Salmonella* spp., *Campylobacter* spp. and *Brucella* were not detected in this study.

## Discussion

In this study, the wide range of bacterial counts between sources could be due to pre-and post-milking hygienic practices because based on the author's observation during sample collection, neither pre-milking nor post-milking hygiene routine were practiced among the milkers in all 3 farms where milk collection was performed by the farm workers. This may result in increased bacterial contaminations from the udder. The practice may also increase the risk of intramammary infections that directly increase the TPC in milk. In farms where the author milked the goat, a very low number of counts were found.

Inferior microbiological quality of the water used for cleaning the utensils could have contributed to the high TPC of the milk samples. However, most of the sampled farms uses tap water, therefore reduces the possibility of water-borne contaminations.

The presence of coliform is associated with faecal and environmental contamination and the counts in raw milk should be less than 50 cfu/mL. The existence of coliform bacteria in the milk may not necessarily indicate a direct faecal contamination of milk, but may indicate poor hygiene and sanitary practices during and after milking. In the present study, we suspect that the high coliform count could be due to the poor hygiene and sanitary practices during milking. This is because the goats were kept in houses with raised-slatted flooring which generally are easily cleaned and remained clean for longer periods. Besides, the faeces of the goat are in pelleted form and are drier as compared to the cow dungs. Thus, contamination due to the direct faecal contact is much reduced as compared to that that would occur in cow's milk.

*Staphylococcus* aureus was found in 35% of raw milk sampled. The finding is in agreement with other studies that reported *S. aureus* isolation rate of 12- 32% in raw goat's milk (Ekici *et al*., 2004) and 37 to 70% in other type raw milk (sheep, cow and camel) (El-Ziney and Al-Turki, 2006).

The author speculate that the high percentage of positive samples might be due to subclinical mastitis as *S. aureus* is the major causative pathogen that causes the disease (Chye *et al*., 2004). As observed during the milking of animals in the present study, the milkers did not perform any basic sanitary precaution before and after milking and the milker did not wash his hands between milking different animals. Therefore, this increase the chances of bacterial transmission during the milking process as contaminated hands and milking equipment come into contact with uninfected mammary glands (Moroni *et al*., 2005). Thus, Oliver and Gillespie. (1999) suggested that post-milking teat disinfection is an effective procedure to reduce the number of contagious mastitis pathogens such as *S. aureus* on the teat skin immediately after milking.

Failure to isolate other pathogens targeted in the study does not necessarily mean that goat's milk is free from the pathogens. However, it may suggest that the prevalence or concentration of the organisms in the milk is low and was not detectable with the study sample size. The low prevalence of the bacteria, the fastidious characteristic of the some of these organisms can affect the success of

its isolation from the raw goat's milk. Rollins and Colwell, (1986) reported that *Campylobacter* might be present in the raw goat's milk, but in a non-culturable state. In addition, for *Brucella*, the sensitivity of the bacteriological culture methods depends on the viability and numbers of the bacteria in the sample, and the nature of other contaminating bacteria in the same samples. Thus, culture methods may not always be successful.

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# Immunoregulatory Response following Fluoranthene Instillation in Embryonated Chicken Eggs

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## Abstract

The immunotoxic effect the air pollutant, fluoranthene in chicken has never been documented. This study was undertaken to determine the possible immunotoxicity of fluoranthene in embryonated chicken eggs. Fifty 9-day-old embryonated chicken eggs were used in this study where 40 were each inoculated with fluoranthene at the dose of 15 mg/kg via the allantoic route. The remaining 10 eggs were inoculated with phosphate buffered saline (PBS) and acted as controls. All eggs were incubated at 37°C and candled every day for evidence of embryonic survival. Dead embryos (before 21 days old) were necropsied while the allantoic fluid and yolk collected for the determination of ND-HI titers. Chicks that hatched were sacrificed and blood was collected for ND-HI titer determination and lymphoid organs were procured for histopathology. Postmortem findings of fluoranthene inoculated embryos were stunted growth, generalized or localized haemorrhages especially at legs and head. Histopathologically, fluoranthene induced lymphoid hyperplasia in the thymus, spleen and bursa. Such change has led to an increase in antibody production compared to the controls. This study has provided evidence that fluoranthene may cross the egg barrier (*in ovo*) in avian species.

**Keywords**: Fluoranthene, embryonated eggs, imunoregulation, ND-HI titer

## Introduction

The air pollutant, fluoranthene is a polycyclic aromatic hydrocarbon (PAH) found in products of incomplete combustion of fossil fuels, main stream cigarette smoke, and in char-broiled foods (Anonymous, 1984). Malaysia and its poultry industry are periodically exposed to episodes pollution, which naturally results in hazardous effects. Unfortunately, there is a dearth of knowledge on the effect of fluoranthene on immunoregulation in embryonated chicken eggs. Thus the main aim of this study is to determine the possibility of '*in ovo*' transmission of fluoranthene and its effect in inducing lymphoid organ changes leading to changes in the regulation of the immunity.

## Materials and Methods

### *Experimental design*

Fifty 9-day old, embryonated chicken eggs were used in this study where 40 were each inoculated with fluoranthene at the dose of 15 mg/kg via the allantoic route. The remaining 10 eggs were inoculated with phosphate buffered saline (PBS) and remained as controls. All eggs were incubated at 37°C and candled every day for evidence of embryonic survival. Dead embryos (before 21 days old) were necropsied while the allantoic fluid and yolk collected for the determination of ND-HI titers. Chicks that hatched were sacrificed and blood collected for ND-HI titer determination and lymphoid organs were procured for histopathology.

### *Inoculation of eggs by the allantoic route*

The volume of fluoranthene and PBS given was 0.1 mL.

### *Newcastle Disease (ND) titer –HA HI antibody test*

Harvested yolk and allantoic fluids were subjected to HA and HI routine test (Allan and Gough, 1974).

### *Histopathology*

Lymphoid organ of the dead embryo and day old chicks was used. All tissue samples were fixed in 10% buffered formalin for at least 24 h and proceed to routine histopathology technique (Ross *et al*., 2006)

## Results

### *Clinical signs*

No significant clinical signs were observed in dead embryonated chicken eggs and day-old chicks.

### *Gross findings/postmortem findings*

Dead embryo exhibited stunted growth and generalized haemorrhage especially at their legs and head (Figure 1). However, no significant gross findings were seen in the lymphoid organs.

### *Newcastle disease hemagglutination-inhibition (HI) antibody titer*

The mean ND-HI titer of yolk of the fluoranthene group (7.89) was not significantly different compared to control (5.80). However, the mean ND-HI antibody titer of day old chicks in the fluorathene group (9.33) is highly significantly ($p<0.05$) higher than that of the control (5.80).

### *Histopathology*

*Thymus*

The ratio of cortical and medullary cell composition remained within normal limits and almost perfect histology was seen in the control group. However, fluoranthene group exhibited compactness of cells indicating possible hyperplasia (Figure 2).

*Bursa of Fabricius*

At all instances, clear demarcation of the medulla and cortex ratio with normal epithelial layer of interfollicular septae was seen in the control group. While with similar morphology, the flouranthene group appeared to exhibit much more compactness (Figure 3).

*Spleen*

As seen in the thymus and bursa, prominent compactness of this tissue is seen in the fluoranthene group (Figure 4). While, normal distribution of red and white pulp was seen in control group.

## Discussion

A single intraperitoneal injection of fluoranthene to pregnant C57/B6 mice on different gestational days produced an increased rate of embryo resorption. Embryotoxic effects included decreased crown-rump length, deformities of the telencephalon, and absence of red blood cell circulation through the yolk sac were also observed in an *in vitro* study in which post implantation rat embryo cultures were exposed to fluoranthene (Irvin and Martin, 1987). Exposure of male and female albino rats to concentrated vapors of fluoranthene for 8 hours produced no mortality (Smyth *et al*., 1962). It is possible by the low mortality rate and mild postmortem lesions, the dose used in this study could be considered as the lowest-observed-adversed-effect-level. However, its effect in yielding immune upregulation indicates that the compound can pass through the in ovo barrier. This is the first study demonstrating the ability of fluoranthene in inducing lymphoid organ changes leading to upregulation of the immunity.

The exposure of high levels of air pollutant due to PAH exposure led to pathologies of many body systems of adult poultry (Latif *et al*., 2009; 2010). However, in this study embryonated eggs yielded the reverse indicating the involvement of a different mechanism, which remains to be investigated.

## Conclusion

Thus, fluoranthene is able to elicit lymphoid organ changes leading to immune upregulation in embryonated chicken eggs.

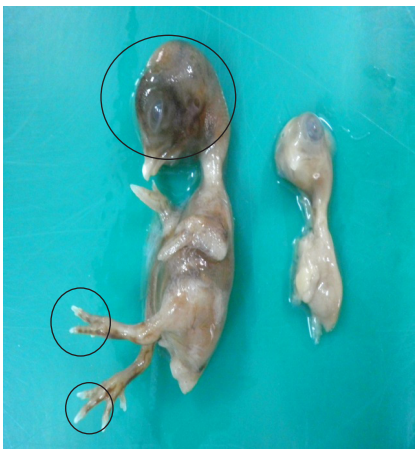

**Figure 1.** Dead embryo exhibiting stunted growth and generalised hemorrhage at legs and head (circle)

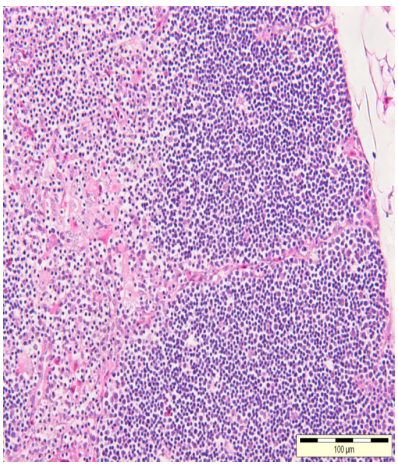

**Figure 2.** The thymus shows compactness of cells indicating possible hyperplasia.

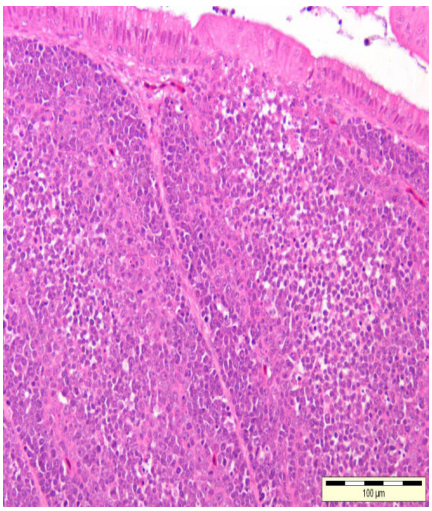

**Figure 3.** The Bursa of Fabricius exhibited much more compact population of cells.

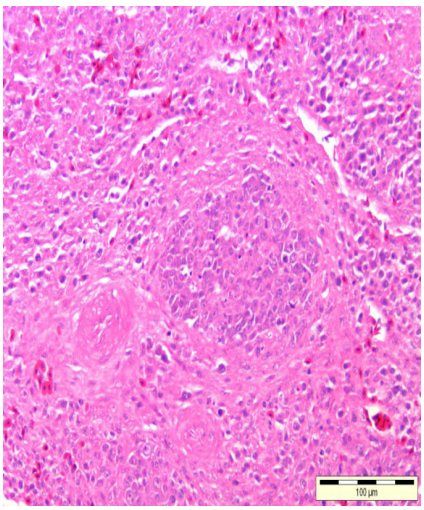

**Figure 4.** The spleen shows prominent compactness of tissue denoting possible hyperplasia.

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6th Proceedings of the Seminar on Veterinary Sciences, 11 – 14 January 2011: 17-21

# Antiviral Properties of Berembang Bukit and Kandis Hutan Against Pseudorabies Virus in Animal Cell Culture

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## Abstract

The tropical rainforest in Malaysia represents an untapped potential source of antiviral compounds. Bioactive compounds in plant species from the same genus as Kandis Hutan such as xanthones, benzophenones, biflavonoids and lupeol had been studied. Eugeniin is an anti-herpesvirus compound which had also been found in Berembang Bukit. This preliminary study was carried out to discover the presence of antiviral properties in Berembang Bukit and Kandis Hutan using different antiviral assays. In this study, MTT cell viability assay was used in addition to microscopic evaluation of pseudorabies virus (PrV)–induced cytopathic effect (CPE) on Vero cells. The cellular toxicity of DMSO was also evaluated. DMSO was less than 10% cytotoxic at concentration of 0.1% to Vero cells and its effect can be negligible. Both plants had demonstrated antiviral properties in ethyl acetate and ethanol extracts. From our findings from all three antiviral assays, the ethanol-extracted Kandis Hutan possessed the most promising antiviral properties. Nevertheless, antiviral potential of ethyl acetate and ethanol-extracted Berembang Bukit and ethyl acetate-extracted Kandis Hutan also merit further investigation.

**Keywords**: Pseudorabies virus, plant extracts, antiviral assays, MTT assay, DMSO, cytotoxicity

## Introduction

All herpesviruses are morphologically similar. Pseudorabies virus (PrV) and other closely related homologs such as BHV-1, BHV-5, SHV-1, CHV-1, EHV-1, EHV-3, EHV-4, and FHV-1 are members of the genus *Varicellovirus* and subfamily *Alphaherpesvirinae*. The viruses have a short replication cycle and can establish latency or recrudescence characteristic in infected animal host. Veterinary important pathogenic herpesviruses are contagious or infectious and affected animals have

poor prognosis for recovery or survival. Specific treatment with antiviral drugs have side-effects and its efficacy may be impaired by resistant virus strains.

Driven to identify compounds with antiviral properties for future clinical use as antiviral drugs or antiviral agents, researchers discovered that plants possess various biologically active compounds with potential therapeutic use (Xu *et al.,* 1999). The preserved biodiversity in Malaysian tropical rainforest allows numerous antiviral medicinal plants to be discovered (Ali *et al*., 1996). In addition, anti-PrV and anti-herpesvirus activity had been studied in numerous plant species (Summerfield *et al.,* 1997; Kurokawa *et al*., 1998).

Berembang Bukit is a medium sized to large tropical rainforest tree. The seeds of the tree had been used to treat abdominal pain, food poisoning and peptic ulcer and the leaves applied on the skin by local folks (Tsukiyama *et al.,* 2010). Berembang Bukit had been found to have anti-aging, anti-inflammatory and antimicrobial activities (Tsukiyama *et al.,* 2010; Othman *et al.* 2011).

Kandis Hutan had been used to treat stomachache and fever by local folks (Jabit*et al*., 2009). The leaves of Kandis Hutan contain cytotoxic xantones (Khalid *et. al.,* 2007). Other compounds such as benzophenones, biflavonoids, biphenyls and alkaloids have also been found in plants of this genus (Chiang *et al*., 2003; Jabit *et al*., 2009). The plants of this genus have selective cytotoxic, anti-inflammatory, free radical scavenging, antimicrobial, larvicidal, and anti-HIV activity (Goh, 2011). Although extensive studies had been conducted on plants of this genus, the antiviral potential of Kandis Hutan against herpesvirus had not been elucidated.

The objective of this study was to evaluate the antiviral potential of Berembang Bukit and Kandis Hutan plant extracts against PrV *in vitro.*

## Material and Methods

### *Crude plant extracts*

Samples of crude plant extracts from the leaves of Berembang Bukit (UNMC 37) and Kandis Hutan (UNMC 45) were obtained from the Faculty of Bioscience, University of Nottingham, Malaysia. Both plant extracts were crude and extracted with 3 organic solvents namely hexane, ethyl acetate and ethanol.

### *Pseudorabies virus (PrV) and Vero cells*

An established strain of PrV was used in this study. One hundred pfu/mL of PrV was inoculated into each experimental flask-well-seeded with Vero cells. Vero cells (ATCC No. CCL-81) were seeded into sterile 96-well flat bottom plates at 1 X $10^4$ cells/well, maintained in RPMI media supplemented with 1% FBS and incubated at 37$^o$C with 5% $CO_2$ humidified atmosphere

### *Cytotoxicity Assay*

Plant extracts from Berembang Bukit and Kandis Hutan were evaluated for Vero cells cytotoxicity effect *in vitro* at a concentration ranging from 1.56 - 100 µg/mL in 0.1% DMSO. Besides, DMSO cytotoxicity was also evaluated. MTT assay was conducted following cytotoxicity assay to determine the remaining number of viable cells in the experimental wells (Goh, 2011).

### *Antiviral Assays*

Three antiviral assays were carried out in this study namely virucidal assay, attachment assay and prophylaxis study (Goh, 2011). MTT assay was also conducted following antiviral assays.

### *Statistical Analysis*

All samples were tested in triplicate and the results are expressed as Mean ± Std Dev. Percentage of cell cytotoxicity was calculated with the formula: % cell cytotoxicity = $OD_{sample}$ / $OD_{Cell\ ctrl}$ X 100%. The percentage of viral inhibition was calculated using the formula: % viral inhibition =($OD_{sample}$ – $OD_{Virus\ Ctrl}$) / ($OD_{Cell\ ctrl}$ – $OD_{Virus\ Ctrl}$) X 100%. One-way ANOVA was used to determine the means difference between samples and controls using SPSS 16.0. The significant value is set at $P< 0.05$.

## Results and Discussion

Crude extracts from both plants exhibited antiviral properties. They inhibited cytopathic effect (CPE) formation at higher concentrations [100 - 12.5 µg/mL] in virucidal and attachment assay. Anti-PrV properties of Berembang Bukit were likely due to the presence of bioactive compound Eugeniin (Tsukiyama *et al*., 2010). Caged xanthones, benzophenones, biflavonoids or lupanes which were found to be anti-HIV may also be present in Kandis Hutan. However, the hexane extraction of this plant contain cytotoxic compound(s) and hence its antiviral property cannot be evaluated in this study. It is noteworthy to mention that at a concentration of ≤0.1% DMSO, less than 10% cytotoxic effect was observed in control cells and can be regarded as negligible.

Both plants demonstrated better virucidal effects than their attachment and prophylaxis ability, with the overall lowest $IC_{50.}$ They exert their virucidal effect by inactivating the virion through stably binding to it (Carlucci *et al.,* 1999).

To conclude, among all the plant extracts, ethanol-extracted Kandis Hutan was the most promising antiviral sample because it had overall high antiviral potential and it was easily dissolved and hence, will be a more economical and less time consuming antiviral agent to manufacture.

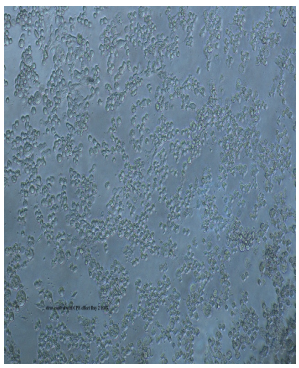

**Figure 1.** CPE formation in positive control well at day-2 post-attachment assay (100X).

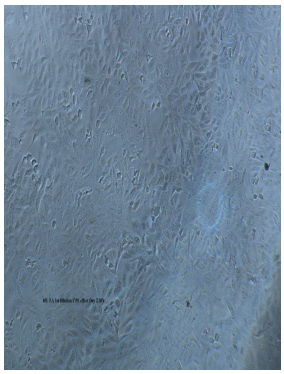

**Figure 2.** Absence of CPE on day-2 post- attachment assay in wells containing ethyl acetate-extracted KandisHutan at $2^{-1}$dilution (100X).

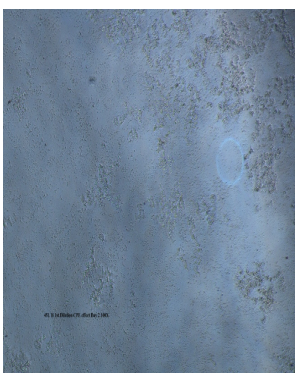

**Figure 3.** Drastic reduction in the number of viable Vero cells on day-2 post-virucidal assay in wells containing hexane-extracted Kandis Hutan at $2^{-1}$dilution due to cytotoxicity (100X).

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6th Proceedings of the Seminar in Veterinary Sciences, 11-14 January 2011: 22-28

# Isolation and Identification of *Riemerella anatipestifer* from Ducks in Malaysia

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## Abstract

*Riemerella anatipestifer* is the primary etiological agent of contagious septicemic diseases among ducks. The study is the first attempt to isolate and identify *R. anatipestifer* from ducks in Malaysia. In this study, ten diseased Khaki-Campbell ducks and forty healthy Khaki-Campbell ducks were selected. A pharyngeal swab was collected from each selected ducks. One strain of *R. anatipestifer* was successfully isolated out of the ten diseased ducks and identified using conventional biochemical tests. *R. anatipestifer*was isolated from the healthy ducks. The *R. anatipestifer* isolate was then subjected to antibiotic sensitivity testing using Kirby-Bauer method. The sensitivity of *R. anatipestifer* to penicillin G, enrofloxacin, oxytetracycline, gentamicin, neomycin, and ceftiofur was determined. *R. anatipestifer* was found to be highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G and ceftiofur.

**Keywords**: *Riemerella anatipestifer*, Khaki-Campbell ducks, conventional biochemical tests, Kirby Bauer method

## Introduction

Economic losses due to *R. anatipestifer* infections in ducks are of significant concern, since the infection can result in significant weight loss, mortality rate of up to 75%, carcass condemnations, and the destruction of colonies as a containment strategy (Zhong *et al*., 2009; Leavitt *et al*., 1997). Mortality can be as high as 95% and is influenced by predisposing viral and bacterial infections (Leavitt *et al*., 1997). Co-infection and adverse environmental conditions can predispose ducklings to disease outbreak (Zhong *et al*., 2009). The disease has a worldwide distribution, and endemic infections are restricted to commercial duck flocks (Singh *et al*., 1983). The infection can be per acute, acute or chronic. The exact route of transmission and the challenge dosage of *R. anatipestifer* are still debatable (Sarver *et al*., 2004).

*R. anatipestifer* is a gram-negative, nonmotile, nonspore-forming, rod shape bacterium that occurs singly, in pairs and occasionally in pairs. *R. anatipestifer* grows well on blood agar and chocolate agar but is usually non-haemolytic. It shows no growth on MacConkey agar. Its growth is enhanced when incubated at 37°C in a candle jar that provides increased carbon dioxide and moisture. The colonies of *R. anatipestifer* on blood agar when incubated at 37°C for 24 to 48 h are 1 to 2 mm in diameter, convex, transparent, and glistening. *R. anatipestifer* is catalase- and oxidase-positive. It is usually positive for gelatinase test, thus is capable of liquifying gelatin. *R. anatipestifer* is negative for nitrate reduction and has no action on glucose. To date, 21 serovars have been detected using the agglutination test method (Pathanasophon *et al*., 1995).

Ocular and nasal discharges, diarrhea, mild coughing and sneezing, tremors of the head and neck and incoordination are common clinical signs of *R. anatipestifer* infection in ducks. Upon postmortem, the most obvious gross lesion in ducks is fibrinous exudates in the pericardial cavity, air sacs and over the liver surface. Mucopurulent exudate is often detected in nasal sinuses. Pneumonia may be seen. Spleen and liver may be enlarged and mottled. Infection of the CNS can result in fibrinous meningitis. A definitive diagnosis can only be established by isolation and identification of *R. anatipestifer*. It is not yet confirmed whether *R. anatipestifer* may localize and persist in the upper respiratory tract of birds without causing any signs and lesions. Isolation and identification of *R. anatipestifer* in ducks in Malaysia has not been established so far.

Antibiotics can be used to treat *R. anatipestifer* infection. *R. anatipestifer* is reported to be sensitive to enrofloxacin, chloramphenicol, lincomycin, streptomycin and neomycin but is resistant to penicillin G, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, kanamycin and gentamycin (Zhong *et al*., 2009). Identification of the suitable antibiotic for treatment of *R. anatipestifer* infection is significant to treat and control the infection, reduce or prevent mortality of the infected ducks and ensure maximum cost effectiveness by diminishing the unnecessary use of antibiotics to which *R. anatipestifer* is resistant.

## Materials and Methods

### *Sampling*

Fifty Khaki Chambell ducks aged one-year were selected from a population of 1000 ducks. Forty healthy ducks and 10 ducks which showed clinical signs suggestive of being diseased such as unsatisfactory or stunted growth, poor feather condition and inactive were sampled. A pharyngeal swab was obtained by encircling the sterile swab around the pharyngeal region for 2 to 3 times. The swab was stored in Aimes® transport medium and transported to Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

### *Isolation and identification of Riemerella anatipestifer from ducks*

The pharyngeal samples collected were cultured onto blood agar on the day of collection and incubated at 37°C for 24 h. The resultant plates were read after 24 h. Colonies that are highly suggestive of being *R. anatipestifer* was selected and subcultured onto blood agar to obtain a pure culture. At the same time Gram-staining was performed on the suspected colonies. Identification of *R. anatipestifer* via conventional biochemical tests was performed only if a particular secondary culture was confirmed to be Gram-negative rods. Biochemical tests that were performed included catalase, oxidase, nitrate reduction, oxidative fermentative and gelatin liquefaction tests.

### *Antibiotic sensitivity testing of Riemerella anatipestifer isolated from ducks*

The isolated *R. anatipestifer* was tested against penicillin G, enrofloxacin, oxytetracycline, neomycin, ceftiofur and gentamycin to determine its sensitivity against each of the mentioned antibiotic. Twenty microliters (20 $\mu$L) of bacterial isolate suspension in 0.5 McFarland concentration was inoculated onto the Muller-Hinton agar surface in at least three directions to obtain uniform growth. A final sweep was made around the rim of the agar. The plates were allowed to dry for 5 min. Commercial antibiotic discs of penicilin, ampicillin, enrofloxacin, tetracycline, kanamycin, and gentamicin were placed onto the bacteria field on the agar plate using sterile forceps. The plates were incubated at 37°C for 24 h. The diameter of the zone of growth inhibition each disk was measured using calipers.

## Results

*R. anatipestifer* was isolated from one out of 10 pharyngeal swabs of diseased ducks and identified via gram-staining and biochemical tests that included catalase, oxidase, nitrate reduction, gelatin liquefaction and oxidative fermentative tests. *R. anatipestifer* was not isolated from the 40 pharyngeal swabs of healthy ducks. *R. anatipestifer* is highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G and ceftiofur.

## Discussion

One *R. anatipestifer* strain was isolated from out of 10 diseased ducks but none from the 40 healthy ducks. Although only one isolate was detected, this finding is significant as the duck carrying that particular isolate may serve as a source of infection and transmission of *R. anatipestifer* to other ducks within the same farm. Under unfavorable stressful circumstances such heat stress, nutrient deficiency, concurrent bacterial or viral infection and other underlying diseases, *R. anatipestifer* can infect and multiply rapidly, resulting in an outbreak of *R. anatipestifer* with a

mortality rate of up to 95%. The farm owner may suffer great economic loss due to weight loss death, and carcass condemnation of the infected ducks.

Based on the findings in this present study, *R. anatipestifer* does not localize in the upper respiratory tract of healthy ducks. Nevertheless, Sarver *et al*. (2001) successfully isolated 44 *R. anatipestifer* strains from 49 clinically healthy ducks. It is vital to take into account that the sample size in this present study is small and sampling was only done in one farm only, hence it is not representative of the duck population in Malaysia.

Knowing the susceptibility of *R. anatipestifer* to various antibiotics is important as it provides valuable information in deciding the choice of effective treatment, control and prevention of *R. anatipestifer* infection. In this present study, when *R. anatipestifer* that was successfully isolated was subjected to antibiotic susceptibility testing using Kirby-Bauer method, it was found to be highly sensitive to enrofloxacin, oxytetracycline and neomycin, intermediately sensitive to gentamicin and resistant to penicillin G as well as ceftiofur.

Upon comparing the findings of present study with that of previous works conducted on antibiotic susceptibility of *R. anatipestifer*, the antibiotic susceptibility of *R. anatipestifer* is found to change with time, thus it is best to perform antibiotic susceptibility test before prescribing and administering the best choice of antibiotic for *R. anatipestifer* treatment, control and prevention.

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6th Proceedings of the Seminar on Veterinary Sciences, 11 – 14 January 2011: 26-29

# Detection of *Mycoplasma hyopneumoniae* and Porcine Reproductive and Respiratory Syndrome in Clinical Samples by Polymerase Chain Reaction

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## Abstract

The aim of this study was to detect Swine Enzootic Pneumonia (SEP) and Porcine Reproductive and Respiratory Syndrome (PRRS) from clinical affected lung samples with PCR technique. In this technique three primers set used for *M. hyopneumoniae* detection were Cai (Forward: GAG CCT TCA AGC TTC ACC AAG A: Reverse: TGT GTT AGT GAC TTT TGC CAC C), Baumeister (Forward: TAG AAA TGA CTG GCA GAC AA: Reverse: GAG GCT TGA TTT TGG AGT C) and Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG). For the detection of PRRS virus the primer sets used were Oleksiewicz (Forward: GAA CCT GCC CAI CAC G: Reverse: TAC CCC TAA TTG AAT AGG GGA) and Suárez (Forward: GGG AAT GGC CAG CCA GTC AAT CAA CTG T: Reverse: TGT AGA AGT CAC GCG AAT CAG GCG CAC T).Nine pigs aged between 6-10 weeks were collected from farms in Selangor, Malaysia. One healthy pig and 8 other pigs with clinical signs of respiratory distress problem were sacrificed and lung bronchoalveolar lavage samples were obtained. Healthy pigs were selected as negative control while samples were harvested from 4 pigs with suspected SEP and 4 with respiratory problems for *M. hyopneumoniae* and PRRS virus detection. Base on the result, the Caronand Caiprimer sets were able to detect SEP from the affected lungs. For PRRS virus, RNA was extracted using easy-BLUE™ Total RNA Extraction Kit and converted it to cDNA with Maxime RT PreMix Kit. The Oleksiewicz primer set was ideally suited for the detection of PRRS virus.

**Keywords:** Swine enzootic pneumonia, PRRS, PCR, primer

## Introduction

Mycoplasma pneumonia, also known as Swine Enzootic Pneumonia (SEP) which cause by *M. hyopneumoniae,* is a highly contagious disease of pigs. The disease occurs worldwide and considers one of the most important diseases. It will disrupt the feed conversion ratio (FCR) and average daily weight gain of the affected pigs. In addition, about 40 to 80% of lung lesion at Malaysia abattoir were related to SEP infection. Swine Enzootic Pneumonia can be considered endemic in most of the farms in the country. The organism normally inhabits the respiratory tract of pigs and can be transmitted between pigs by direct contact. The severity of disease varies from farm to farm and this is influenced by factors such as husbandry, farm biosecurity, pig density and secondary bacterial infections. Porcine Reproductive and Respiratory Syndrome (PRRS) is an important viral disease in the pig industry worldwide. This disease, especially that caused by the highly pathogenic PRRS virus, has caused great losses to the pig production worldwide. For the diagnosis of the disease, bacterial culture and isolation are the most common used diagnostic methods; however this method is not ideal for *M. hyopneumonaie* because it is very fastidious and difficult to culture. With the PRRS viral pathogen, a series of viral passage is needed before it can be isolated and identified. Therefore, the objectives of this study were to select suitable a primer set using an optimized PCR technique as an alternative for traditional diagnostic procedure for SEP and PRRS.

## Materials and Methods

Nine pigs aged between 6 to 10 weeks were collected around farms in Selangor. One healthy pig (as negative control) and 8 other pigs with clinical signs of respiratory distress problem were sacrificed and postmortem performed and lung samples bronchoalveolar lavage fluid collected for analyses. The DNA was extracted from the lung samples using i-genomic CTB DNA extraction Mini Kit (iNtRON Biotechnology) according to the method described by the manufacturer. RNA was extracted using easy-BLUE™ Total RNA Extraction Kit (iNtRON Biotechnology) according to the manufacturer protocol. cDNA was synthesis by using Maxime RT PreMix Kit, (iNtRON Biotechnology). In this technique three primers set used for *M. hyopneumoniae* detection were Cai (Forward: GAG CCT TCA AGC TTC ACC AAG A: Reverse: TGT GTT AGT GAC TTT TGC CAC C) (Cai *et al*., 2007), Baumeister (Forward: TAG AAA TGA CTG GCA GAC AA: Reverse: GAG GCT TGA TTT TGG AGT C) (Baumeister *et al*., 1998) and Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG) (Caron *et al*., 2007). For the detection of PRRS virus the primer sets used were Oleksiewicz (Forward: GAA CCT GCC CAI CAC G: Reverse: TAC CCC TAA TTG AAT AGG GGA) (Oleksiewicz *et al*., 1998) and Suárez (Forward: GGG AAT GGC CAG CCA GTC AAT CAA CTG T: Reverse:

TGT AGA AGT CAC GCG AAT CAG GCG CAC T) (Suárez *et al*., 1993).The amplification was performed in the Swift™ Maxi Thermal Cyclers, (ESCO) and the samples were subjected to gel electrophoresis for band size detection.

## Result and Discussion

For SEP, all 3 set of primers used in the study were able to detect the positive control which was extracted from a commercial vaccine. At 5ºC above and below $T_m$ of primer did not show any significant different in result. The Cai primer set targets the 16s rRNA gene in small 30S ribosomal subunit. Based on the result, only 50% of samplesfrom pigs with typical *M. hyopneumoniae* lesion were positive. The Baumeister primer was used to detect *M. hyopneumoniae* in the bronchoalveolar lavage fluid of pigs. However, none of the lung samples produced specific bands. The Caron primer set targets antigenic determinants of 36 gene from cytosolic protein. All 4 samples with typical lung lesions were positive for *M. hyopneumoniae* infection, producing the 948 bp band (Figure. 1). From the results, the Caronprimer set was able to detect all *M. hyopneumoniae*-positive clinical lung samples. However, the bands obtained were very weak suggesting that the PCR protocol need to be optimized further. Meanwhile, for PRRS virus, the Oleksiewicz primer set, which targets the ORF 7 gene, was able to detect positive controls using the vaccine as DNA template. While Suárez primer set, which targets ORF 7 encoding nucleocapsid protein was not suitable as the positive control. For Oleksiewicz primer set, 4 out of 5 lung samples from pigs with respiratory distress problems produced a 660 bp band and some non specific bands. This suggests that the Oleksiewicz primer set still needs to be optimized further to ensure reliability of results. The Suárez primer set was unable to form the positive control with extracted DNA from vaccine, suggesting that this primer set was not ideal for the detection of PRRS virus.

## Conclusion

Based on the result, the Baumeister primer set was not an ideal for the detection of *M. hyopneumoniae* in clinical lung samples. Further study was needed to determine the effectiveness of this primer set for *M. hopneumoniae* detection in the porcine bronchoalveolar lavage fluids. This study also demonstrated that the Cai and Caron primer sets were able to detected SEP in clinical lung samples. The Baumeister primer set was more ideal for *M. hopneumoniae* detection in clinical lung samples. For PRRS, the Oleksiewicz primer set was more ideally suited for PRRS virus detection in lung samples, while the Suárez primer set did not produce any result when the PRRS vaccine was used as the DNA template.

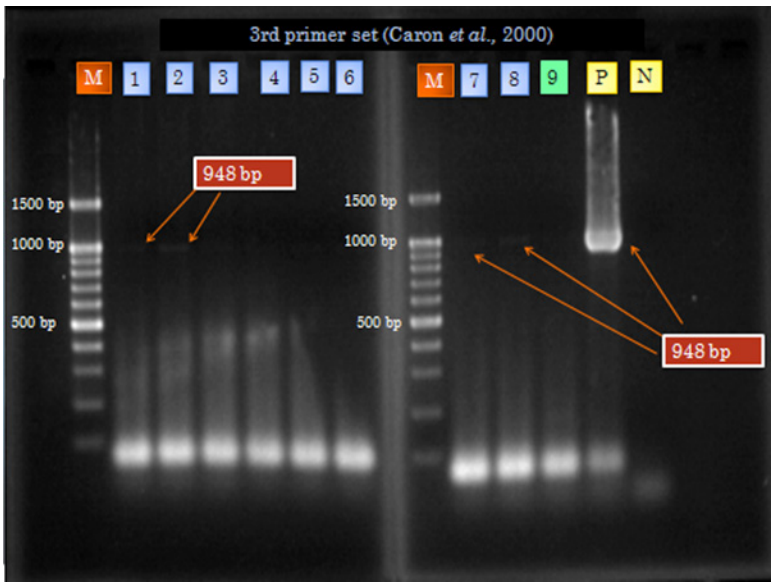


**Figure 1.** Electrophoresis of polymerase chain reaction product of porcine lungs samples. Primer set [Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG)]. M is 100 bp BLUE extended DNA Ladder. Columns 1, 2, 7, and 8 were sample from pig withrespiratory distress and gross lung lesions. Columns 3, 4, 5 and 6 are samples from pigs with respiratory distress without clearly demarcated lung lesions. Column 9 is form from a clinical healthy pig. P = positive control with DNA from vaccine; N = negative control with distilled water. Columns 1, 2, 7, 8 were positive control showing the 948 bp band.

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6th Proceedings of the Seminar on Veterinary Sciences, 11 – 14 January 2011: 30-36

# Anti-epileptic Properties of Terpineol Extracted from *Myristica fragrans* Houtt. Essential Oil in the Epileptic Rat Model

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## Abstract

Epilepsy is the manifestation of the disease due to overstimulation of the brain. The common signs of this disease are chronic, episodic and recurrent seizures. This problem usually occurs in dogs and cats with the prevalence of the problem estimated about 5.0-5.7 % in dog population and about 0.5 % in the cat population. Today, there is a lot of studies that had been done to search for the new alternative anticonvulsant and antiepileptic using allopathy or traditional medicine, one of them that had been done was *Myristica fragrans* Houtt. The volatile oil from the *Myristica fragrans* Houtt. had been studied and proven to have anticonvulsant effect and one of the constituents suspected to contribute to the anticonvulsant activity was terpineol. This study was done to screen the anti-epileptic effect of terpineol that had been extracted from *Myristica fragrans* Houtt. essential oil to the kainic acid induced epileptic rat model by determining the effect of terpineol to the behavioural seizure activity and electroencephalogram (EEG) of this model. In this study, 3 adult male SpraqueDawley rats were established with a radiotelemetry system by implanting with electroencephalogram telemetry device and were induced for epilepsy using kainic acid and then treated with the terpineol with doses of 20 mg/kg and 50 mg/kg. The rats were observed for behavioral seizure activity and electroencephalograms were done qualitatively and quantitatively based on the Racine Scale and EEG semiology. The result of this experiment indicated that terpineol had the antiepileptic properties by inhibiting the behavioral seizure activity and seizure electroencephalogram. The effect of antiepileptic of terpineol also correlated with the doses that were used in this experiment. The possible mechanism of antiepileptic effect of terpineol is by enhancing the gamma-aminobutyric acid (GABA) inhibition mechanism.

**Keywords:** Terpineol, *Myristica fragrans* Houtt., epilepsy, antiepileptic, behavioural seizure activity, electroencephalogram

## Introduction

Epilepsy is not a single disease but it is a manifestation of the disease that results from over-stimulation of the brain. The signs include chronic, episodic and recurrent seizures, with or without any detectable underlying brain lesion (Blood *et al*.,2007). Epilepsy is a common term used interchangeably with seizure and convulsion. The problem can occur in cats and dogs with the prevalence of 5-5.7 % in the general population of dogs and 0.5 % in cats (Berendt, 2008). Epilepsy can progress to a severe condition which is status epilepticus leading to euthanasia of the animals. Today, there are a lot of studies that had been done using the human traditional or allopathy medicine as anticonvulsant or alternative antiepileptic agent. One of them is *Myristica fragrans* Houtt. or nutmeg or mace. Nutmeg or mace also known as "Buah Pala" in Malay is the seed of an apricot-like fruit of the nutmeg tree which is native to Moluccas or Spice island of Indonesia (Bewley *et al*., 2006). Previous report had indicated that pharmacological activity of *Myristica fragrans* Houtt.exists in its volatile oil fraction extracted from the kernel. This oil was proven to have anti convulsant activity. Terpineol is a naturally occurring monoterpene alcohol that has been isolated from the nutmeg. terpineol is one of the compounds that issuspected to give anti-epileptic effect (Wahab *et al*., 2009). An experiment was conducted to screen the antiepileptic properties in terpineol extracted from nutmeg or *Myristica fragrans* Houtt. essential oil in the epileptic rat model by observing the antiepileptic effect on behavioral seizure activity in kainic acid induced epileptic rat model and to determine the antiepileptic effect of terpineol via electroencephalogram recording using a radiotelemetry system.

## Materials and Methods

The experiment was performed on 3 male Sprague-Dawley rats, weighing 400-420 g, at Animal Laboratory House, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. Experimental procedures were done with the permission from the Animal Research Committee of Universiti Sains Malaysia. The rats were divided into three groups: untreated group, terpineol 20 mg/kg treated group, and terpineol 50 mg/kg treated group.

In this study, EEG was obtained from the normal rat, during epilepsy and after treatment via a radiotelemetry system developed by the Division of Transomal Medical (DSI), United States. All rats used in this experiment were implanted with transmitters by surgical implantation. The injection of kainic acid was performed according to the protocol described previously by Dudek *et al*. (2006). The injection of 5 mg/kg kainic acid was performed intraperitoneally at lateral to linea alba. Treatment of epilepsy was performed by an intraperitoneal injection of terpineol 1 h after of the kianic acid induced epileptic rat model was established. The doses were terpineol at 50 and 20 mg/kg, respectively, for each group. The treatment was performed after the rats were confirmed in the status epilepticus.

Seizure behavior activity was determined using a scoring system as described by Racine (1972). Monitoring of seizure was conducted by visual observation and video recording. The behavior seizure activity was monitored qualitatively for 12 hours and quantitatively for 3 h. Methods of observation and interpretation of EEG were conducted based on the EEG semiology as described by Dr. Tahamina Begum of Universiti Sains Malaysia. The pattern was categorized into the four distinct EEG morphological patterns. Based on these data, EEG was monitored qualitatively for 12 h and quantitatively for 2 h. The EEG patterns were calculated based on the semiology.

Data analysis was performed on EEG and behavioral seizure activity for qualitative and quantitative data. Qualitative data were behaviour video recordings and EEG data were analyzed by visual observation and quantitative data were analyzed by manual calculation.

## Results

### *Qualitative Behavioural Seizure Activity*

#### *Behaviour Characteristics*

After kainic acid was injected to the rat, the behavioural seizure activities such as head nodding or wet dog shakes, forelimb clonus, lordosis posture, rearing behaviour and loss of righting reflex were scored using Racine Scale (Racine, 1972) and were present in all rat groups in this study. But without treatment the condition continued and became severe and with treatment with terpineol the condition was absent.

#### *Survivability*

The rats with the treatment had a much better survivability compared to untreated rats. Treated rats for both 20 and 50 mg groups survived the whole 12 h observation period but untreated rats died within 4 hours after kainic acid was injected.

#### *Severity of Seizure*

The severities of the seizure in treated groups compared to before and after treatment were significantly reduced. For terpineol 20 mg there were only 2 seizures observed which was of class 2 in the early minutes after treatment and the seizure did not occur after that. For terpineol 50 mg/kg there was no seizure activity observed immediately after the treatment was given. However, for untreated rats all seizures were still present and most of the seizure were of class 5 seizure and similar to the severity before treatment.

### *Quantitative Behavioural Seizure Activity*

There were reductions in total number of behavior seizure activities in treated groups compared to untreated group. Figure 1 presents the total behavior seizure activity for these experimental groups.

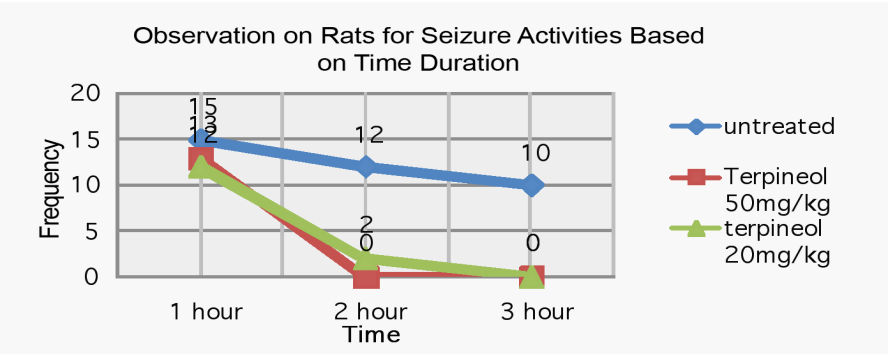


**Figure 1.** Total Behavioural Seizure Activity for Experimental Groups

### *Qualitative Electroencephalogram*

Figure 2 presents the portion of the EEG wavelength taken from each group during normal, before and after treatments. In normal rats, EEG was stable with straight wave observed. The amplitudes were less than 0.2 mv and no spike was present. After kainic acid induced seizure, all 4 distinct patterns of epileptiform discharges were present. The amplitudes of the wave were variable in the range of 0.5 -0.8 mv and spike frequency was in the range of 3 – 5 hertz. This pattern of amplitudes and spike frequency occurred continuously when no treatment was given. However, for the treated group, there was a decrease in the pattern from severity to lesser severity.

### *Quantitative Electrocephalogram*

There was a reduction of the EEG seizure patterns after the treatment was given in the terpineol groups compared to untreated group as showed in the Figure 3. Based on this result we concluded that terpineol had decreased the severity of seizure EEG pattern and total number of seizure pattern in EEG.

## Discussion

Based on the results, we concluded that terpineol has a potential in minimizing the behavioral seizure activity and seizure electroencephalogram. This finding actually is novel because there are no studies prior to this to test terpineol as an antiepileptic and to examine the antiepileptic effects of terpineol extracted from the nutmeg.

The effects of terpineol as found in this study were consistent with other similar studies using a similar model to examine antiepileptic or anticonvulsant (Cepeda *et al*., 1984; Hashizume *et al*., 2000; Grabenstatter *et al*., 2007). All of these studies reported inhibition of seizure activity and electroencephalogram. Based on these studies, we can suggest that terpineol also has an anti-epileptic effect to behavioral seizure activity and seizure electroencephalogram.

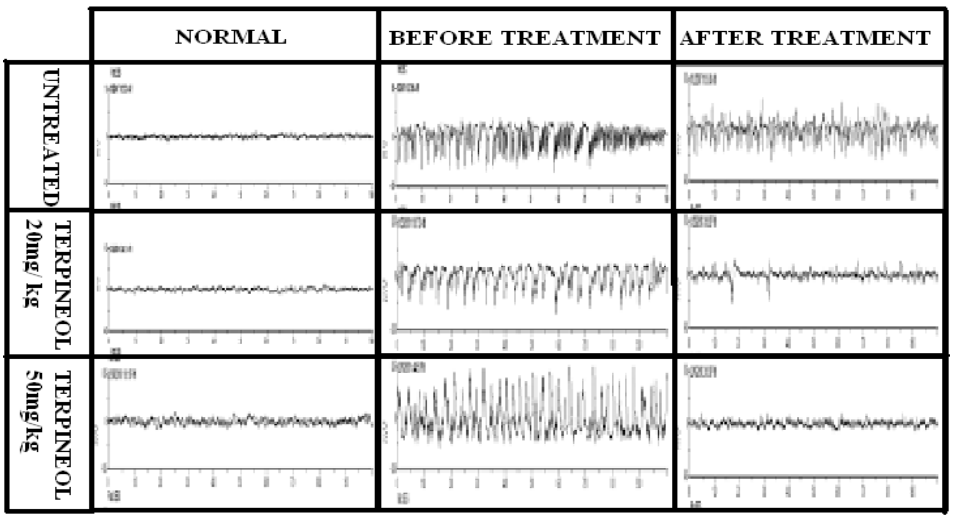


**Figure 2.** Portion of EEG wavelength taken via radio telemetry system from each group during normal, before treatment and after treatment

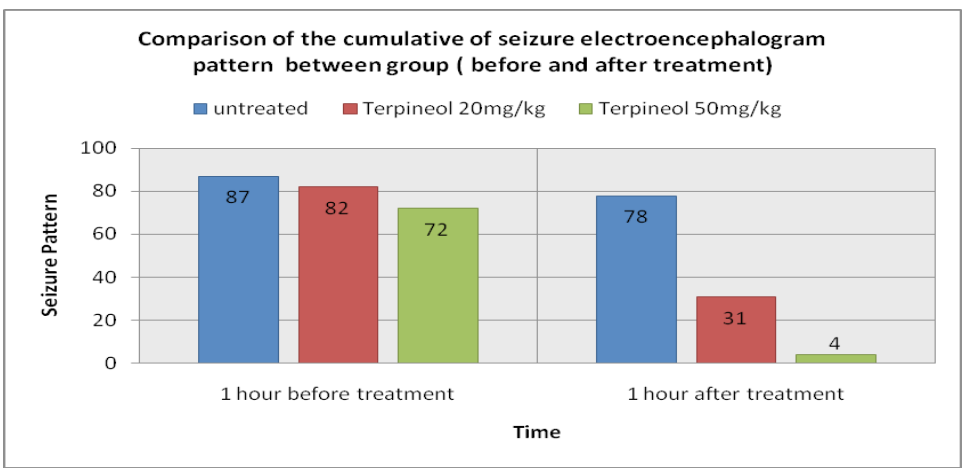


**Figure 3.** Total seizure electroencephalogram (EEG) pattern for experimental groups

Qualitative observation in this study had also indicated that terpineol may be beneficial to reduce motor activity in the rat and has some effect of anesthesia such as sleep when injected systemically. This may correlate with the effect of terpineol having one of these anesthesia activities (Moreira *et al*., 2001). Qualitatively, we have also found that terpineol may be possible to enhance the survivability in the rat with seizure but due to a limited number of animals used in this experimental study, the result obtained was cogent.

The rats in untreated group died at the end of experiment and were a common occurrence in the kainic acid induced epilepsy rat. This is consistent with the previous study of kainic acid induced epileptic model. Rats usually will have continuous episodes of seizure and some studies indicated that rats died within 24 hours after kainic acid induced seizure. This may bedue to neuronal damage that occurs after induction by kainic acid (Berg *et al*., 1993).

The seizure activity was different between the groups and could be due to various individual factors of the rat. As described in the literature, the aim of the kainic acid induced epileptic model is to have repetitive convulsive motor seizure activities longer than 3 hours. Generally, an animal should have a minimum of 1 obvious seizure per hour and typically an animal will have 7 to 22 episodes of seizure every hour. Seizure activity may last for 72 hours after kainic acid injection (Dudek *et al*., 2006).

The mechanism of the terpineol to reduce seizure in this study is most likely the reversing of the effect of kainic acid through enhancement of GABA inhibition mechanism of the neuron. As mentioned earlier, kainic acid is an excitatory neurotoxin that was proven to reduce GABA inhibitory mechanism by disinhibiting the GABAB receptor (Haas *et al*., 1996). GABAB is presynaptic receptor for GABA, which is important in regulating the secondary messenger system. Activation of this receptor will decrease the neurotransmitter release. Kainic acid will inhibit the function of GABAB, resulting in constant activation of the receptor which leads to a decrease of GABA release, impaired GABA inhibition mechanism, excitation of the brain and finally, epilepsy. However, when rat was treated with terpineol, this effect was reversible, as evident of inhibition of seizure activity and seizure electroencephalogram. This could be due to the effect of terpineol by enhancing the GABA release and lead to the increase of the GABA inhibition effect. Further studies need to be conducted to confirm the enhancement of GABA inhibition effect by terpineol.

## Conclusion

terpineol extracted from the *Myristica fragrans* Houtt. essential oil may be beneficial for the inhibition of behavioral seizure activity and seizure electroencephalogram. The antiepileptic effect of terpineol was correlated to the dose of terpineol as

used in this experimental study. Possible mechanism of antiepileptic properties of terpineol is by enhancing GABA inhibition mechanism.

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# Stress Levels in Bulls during and after Electroejaculation

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## Abstract

The Animal Welfare Society has expressed its concern on the use of electroejaculator as a semen collection device. The Society believes that electroejaculation will incur pain to the animal and thus causing stress. Thus, this will compromise the quality of semen collected from the animal. A study was conducted to observe if bulls were stressed when the electroejaculation technique was applied for semen collection. A serum blood sample was collected before, immediately after and after semen collection using the EE technique. Changes in serum cortisol concentrations in serial blood samples were used to quantify stress response in the bulls. Eight bulls aged from 3 to 8 years and weighing between 320 and 830 kg were randomly assigned to one of the two treatments. The first treatment group consisted of bulls that were inserted with a probe and given electrical stimuli (ES) while the second treatment group comprised of bulls that were inserted with a rectal probe but without electrical stimulus (WES). Blood samples were collected by venipuncture at rest (first before the rectal probe was inserted), immediately after ejaculation, 10 and 15 min postejaculation. There was no significant difference ($p \geq 0.05$) between the group with electrical stimulus and the group without electrical stimulus**.** However, there are significant differences between bulls in each group. The use of probe with electrical stimuli did not significantly increase serum cortisol concentrations.

**Keywords**: Electroejaculation, semen, bull, electrical stimuli

## Introduction

Electroejaculation (EE) is one of the two common methods used to obtain semen from bulls and goats. The procedure is generally used for breeding programmes and research purposes. This method has been used in rams that lacked libido. However, this technique can cause pain and stress to the animals (Mosure *et al*., 1998).

Stress can be defined into three different ways; first definitionis a constraining or impelling force, it is an effort or demand on energy and third definition it is a force exerted on a body. Exposing the animals to stress can induce changes in physiological and immunological behavior. In fact, prolonged stress reduces productivity and performance of animals. Serum cortisol concentration has been used as an indicator for stress. Thus, when an animal is in stress, the cortisol level in the body is increased. Pain is often associated with stress, thus when an animal is in pain it is under stressed. Pain caused by restraining and handling, palpation *per rectum*, and venipuncture leads to elevation of serum cortisol concentrations. During EE, the clinical signs that indicate an animal is in pain are vocalization, struggling, lying down, generalized muscular contraction, and spasms of the hind limb.

Currently, there is no information on the relationship between stress in by EE and cortisol concentrations in bulls. Therefore, the objective of this study was to determine the concentration of serum cortisol before, during, and after EE in breeding bulls.

## Materials and Methods

### *Animals*

The animals used were 8 bulls from 5 different breeds (KK-X, Braford-X, Friesian-X, Draughtmaster-X, and Brangus) aged from 2 to 8 years and weighing between 320 and 830 kg. All bulls were raised under free grazing system and fed with commercial concentrates daily consistingmainly of palm kernel cake (PKC).

### *Electroejaculator Set*

The rectal probe used in EE was 47.7 cm long and 10.0 cm in diameter. The electrodes were separated by an angle of arc on the body of the probe. The handle was smaller in diameter so the probe when fully inserted into the rectum did not cause stretching of the anal sphincter.

### *Electrical control panel*

The machine was used on manual control, which allowed the operator to control the amount of voltage from 0 to 15 volts that was channelled to the probe. In electroejaculation, multiple electrical stimuli were applied for an interval of 3 to 5 sec until ejaculation of semen.

### *Blood Sampling*

An approximate 5 mL blood sample was collected by venipuncture from the jugular vein into a plain tube using an 18-gauge venojet needle. After the collection, the

blood samples were kept in ice and immediately transported to the laboratory. The samples were then centrifuged at 5000 rpm for 15 min. Serum was aspirated after centrifugation and stored below -20°C pending analysis.

***Experimental design***

In this study, 8 bulls were divided equally into two groups. The treatment group received the probe with electrical stimuli (ES) while the control group received the probe without electrical stimuli (WES). For the treatment group, electrical stimuli were continued until ejaculation occurred, while for the control group, the probe was inserted into the rectum for 10 min without electrical stimulus.

Blood samples were collected from the treatment group at four different occasions. First, at the end of the 15 min after the bull entered the crush, one immediately after ejaculation, followed by 10 and 15 min after ejaculation.On the other hand, for the control group, the blood samples were collected first at the end of the 15-min resting period, and second immediately after removal of the probe, 10 min after it had been in the rectum, the third and fourth samples were collected 10 and 15 min later.

***GammaCoat Cortisol Radioimmunoassay Kit***

For the quantitative determination of serum cortisol concentration, the cortisol was measured using GammaCoat™ Cortisol Radioimmunoassay Kit produced by DiaSorin Company. Serum samples were thawed at room temperature and 10 µL of serum sample was added into each GammaCoat tube. Subsequently, 1.0 mL of tracer-buffer reagent was added into each tube and mixed gently with a vortex mixer. The tubes were incubated in a dryer oven at 37°C for 45 min. After that, all tubes were decanted, and put into an inverted position for 3 to 5 minutes to allow tubes to drain. Finally, all tubes were placed in 1470 Automatic Gamma Counter for cortisol concentration measurements.

***Statistical analysis***

Data were analyzed using Repeated Measures Analysis of Varianceof the SPSS Version 16.0 to measure differences of serum cortisol concentration between and within ES and WES groups over time.

## Result and Discussion

The mean serum cortisol concentrations at rest before the treatment were higher in electrical stimuli (ES) bulls than the without electrical stimulus (WES) bulls. After receiving the electrical stimuli from the electroejaculator, all ES bulls showed drastic increase in mean serum cortisol concentration level from rest to immediately after ejaculation. Subsequently, the concentration of serum cortisol

declined over time. However, an increased serum cortisol concentration was observed in the WES group at 10 min after the probe was inserted into the rectum. In addition, the concentration of serum cortisol continued to increase until the end of predetermined time 15 min after probe was inserted.

There was no significant difference ($P > 0.05$) between the ES group and the WES group at four different occasions. There are significant differences among bulls in each group.

## Conclusion

The present study proved that the use of probe with electrical stimulidid not significantly increase cortisol concentration.

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6th Proceedings of the Seminar on Veterinary Sciences, 11 – 14 January 2011: 41-45

# Detection of Heavy Metal Residues in the Muscle and Skin of Tilapia

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## Abstract

A study to detect the presence of heavy metals, which are Lead (Pb), Copper (Cu) and Zinc (Zn) in Tilapia caught from 3 waterways in Universiti Putra Malaysia, Serdang, Selangor was conducted. Water samples from the study sites were also sampled and analysed. These locations were in the academic areas of Universiti Putra Malaysia, Serdang campus near the agricultural and housing areas. Results showed that the mean concentration of Cu, in the water was 0.04 µg/mL and Pb and Zn were 0.03 µg/mL. The concentration of the elements tested was found to be lower than the recommended limits set by FAO/WHO but the concentration of Pb almost breached the limit of 0.05 µg/mL. The mean concentration of Pb in the fish muscles (0.64 µg/g) did not exceed the permissible limits set by FAO/WHO (1.5 µg/g) and the Malaysian Food Regulation (2.0 µg/g). The concentration of Cu in the fish muscle was much lower (2.13 µg/g) than the permissible limits set by the WHO (10.0 µg/g) and the Malaysian Food Regulation (30.0 µg/g). The highest element that had accumulated in the fish muscle was Zn (8.28 µg/g). However, the concentration of Zn did not exceed the permissible limits set by FAO/WHO (150.0 µg/g) and the Malaysian Food Regulation (100.0 µg/g). In the fish skin, the concentration of Cu and Pb were quite high. The concentration for Pb in the skin (6.77 µg/g) exceeded both the permissible limits set by FAO/WHO (1.5 µg/g) and the Malaysian Food Regulation (2.0 µg/g) while the concentration of Cu in the skin only exceeded the permissible limits set by FAO/WHO (10.0 µg/g). Among the three elements studied, Zn concentration was highest in the fish skin (45.8 µg/g). However, the Zn levels did not exceed the permissible limits of FAO/WHO and the Malaysian Food Regulation. Therefore, it can be concluded that the Tilapia caught from the waterways were not suitable for animal and human consumption because the concentration Pb in the fish skin was too high. Copper was also found to be present in the skin at concentration that can pose health hazard. However, these fishes can be said to be safe to be consumed if the skin were to be removed.

**Key words:** Heavy metal, residue, skin, muscle, Tilapia, waterways, permissible limits

## Introduction

Pesticides and herbicides derived from agricultural operations and industrial effluents such as metals can ultimately find their way into a variety of different water bodies and produce toxic effects in aquatic organisms. Toxic metals that accumulated to hazardous levels in aquatic biota can pose health problem of public concern. Drinking of water and/or consumption of fish from excessively polluted water could lead to health hazards to man. Pollutants can enter fish through five routes via gills, oral consumption of food and water, skin, and food or non-food particles.

Following absorption, the pollutant is carried in the blood stream to the liver for transformation and/or storage, or either stored in a storage point (tissues) inside the fish's body. The rate of absorption and the dynamic process associated with the pollutant's elimination by the fish determines the concentration of the pollutant at any given tissue.

Heavy metals in the aquatic environment are ranked as major polluting chemicals in both developed and developing countries. It is also a major concern worldwide due to their threat to plant and animal life, thus disturbing the ecological balance of nature.

Tilapia was chosen for this study because of its abundance and ubiquitous distribution. Other than that, the public is seen catching these fishes at the study areas for consumption using cast nets and/or rod and line where this species is abundant together with other fishes such as Suckermouth Catfish (*Hypostomus plecostomus*). Furthermore, Tilapia can successfully spawn, is fast growing andhighly resistant to diseases. These superior characteristics lead to its wide distribution in rivers, reservoirs and fishponds which attract fishing enthusiasts to fish this fish.

This study highlights the public health significance upon consumption of fish with heavy metal residue from polluted waterways. Previous studies on heavy metal residues in fish were on the residues of metals in tissues that were inedible such as gills, intestines, liver, and tail of fish (Zheng Zhang *et al*., 2007, Olowu *et al*., 2010). Studies on heavy metal residues in the muscle and skin of fish are very limited.

The specific objective of this study was to detect the presence of heavy metals (Cu, Pb, Zn) in the muscle and skin of Tilapia and to compare the concentration of these heavy metals in the fish muscle and skin with available standards.

## Materials and Methods

### *Experimental Animals*

Ten juvenile Tilapias were caught with a rod and line from three waterways, two at Jalan Sapucaya and one at Ladang 2 in the Serdang campus of Universiti Putra Malaysia. These waterways drain water from the academic areas which include Faculty of Veterinary Medicine, Faculty of Biotechnology, Faculty of Environmental Studies, Faculty of Agriculture, and Faculty of Forestry and nearby housing areas including farms and orchards. It was a rainy season at the time of

sampling. The water pH and temperature were 6.62 and 29.8°C, respectively, in the three waterways. The fishes were later transported to the Analytical Laboratory of Department of Soil Science, Faculty of Agriculture, Universiti Putra Malaysia.

### *Sampling*

In the laboratory, the fish were weighed using an electronic balance and the values were recorded to the nearest gram before 10 muscle samples and 10 skin samples were dissected from the fish. Water samples were also collected from each waterway using plastic containers. Locations of water samplings were based on evidence of local public seen fishing there. The temperature and pH of the water in the waterways were also taken on the same day. All samples were then analysed for heavy metal concentration using a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer.

### *Sample Storage*

The samples were thoroughly cleaned with distilled water to remove debris that adhered on the surface of the fish body and gills. Each sample was then wrapped in aluminium foil and kept overnight in a freezer at -10°C (Olowu *et al*., 2010).

### *Sample Preparation*

The samples were then thawed at room temperature after been frozen for 24 h and defrosted before the samples were unwrapped. The scales of the fish were removed using scale remover before the fish were dissected using stainless steel scalpel blades attached to a scalpel holder (Zheng Zhang *et al*., 2007). Muscle tissues at the trunk, caudal peduncle and also the belly were dissected and removed from the bones. After removing the whole chunk of muscle tissue from the bones, the skin was separated from the muscle using scalpel blades and forceps. Dissected samples were placed in small air tight plastic containers (5 3cm) and labeled accordingly. Later, all the samples were chilled overnight at 4°C.

### *Sample Digestion*

Three grams of fish muscle tissue and 0.4g of fish skin were weighed using an electronic balance. The samples were oven dried for 1 h at 150°C to obtain the dry weight. Later, the samples were placed in 200-mL beakers containing 100 mL HNO and 300 mL HCl acids. The samples were then left overnight. All the procedures were conducted in a fume cupboard.

After 12 h, the samples were placed on a stirring hot plate and heated at 150°C for 1 h. After 1 h, the samples were left to cool for half an h at room temperature and later poured into 100 mL volumetric flask. Distilled water was added until the final volume of 100 mL and the volumetric flask was covered with "Parafilm". The flask was shaken gently and labelled.

The sample digests were later transferred into 100-mL plastic bottles prior to analysis with a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer to detect the presence of heavy metals in the tissues.

### *Detection of Heavy Metals*

The Atomic Absorption Spectrophotometer (AAS) used for this study is a Perkin Elmer 5100 PC, connected to a computer equipped with software (AA WinLab Analyst) for heavy metal detection. Before operating, the AAS was calibrated with three standard solutions and a blank solution which was distilled water. The three standard solutions were T1, T2 and T3, standards for Cu, Zn and Pb respectively, in 100-mL volumetric flasks. After calibrating with the standard solutions, the machine was calibrated with the blank solution before analyzing the prepared samples. A cathode lamp, depending on the element to be tested was fixed on the machine.

A capillary aspiration tube connected to the machine was dipped into a sample to be analyzed and the sample was aspirated into a flame, atomized and detected by the machine.

## Results

The present study showed that there were heavy metal residues in Tilapia in the waterways in Universiti Putra Malaysia, Serdang, Selangor. All the elements studied (Cu, Pb and Zn) had been successfully detected in the fish muscles and skin. Copper and Pb were observed to be present at high concentrations in the fish skin of the Tilapia. However, the results revealed that the Tilapia studied can be considered safe for consumption if the fish skin is removed.

## Discussion

Tilapias is a type of fish that eats almost everything and most of the time scavenge food from the river beds and the water column. Aquatic microflora and fauna which are constituted as fish food have capabilities of accumulating or incorporating heavy metals into their living cells from the environment (Fostner and Wittman, 1981, Ibrahim and Sa'id, 2010). The mean values of Cu, Zn and Pb in the water samples were 0.04 μg/mL, 0.03 μg/mL and 0.03 μg/mL, respectively. None of these values exceeded the permissible limits for human consumption by the World Health Organization. For this study, only surface water samples were collected. The results could have differed if water samples were collected from the bottom of the waterways and analyzed.

Food for the fishes is mainly found at the bottom and there are possibilities that the heavy metals can accumulate at this site of waterways due to their large atomic numbers. Heavy metal concentration is usually high in sediments or riverbeds and this may also be attributed to human activities such as discharge of untreated sewage and uses of metals and industrial materials that contain metals as well as the ability of the sediment to act as sink (Okeye *et al*., 1991; Olowu *et al*. 2010).

Bioaccumulation is a process where an organism concentrates metals in its body from surrounding food or medium, either by absorption or ingestion (Fostner and Wittman, 1981). When bioaccumulation occurs, fish can regulate metal concentration to a certain limit (Health, 1991). Results from this study showed that the heavy metals tended to be bioaccumulated in the skin rather than the muscle.

Lead had the least bioaccumulation in the fish muscle studied. The content of Pb in the fish muscle was lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation although the level of Pb in the fish tissue was the lowest; it was close to the permissible limits set.The second highest metal bioaccumulated in the fish muscle was Cu. The content of Cu in the fish muscle was much lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation. Zinc was the element highly bioaccumulated in the fish muscle. Even though the content of Zn in the fish muscle was the highest, its level of bioaccumulation was very much lower than the permissible limits set by FAO/WHO and the Malaysian Food Regulation.

Lead was the least bioaccumulated element found in the skin when compared to other elements tested. Although the content of Pb was lowest in the fish skin, it exceeded the permissible levels set by the FAO/WHO and the Malaysian Food Regulation. The second highest metal bioaccumulated in the fish skin was Cu and it also exceeded the permissible levels set by FAO/WHO and the Malaysian Food Regulation. Zinc was also the highest bioaccumulated element in the fish skin studied. Eventhough Zn had the highest level of bioaccumulation in the fish skin, it still did not exceed the permissible limits set by FAO/WHO and the Malaysian Food Regulation.

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# Study on Coccidia Infection and Species in Cyprus Shami Goat Population

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## Abstract

A survey was undertaken to determine the prevalence of coccidian and helminth infections in the Cyprus Syami goats in two goat farms in the states of Pahang and Negeri Sembilan. The burden of coccidiaoocysts and helminth eggs were determined by the McMaster technique. Identification of *Eimeria* species was carried out following oocyst sporulation in 2.5% Potassium dichromate solution. The burden of helminthes and coccidia in terms of egg and oocyst counts per gram of faeces was high especially in young animals under the extensive management system. Eimeriaoocysts were found in all faecal samples examined. The species of coccidia identified were *E. ninaekohlyakimovae, E. arloingi, E. christenseni, E. hirci, E. alijevi, E. jolchijevi, E. caprina, E. caprovina and E. pallida*. The most prevalent species identified was *E.arloingi*, found in 71% of the samples followed by *E. Ninakohlyakimovae* (67%), *E. christenseni* (63%) and *E. alijevi* (61%). Other species present were *E. hirci, E. jolchijevi, E. caprovina, E. caprina* and *E. pallida* in 34, 22, 12, 9 and 4% of the faecal samples examined respectively. Oocyst counts were significantly higher in animals below 8 months and in animals kept under extensive management system ($P<0.05$). High oocyst counts were mainly of non-pathogenic species. High coccidial infection was found to be directly related to poor hygienic conditions in the management system. Morbidity rates in kids could not be related to the intensity of coccidial infections

**Keywords:** McMaster technique, Eimeriaspp; Goat; Cyprus Shami

## Introduction

Coccidiosis is a parasitic disease of the intestinal tract caused by microscopic organisms called coccidia, causing economic losses in livestock productions especially goats kept in large numbers under various management systems.

Seventeen species of Eimeria are known to infect goats throughout the world of which nine species *E. arloingi, E. christenseni, E. ninacohylakimovae, E. alijevi, E. jolchijevi, E.hirci, E. aspheronica* have been recorded in Malaysia. The Cyprus Shami goat which is native to the Middle East countries is a hardy breed and highly adaptive to new environments. The doe is noted for its high milk production and its ability to produce triplets or even quadruplets. High milk production, averaging 4 – 5 litres, is attained 3 – 4 days following parturition and with the current program to improve the goat sub-sector it is envisaged that this breed could replace some of common goat breeds in Malaysia such as the Boer and Jamnapari for meat or milk production.

The objective of this study was to evaluate the prevalence of coccidian and helminth infections in two newly established Shami goat farms in the states of Pahang and Negeri Sembilan. The study takes into consideration the management practices adopted by the farms as it is a well known generality that parasitic infection is closely related to management practices.

## Materials and Methods

A Shami goat farm in Pahang state which adopts the extensive management system and a similar farm in Negeri Sembilan which practices the intensive management system were selected for this study. In both farm the goats were divided into two age groups. Group 1 was animals below one year old while group 2 were adult goats more than two years old. Faecal samples from 81 animals were collected for coccidian oocysts and helminth eggs count using modified McMaster technique. Samples positive for coccidian oocysts were subsequently incubated in 2% Potassium dichromate for species identification based on their morphological features described by Anonymous, (1986).

## Results

The prevalence for coccidia in relation to management practices and animal age groups are shown in Figures 1 and 2. Shami goat farm practicing the intensive management system showed that 85% of animals in the farm had low oocyst count while the remaining 15% had high to very high oocyst count. The farm which practices the extensive management system had high oocysts count with 19.5% of the goats having very high (>4500) counts, 43.9% animals had high (3001-4500) counts, 19.5% had moderate (1501-3000) counts, and 17.1% had low (0-1500) counts. *Eimeria arloingi* was the most common species identified with an overall prevalence of (71%), followed by *E. ninakohlyakimovae* (67%) and *E. christenseni* (63%). Other species identified were *E. alijevi* (61%), *E. hirci* (34%), *E. jolchijevi* (22%), *E. caprovina* (12%), *E. caprina* (9%), and *E. pallida* (4%).

## Discussion

The prevalence of coccidia infections was highest in young Shami goats based on the high oocyst counts. The higher infestations in kids of 6–7 months could be explained by the lack of passive immunity provided by the colostrum during the first few weeks following birth (Taylor, 2007). However high infestation could be attributed to the management practices of keeping young kids alongside their does in and near the goat sheds thus exposing them to infection. Goats more than one year old showed a lower oocyst counts indicating the possibility of the development of acquired immunity. The role of coccidiosis in mortality cases in the present study is difficult to assess. Yvore´ *et al*, (1980) claimed that severe enteric lesions and very high oocyst count were associated with diarrhoea. In the present study, diarrhoea was not a common clinical sign among kids and high oocyst counts were rarely associated with diarrhoa.

*E. ninakohlyakimovae* is considered to be the most pathogenic *Eimeria* spp. (Pellerdy, 1974) Yvore *et al*. 1980) as counts of 200,000 *E. ninakohlyakimovae* oocysts per gram faeces are associated with severe diarrhoea, depression and death. In the case of *E. arloingi* counts up to 24-106 OPG were only associated with mild, transient diarrhoea. Although *E. ninakohlyakimovae* in the present study was widespread, differential oocyst counts showed that the intensity of infections caused by this species was low in most cases. Thus, high oocyst counts were mainly attributed to species of lower pathogenicity and not by *E. ninakohlyakimovae*.

One of the most common factors that precipitated in coccidiosis is heavily contaminated environment (Schillhorn van Veen, 1986.). According to Catchpole *et al*. (1993), clinical coccidiosis is mainly seen in intensive management system; in less intensive systems disease rarely occurs because the young animals are exposed to the parasites rather gradually and are able to gain effective immunity as the animal ages. A typical elevated, slatted floor Malaysian goat shed reduces the threat of parasitic infection through efficient waste evacuation approach. In this study the intensive management system goats had a lower oocyst counts compared to the extensive system. Other factors such as nutrition could contribute to the animals' health status.

**Table 1.** Eimeria spp identified in Cyprus shami goats

| EimeriaSp | Number of Animal (Total = 81) | Percentage of Animals affected |
|---|---|---|
| *E. christenseni* | 51 | 63 |
| *E. ninakholyakimovae* | 54 | 67 |
| *E. pallida* | 3 | 4 |
| *E.caprina* | 7 | 9 |
| *E.hirci* | 27 | 34 |
| *E. alijevi* | 49 | 61 |
| *E. caprovina* | 9 | 12 |
| *E. jolchijevi* | 17 | 22 |
| *E. arloingi* | 57 | 71 |

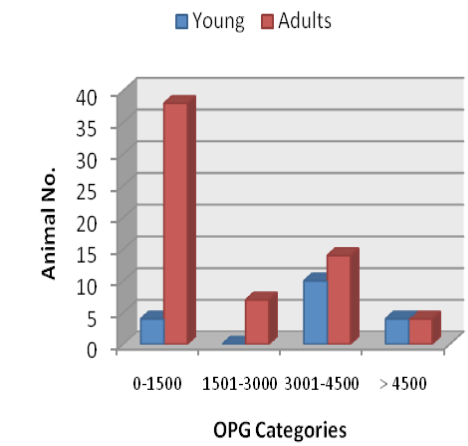


**Figure 1.** Oocyst burden based on age differences

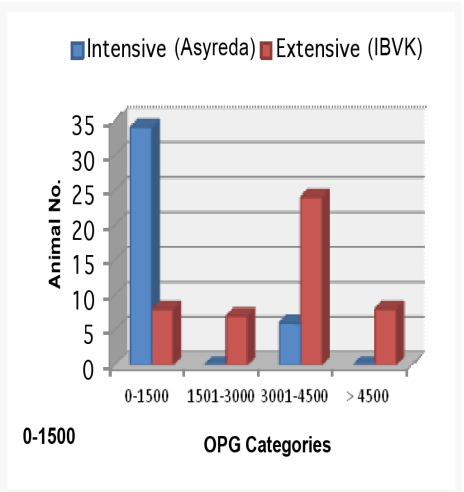


**Figure 2**. Oocyst burden based on management differences

## Conclusion

It is concluded from the present study that there is a prevalence of coccidian infection among kids in the newly established Shami goat farm. The high prevalence appears to be attributed to the management system, in this case the extensive management system. Among the nine *Eimeria sp*. identified *E. arloingi* was the most common. The high oocyte count among the kids is from species of low pathogenicity. The present study also showed that the raised slatted floor goat sheds appear to contribute towards reducing the prevalence of coccidia in these farms.

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# Effect of Short-Term Ingestion of the Methanolic Extract of *Mitragyna Speciosa* on Sperm Quality in Mice

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## Abstract

*Mitragyna speciosa* (MS) which is known as '"Ketum" in Malaysia and "Kratom" in Thailand is a tropical plant indigenous to Southeast Asia. The leaves of MS have been used by natives of these countries for their opium-like effects and cocaine-like stimulant activities to overcome fatigue, enhance tolerance to hard work, prolong the duration of sexual intercourse and increase libido in males. However, no scientific studies have been carried out to assess the effect of MS consumption on the quality of sperm in animals or man. In this study, the effect of short-term ingestion of MS on the quality of sperm in mice was investigated. Forty mice were divided into 5 groups; 2 controls and 3 treatments. The negative control group received 0.9% sodium chloride (NaCl, 10 mL/kg) while the positive control received clomiphene (25 mg/70 kg). The respective treatment groups received either 50, 100 and 200 mg/kg of MS extract. The drugs and extracts were administered orally once daily for 14 d. The results showed an increase in the number of sperms in groups treated with MS. The morbidity rates of the sperm in groups treated with MS were markedly lower than that of the control groups. In addition, marked deformity in the sperm in the form of swelling at the tail was observed in the groups treated with MS. In conclusion, mice treated with MS showed an increase in the number of sperm count in spite of defect in sperm morphology and reduced sperm motility.

**Keywords:** *Mitragyna speciosa*, clomiphene, sperm quality, mice

## Introduction

In humans, male infertility accounts for an estimated 40-50% of the factors responsible for sexual dysfunction and failure of pregnancy in women (Brugh and Lipschultz, 2004). Examples of available treatments currently used to correct this

condition include Vitamin E prescription to counter the oxidative stress associated with sperm DNA damage and reduced sperm motility, a hormone-antioxidant combination and off-label use of clomiphene citrate, an anti-estrogen drug used to treat fertility problem in females.

Clomiphene citrate for example, has been shown to correct hypogonadism produced by functional suppression of pituitary gonadotropin with a modest effect on sexual function**.** In addition, patients treated with clomiphene citrate showed sexual improvement with significant increases in the luteinizing hormone (LH) and level of free testosterone (Guay *et al*., 2003)**.** However, clomiphene citrate is currently under trials for use in men and has not been approved by the U.S Food and Drug Administration for use in humans. Moreover, any enhancement of fertility in men could only be observed after 3-6 months of continuous clomiphene citrate consumption. Therefore, the need to find an alternative drug or herb which may enhance fertility rate in males in shorter period remains the focus of many reproductive researches worldwide. *Mitragyna speciosa* (MS) plant, for example, has been used in folklore medicine to treat such cases.

*Mitragyna speciosa* is a tropical plant indigenous to Southeast Asia especially in Thailand, Peninsular Malaysia and Indonesia. It has been reported that, MS taken as concoction may prolong the duration of sexual intercourse in humans although the claim has not been scientifically proven. In addition, consumption of MS leaves has been reported to increase the energy level in labourers and libido in male subjects. However, no scientific studies have been done to assess the effect of MS consumption on the sperm quality of males in general. Thus, the aim of this study was to evaluate the quality of sperm in mice treated with the crude methanolic extract of *Mitragyna speciosa* to provide pharmacological basis for treatment of male fertility disorder.

## Materials and methods

### *Experimental animals*

Mice were kept under standard laboratory conditions (12h-light: 12h-dark) with an environmental temperature of 24-25˚C. Feed and water were supplied *ad-libitum*. The animals were acclimatized for one week prior to the experiment. Fifty healthy male BALB/c mice (3-4 weeks old, weighing 20-25 g) were divided randomly into five groups. The negative control group received sodium chloride (0.9%, 10 mL/kg), positive control group received clomiphene (25 mg/70kg) and another three groups were treated with MS methanolic extract (MSM) at the dosage of 50, 100 and 200 mg/kg, respectively. Drugs and extracts were administered orally once daily for 14 consecutive days via intra-gastric route using an oral gavage needle attached to a syringe. Mice were euthanized on Day 14 and sperm samples were harvested from each mouse immediately after euthanasia. The quality of each sperm samples was evaluated using standard evaluation techniques.

### *Sperm evaluation*

One drop of sperm suspension was placed on a microscope slide using a pipette. The slide was then covered with a 22 x 22 mm coverslip. Microscopic fields were observed at 400x magnification using a standard microscope and the percentage of sperm motility was determined. Sperm numbers were calculated by using a Haemacytometer and the concentration was expressed as $x10^6$ sperm/mL sample. In order to observe the effect of various treatments on sperm morphology, one drop of sperm suspension was smeared onto a slide and stained with eosin-nigrosin. Samples were observed under the light microscope for presence of deformities and other abnormalities.

### *Statistical analysis*

The one-way analysis of variance (ANOVA), followed by Tukey's test, was used to compare differences between treatments. The student's *t*-test was used to compare differences between 2 groups. Data are expressed as mean ± SEM. Differences were considered to reach statistical significance when $P<0.05$.

## Results

The effect of various treatments on the sperm count of mice is shown in Figure 1. Results showed that mice treated with 100 mg/kg of extract produced marked increase in sperm count ($1.96 \pm 0.164 \times 10^6$ sperm/mL) as compared to negative (0.9% NaCl) and positive (clomiphene) control groups. However, the group that was treated with 200 mg/kg showed a sperm count of $1.8 \pm 0.07 \times 10^6$ sperm/mL, which is almost similar to the group treated with 100 mg/kg extract. Despite this, there was no significant difference between these groups as indicated by the *t*-Test.

The effects of various treatments on mice sperm motility is shown in Figure 2. The group that received 100 mg/kg of extract showed a 79 ± 1.01% sperm motility. There was no significant difference in the percentage of sperm motility as compared to negative (0.9% NaCl) and positive (clomiphene) groups except for the group treated with extract at the dosage of 50 mg/kg.

In Figure 3, the group that was treated with 100 mg/kg MSM showed an increase in the weight of testis (0.1 ± 0.0 mg) when compared to the group treated with 0.9% NaCl (0.015 ± 0.002 mg). However, there was no significant difference between groups treated with 100 and 200 mg/kg of extract.

In Figure 4, no apparent abnormal findings on sperm were observed in groups treated with clomiphene and 0.9% sodium chloride. However, the groups of mice that were treated with 50, 100 and 200 mg/kg of extract showed an apparent deformity in the form of swelling at the middle tail region as shown in Figure 5.

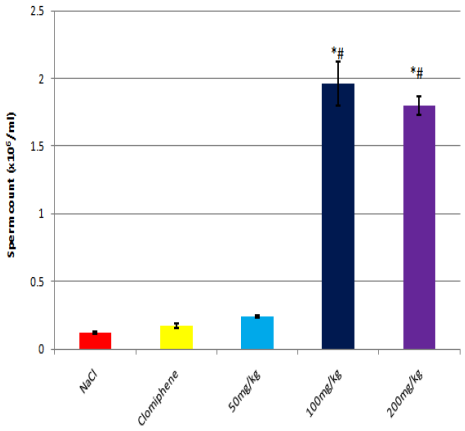


**Figure 1.** Effect of short-term ingestion of the methanolic extract *Mitragyna speciosa* on sperm count (x$10^6$ sperm/mL) in mice. Values are mean ± S.E.M ($n$=10). * Significantly different from 0.9% NaCl-treated group ($P$<0.05) and # significantly different from clomiphene-treated group ($P$<0.05), as determined by ANOVA followed by Tukey's test.

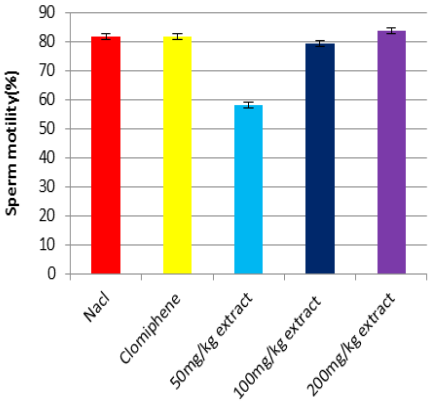


**Figure 2.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on sperm motility (%) in mice. Values are mean ± S.E.M ($n$=10). * Significantly different from 0.9% NaCl-treated group ($P$<0.05) and # significantly different from clomiphene-treated group ($P$<0.05), as determined by ANOVA followed by Tukey's test.

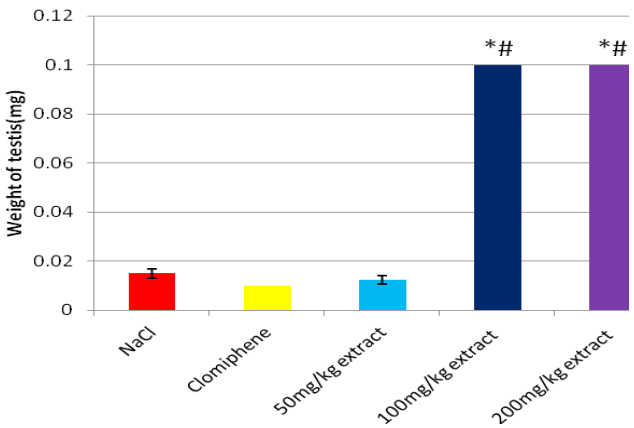


**Figure 3.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on the testicular weight (mg) of mice 14 days post-treatment. Values are mean ± S.E.M ($n$=10).
* Significantly different from 0.9% NaCl-treated group ($P$<0.05) and # significantly different from clomiphene-treated group ($P$<0.05), as determined by ANOVA followed by Tukey's test.

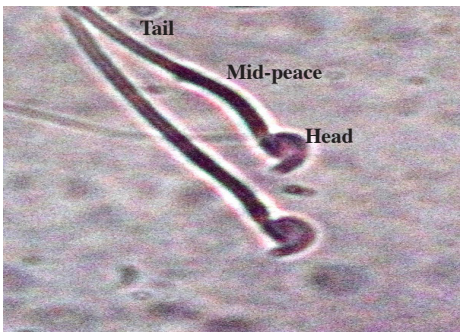


**Figure 4.** Effect of short-term ingestion of 0.9% NaCl or clomiphene on the morphology of mouse sperm. Eosin-nigrosin, (400x). Sperms appeared normal with no marked change in morphology

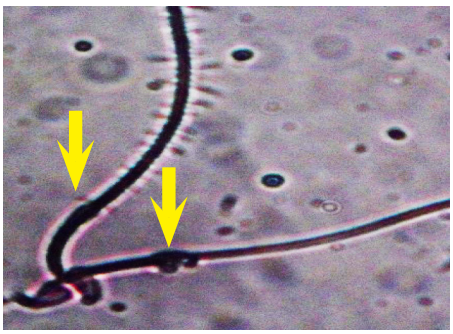

**Figure 5.** Effect of short-term ingestion of the methanolic extract of *Mitragyna speciosa* on the morphology of mouse sperm. Eosin-nigrosin, (400x). Arrow indicates the deformity in the form of swelling at the middle tail region of mouse sperm.

## Discussion

In this study, the effect of short-term ingestion of MS extract showed an increase in sperm count in mice compared to control groups fed with NaCl (0.9%) and clomiphene. The results from this study also showed that there was a slight difference in weight of epididymes in control and treatment groups. Increase in the sperm count and weight of epididymes could be due to stimulation of endocrine system specifically the testis which resulted in increased spermatogenesis and consequently increased testosterone level. In addition, there may be a possible simultaneous stimulation of the hypothalamus and the pituitary gland to produce luteinizing hormone and follicle-stimulating hormone (Guay *et al*., 2003) and thus, signals the testicles to produce testosterone and possibly more sperm through the hypothalamus-pituitary-gonadotropin axis. A possible explanation for the observed effect could be due to the synergistic effects of various secondary metabolites present in the MS leaves on these structures and not due to the effects of MS on opioid receptors. It is well-known that opiates inhibit gonadotropin secretion in experimental animals and humans (Pfeiffer and Herz, 1984). In addition, exogenous and endogenous opioids inhibit sexual arousal and erectile function in experimental animal and humans (McIntosh *et al,* 1980: Cushman, 1972). Morphine addiction for example, has been associated with suppression of male and female LH-gonadal axis (Cicero *et al*., 1979). Moreover, chronic exposure to morphine in cell cultures showed reduced basal and GnRH (gonadotropin releasing hormone) – stimulated LH release (Blank *et al*., 1986). Since MS has been classified as an opioid and resembles morphine in terms of pharmacological properties and function, it is believed that the effect on sperm produced by short-term intake of MS could be

mediated by a similar mechanism. However, results obtained from this study were in contrast to this theory. However, the possible explanation for the opposite effects observed could not be determined through this present study.

Conversely, the unexpected results showed by short-term ingestion of clomiphene on the sperm motility and count analyses could be due to the short duration (14) of clomiphene intake. A more accurate result may be obtained from the study if this drug given for a longer period as clomiphene has been shown to increase fertility rate only after 3-6 mo of daily intake (Srivannaboon *et al*., 1992; Schellen, 1982).

Human sperm cells contain $\mu$-opioid receptors (MOR) (Albrizio *et al*., 2006). In males, MOR-1 gene mutation results in decreased mating behavior, sperm motility and reduced litter size (Agirregoitia *et al*., 2006). Mitragynine, an alkaloid from MS leaves has been shown to possess an affinity towards $\mu$-, $\varkappa$- and $\delta$- opioid receptors (Yamamoto *et al*., 1999). Moreover, studies have demonstrated that gonadotropin have opiate receptors of relative high affinity and high capacity (5 x $10^4$ sites/cell) (Fabbri *et al*, 1985). It is probable that the compounds present in MS extract may bind to the $\mu$- opioid receptor and thus inducing mutation in the gene and eventually caused a deformity in the morphology of the sperm at the mid tail region. However, the exact mechanism by which MS extract induces sperm deformity could not be determined at present. Despite this, the motility of sperm especially in groups treated with 100 and 200 mg/kg of MS extract was not affected.

## Conclusion

In this study, the groups of mice treated with the methanolic extract of *Mitragyna speciosa* at the dosage of 100 and 200 mg/kg showed a marked increase in sperm count and sperm motility. The effects seen could be due to the enhancement of spermatogenesis through the stimulation of hypothalamus-pituitary-gonadotropin axis by the compounds or metabolites present in MSM. A marked increase in testicular size and weight could also be explained by the increase in sperm concentration which may be stimulated by similar mechanism stated previously. However as the doses increased, the occurrence of the deformity of the sperm at the middle of the tail region increased simultaneously. The precise mechanism underlying this effect has yet to be determined and currently under investigation, but it is likely to be associated with genetic mutations involving the interaction of MSM with the opioid receptors on the surface of each sperm.

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# Relationship of Colic Occurrences with Nutrition, Management and Work in Horses in the Klang Valley, Malaysia

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## Abstract

Colic is one of the most important conditions in horses. At the Universiti Veterinary Hospital (UVH), Universiti Putra Malaysia, 11% of equine cases treated between 2005 and 2009 were colic cases. In this study, questionnaires were distributed to stable managers of 10 selected stables in the Klang Valley, Malaysia. The questionnaires were designed to determine the association between colic incidences and nutrition, management and work. The studies showed that the total prevalence of colic was 28.19% where almost half the cases were from one stable. Stable management contributed the highest incidence of colic (50%) of which 74% was seen in horses that had dental rasping. Long working hours was the second most important predisposing factor, while high protein diet can also contribute to the risk of colic in horses. Other factors were the changed in the type of feeds, use of feed brands and the size of the cubes.

**Keywords**: Colic, horse, Klang Valley

## Introduction

Colic is defined as a severe paroxysmal pain in horses due to diseases which can be fatal as a result from shock and circulatory collapse (Blood *et. al*., 1988). Colic is a manifestation of an abdominal pain. The causes of this condition can either be specific or non-specific (Bentz, 2004). The USDA National Animal Monitoring System (NAHMS) survey showed that from spring 1998 until spring 1999, the incidence of colic was 4.2 events/100 horses per year which was the second highest cause of mortality after old age. This had incurred treatment cost reaching USD115 million. In North Carolina, 5 to 6 colic cases/100 horse was seen each year (Uhlinger, 1992). In Malaysia, from 2005 to 2009, 11% incidence of

colic cases was recorded by the Universiti Veterinary Hospital (UVH) Universiti Putra Malaysia (UPM) (Fauziah, 2010). It was shown that the risk factors that are highly suspected as the cause of colic in horses in the Klang Valley were nutrition, management and work.

Since colic is one of the most important problems in horses, a number of epidemiological studies were done to determine its risk factors (Cohen *et. al.*, 1999). Goncalves *et al.* (2002) suggested, after 12 epidemiological studies, that management and food were the initiators of colic in horses. It is difficult to determine the exact cause of colic, least of all the mechanism of colic development (White II, 2005). A few predisposing factors of colic in horses were identified, which were signalment, management and transportation (Cohen, 2003). History of previous colic may also be a predisposing factor of repeated episodes of colic (Tinker *et. al.*, 1997a).

It is difficult to compare the incidence of colic between stables due to differences in management and practice (White II, 2005). However, Goncalves *et al.* (2002) showed that there was 3.6 times higher risk of horses developing colic when there was a change in feed, which involved changes in the cube size and frequency of feeding.

## Materials and Methods

One hundred and forty nine adult horses (mainly mare and gelding) consisting of Thoroughbreds, Arabians and Criollos were sampled from 10 stables. The selected stables were from different locations such as in the city centre, suburban area, recreational park and institute of higher education around the Klang Valley. These horses were kept in stables and were not allowed to graze. The horses treated for colic in UVH from January 2005 until October 2010 were included. The questionnaires used reflected the relevant scopes of the study such as the management, the type of feed, changes of feed, dental rasping, drinking water, deworming regime, types and frequency of change of bedding and previous history of colic. The working group consisted of horses kept at the stables and the duration of working hours were recorded daily. The last part of the questionnaire included the nutritional aspect where the protein content of the feed was noted. The stable manager was also interviewed on the overall management and the condition of the stables.

**Table 1.** Prevalence of colic in Klang Valley (January 2005 – October 2010)

| Stables | Prevalence (%) |
|---|---|
| Stable 1 | 45 |
| Stable 2 | 40 |
| Stable 3 | 38 |
| Stable 4 | 0 |
| Stable 5 | 27 |
| Stable 6 | 17 |
| Stable 7 | 40 |
| Stable 8 | 33 |
| Stable 9 | 20 |
| Stable 10 | 0 |

**Table 2.** Risk Factors Associated with Colic

| Risk Factors associated with colic | % |
|---|---|
| **Management** | |
| dental rasping | 73.8 |
| Change of feed | 59.9 |
| Feeding 3 times/d | 73 |
| History of previous colic | 52.4 |
| **Work** | |
| More 1½ h | 56.2 |
| 1- 1½ h | 31.2 |
| Less than 1 h | 12.5 |
| Patrol | 56.2 |
| Leisure | 26.2 |
| Sports | 21.4 |
| **Nutrition** | |
| High protein diet | 66.7 |
| Low protein diet | 33.3 |

The feeding frequency and pattern contributed up to 59.5% of total cases of colic and 73.8% of horses that was rasped. Horses that were fed three times a day showed higher incidence of colic (73%). These horses were fed with two heavy meals and hay at night for chewing activities. The result also showed that 52.4% had a previous history of colic.

Table 2 showed 56.2% of horses that was worked more than $1^1/_2$ h had colic compared to 31.2% that worked between 1 and $1^1/_2$ h and 12.6% less than 1 h. Patrol horses (52.4%) were more susceptible to colic than horses for leisure (26.2%) and sport (21.4%). Horses fed high protein diets showed higher incidences of colic than those fed low protein diet.

## Discussions

The total prevalence of colic in Klang Valley was 28.19% which was much higher than those found by others (Kaneene *et al*., 1997; Tinker *et al*. 1997a; Hillyer *et al*. 2002; Archer and Proudman 2006; Cohen, 2003) which were between 3.5 and 10.6%. Environmental factors such as temperate and tropical climate may influence the incidence of colic. This was seen in horses that were brought into this country from a temperate climate. Although there seems to be no correlation between occurrence of colic and climate (Goncalves *et al*. 2002), these imported horses still developed colic.

A high correlation 73.8% was found in this study between colic and dental rasping although study done by Cohen *et al*. *(*1999), Goncalves *et al*. (2002) and Cohen (2003) show no significant correlation but Hillyer *et al*. (2002) did show that horses given dental care at least once a year had reduced chances of developing colic. The high percentage of colic in horses that had dental rasping in this study might be due to the improper rasping technique (underdone or overdone, one-sided rasping or no routine schedules for rasping).

Changes of feed contributed the second highest cause of colic at 59.5%; these included change in feed, brand and sizes of the cubes that were given. Changes of feed including abrupt change from a normally given diet feed to a new type of feed can cause gastric ulceration (Archer and Proudman, 2006). Thus if changed was necessary, it should be done gradually. These results from this study are similarly to that of Goncalves *et al*. (2002). Feed changes may lead to increase chance of colic. However, Cohen *et al*. (1999) did not find any significant difference between change in feed and colic.

According to a study by Traub-Dargatz *et al*. (2001), 43.5% of horses that had previous histories of colic would experience recurrence. In the current study the recurrence of colic was higher (52.4%) that that reported by Cohen *et al*. (1999) at 70.6%. Long working hours too lead to the development of colic (Hillyer *et al*., 2002). Also there is an association between colic and stage or level of intensity

or work. In one study, it was shown that there was 2.2 times higher risk of colic when the activity of the horses changed (Goncalves *et al*., 2002). However the association between work and colic in horses is debatable (Cohen *et al*. (1999).

Studies by Cohen *et al*. (1999) and Hudson *et al*. (2001) indicated that feeding a low protein diet and low digestible feed predispose to colic. Contrastingly this study found that high instead of low protein diet increases risk of colic. These might due to the fact that, although the horses was kept in and rested, they were still given the same ration of diet as working horses.

Although the incidence of colic cases that was treated by the UVH was 11%, there might be more cases of colic that was not referred to UVH as some stables have their own veterinarians. This study should serve as a basis for further studies on colic cases in Malaysia.

## Conclusions

The incidence of colic is mainly governed by management practices which include changes in the type of feeds, use of feed brands and the size of the cubes. Long working hours were the second factor most important predisposing factor, while high protein diet can also contribute to the risk of colic in horses.

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# Prevalence of *Dirofilaria immitis* in Dogs in Johor Bahru, Malaysia

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**Abstract**

This study was conducted to determine the prevalence of *Dirofilaria immitis* in dogs in Johor Bahru, Malaysia. The diagnostic techniques employed were wet blood mount, Knott's concentration test and two heartworm antigen test kits (IDEXX Canine SNAP® 4Dx and RapiGEN®). This study also attempted to compare the two test kits used and identify the microfilaria isolated. Blood from 100 pet and 50 stray dogs in Johor Bahru were examined. The prevalence of heartworm in pet and stray dogs in this study were 1 and 2% respectively. The overall prevalence of *Dirofilaria immitis* in dogs in Johor Bahru was 1.33% (2/150) and the microfilaria was identified as *Dirofilaria immitis*. Since there was only one false-negative result from RapiGEN® test kit, sensitivity between the two test kits could not be compared. The low prevalence of *Dirofilaria immitis* found in this study confirmed anecdotal evidence that the prevalence of *Dirofilaria immitis* in Dogs is low in Johor Bahru.

**Keywords:** Dogs, *Dirofilariaimmitis*, prevalence

## Introduction

Heartworm affecting the canine population around the world is of considerable economic importance. It is caused by the parasitic worm, *Dirofilaria immitis*. Mosquitoes are the intermediate host for *Dirofilaria immitis*. Currently more than 70 species of mosquitoes have been recorded to transmit *Dirofilaria immitis*. The type of mosquito present depends on locality. In Malaysia, the common genera are *Armigeres* sp., *Culex* sp. and *Aedes* sp. (Vythilingam *et al*., 2005).

Limited information is available in the literature on the occurrence and prevalence of heartworm disease in Johor Bahru, Malaysia. However,Yap and Ong

(2008) reported the prevalence of heartworm in 2 out of 129 samples collected from clinics in Negeri Sembilan, Melaka and Johor, Malaysia. In Johor Bahru, although most dog owners do not put their dogs on heartworm prevention programme (personal communication, Dr Tan Check Nam) the prevalence of this parasite was reported to be low. Thus this study was undertaken to determine the prevalence of *Dirofilaria immitis* in dogs in Johor Bahru, Malaysia.

## Materials and Methods

One hundred and fifty dogs were sampled; 100 pet dogs from four veterinary clinics and 50 stray dogs from one animal shelter in Johor Bahru. The criteria for selection were; the dogs were more than 7-months old and not on any heartworm prevention programme.

Approximately 2 mL of blood were collected from each via cephalic venipuncture. Each blood sample was tested for heartworm antigen using the SNAP® 4Dx test kit and RapiGEN® Canine Heartworm Antigen Test. Each blood sample was also examined for microfilaria using wet blood smear and Knott's concentration test (KCT). A thick blood smear was done and the KCT sediment smear was examined for microfilaria in samples that were positive in serology as well as KCT.

## Results

A microfilaria was detected from one sample among the pet dogs using Knott's concentration technique. However, all 100 samples tested for the presence of heartworm antigen were negative for both antigen test kits. Thus, the prevalence of dirofilariasis in pet dogs in Johor Bahru was 1%. Among the stray dogs, several microfilariae were detected in one sample using Knott's concentration test and the same sample was also the only one out of the 50 samples positive by the antigen test kit (Canine SNAP® 4Dx). Thus the prevalence of dirofilariasis in stray dogs in Johor Bahru was 2%.

## Discussion

This *Dirofilaria immitis* prevalence study is believed to be the first-ever conducted specifically in Johor Bahru. The study showed that the overall prevalence of the parasite in dogs in Johor Bahru was 1.33% (2/150). Other similar studies have been conducted elsewhere. For example, Yap and Ong (2008) conducted a study on heartworm antigenemia in the southern states (Negeri Sembilan, Melaka and Johor) of Peninsular Malaysia and they recorded two positive cases out of 129 samples. However, no positive case was reported among dogs in Johor.

The detection of heartworm in this study showed that it is prevalent in Johor Bahru. However, the prevalence rate was low compared to that in Klang Valley, Malaysia. In Kuala Lumpur, the prevalence of *D. immitis* in dogs in 1970 was 30.4% (Mullin, 1970), in 1987 it was 42% (Dhaliwal, 1987) and in 2004 it was 33.34% (Yap 2004). Toh (2002) recorded a 31.7% prevalence in Selangor, while Retnasabapathy (1976) reported a prevalence of 25.8% in Malaysia. The low prevalence observed among owned dogs in Johor Bahru is probably due to a number of reasons. Firstly, most of the dogs sampled in this study were pet dogs, hence kept indoors most of the time. As such, they were less likely to be bitten by infected mosquitoes. Secondly, it could be related to lower mosquito abundance in this region. According to a press statement released by the Ministry of Health, Malaysia (2010) there wasa higher incidence of dengue fever cases in Selangor (16,179 cases) which was almost four times the number of cases recorded in Johor at 4,355 cases. Dengue fever is transmitted by *Aedes* mosquitoes. This is suggestive of a lower abundance of mosquitoes in Johor than in Selangor. The higher mosquito population in Klang Valley could be largely due to the more rapid socio-economic development in Klang Valley compared to Johor Bahru. There are more construction sites with stagnant water bodies and clogged up drains contributing to the abundance of mosquitoes in Selangor.

In Malaysia, few studies have been done to identify the principal vector of *Dirofilaria immitis*. In one study conducted in two urban areas in Kuala Lumpur, *Armigeres subalbatus* was incriminated as a potential vector of *Dirofilaria immitis* mosquito (Vythilingam*et al*., 2005) because the parasite can develop to infective stage in the head, thorax and abdomen of the mosquito. This finding was also in agreement with Cheong *et al*. (1981) who incriminated *Armigeres subalbatus* as an important vector of *D. immitis* in urban Kuala Lumpur.

Based on the one false-negative result alone using the RapiGEN® Canine Heartworm antigen test kit, it may suggest that this lateral immunochromotographic test is less sensitive than Canine SNAP® 4Dx in heartworm antigen detection. The false-negative result by RapiGEN® Canine Heartworm antigen test in this study may be due to the presence of immature female worms. However, it must be cautioned that one false-negative result does not necessarily suggest that RapiGEN® Canine Heartworm antigen test is less sensitive than the Canine SNAP® 4Dx in heartworm antigen detection.

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# Relationship between Body Weight and Linear Body Measurements in Boer Goats

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## Abstract

Fifty-five Boer goats from a private farm located at Kg Pulau Meranti, Mukim Dengkil, Puchong, Selangor was sampled to examine the relationship between body weight and linear body measurements (body length, height at withers and heart girth). The goats were of four age groups: Group 1 (4-12 mo), Group 2 (13-18 mo), Group 3 (19-24 mo) and Group 4 (25-36 mo). Body weight and linear body measurements (body length, height at withers and heart girth) were taken during a 7-d period in December 2010. The body weight and linear body measurements were not significantly ($p>0.05$) different between male and female goats but were significantly($p<0.05$) different among age groups. Male goats were heavier in weight, longer in body length, taller in height at withers and larger in heart girth compared to the female goats. Pearson's coefficient of correlation showed positive, high and significant correlation between body weight and linear body measurements and also among the body measurements.A simple linear regression equation with a high coefficient of determination ($R = 0.948$) using heart girth to predict body weight of Boer goats was also derived.

**Keywords**: Boer goats, body weight, body length, height at withers, heart girth

## Introduction

Goat is a multifunctional animal and plays a significant role in the economy and nutrition of small farmers in Malaysia. Boer goat farming in Malaysia recorded an annual growth rate of more than 6% between 2006 to 2010. With the support from the Ministry of Agriculture and Agro-based Industry, Malaysia and its associated financial and development agencies, the future seems bright for Boer goat farming. The National Goat Production Programme targets to have 2 million goats in Malaysia by 2015 (Aziz, 2009) and by 2020 the country is envisaged to have 3.2

million goats for breeding. These goats will be the production units to supply 68% of the national requirement for goat meat by 2020. Boer goat is considered to be one of the most desirable goat breeds for meat production. It has gained worldwide recognition for its excellent body conformation, fast growing rate and good carcass quality. Its popularity as a meat goat breed soared during the last decade due to its availability in Australia, New Zealand and later in North America and other parts of the world. It has been demonstrated that Boer goats can improve productive performance of many indigenous breeds through crossbreeding (Lu, 2002). One study conducted in Pakistan showed that the relationship between linear body measurements, such as body length, heart girth and height at withers, and body weight is found to be positive in goats and could be used as selection criteria for the goats (Khan *et al*., 2006). However, information on body weight and linear body measurements of goats in Malaysia is scarce. This current study is performed to obtain more information on the characteristics of Boer goats in Malaysia. The information on the relationship between body weight and linear body measurements of Boer goats is important for the estimation of size and shape of goats suitable for breeding and slaughter. The objectives of this study were to determine the relationship between the body weight and linear body measurements of Boer goats and to predict body weight from linear body measurements in goats.

## Materials and Methods

### *Animals*

Fifty-five healthy Boer goats were selected at random from a private farm (De Kebun Enterprise) located at Kg Pulau Meranti, Mukim Dengkil, Puchong, Selangor. The animals were divided into four age groups: Group 1 (4-12 mo), Group 2 (13-18 mo), Group 3 (19-24 mo) and Group 4 (25 -36 mo). The age of the animals was obtained by referring to the birth records when available or estimated by the number of permanent incisors present. The farm practiced intensive rearing system where the goats were managed indoor with feeds provided in feeding troughs twice a day. The goats were not allowed to graze and feeds were brought to the goats. The goats were fed with cut native grass (*Paspalum conjugatum, Axonopuscompressus)*, commercial goat pellet (14% crude protein) and soya bean waste from the processing of tofu and soya drinks.

### *Body Measurements*

Body weight and linear body measurements were taken to determine the body conformation of the goats. Body weight (BWt) was determined using a hanging weighing scale (100 kg), early in the morning before feeding. The body weight was measured in kilograms to one decimal point. The goats were fasted overnight before weighing.

Height at witers (HAW) was measured from the bottom of the front foot (phalanges) to the highest point of withers between the shoulders by using a one-metre ruler. The measurement was in centimetres, to the nearest whole number.

Heart girth (HG) or circumference of chest was measured as the circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body and measured by using a girth tape. The measurement was in centimetres, to the nearest whole number.

Body length (BL) was measured as the distance from the point of shoulder (dorsal spine of scapular) to the posterior edge of the pin bones (tuber Ischia) using a measuring tape. The measurement was in centimetres, to the nearest whole number.

### *Statistical Analysis*

Data collected were classified on the basis of sex and age. Statistical analysis for body weight and linear body measurements was carried out with SPSS Statistical Software Program version 16.0 (2008). The data were examined for normality in distribution. The relationship between body weight and linear body measurements was measured using Pearson's coefficient of correlation. ANOVA for 2 factors (Sex and Age Groups) was done for body weight and linear body measurements and comparison of means between groups was carried out using Tukey test upon significant F value for the factors of the ANOVA. Simple linear regression analysis was done to predict the body weight of Boer goats using linear body measurements as independent variables (IV).

## Results and Discussion

From the Pearson's coefficients of correlation, there were positive, high and significant ($p<0.01$) correlations between BWt and BL, HAW and HG with correlation coefficients (r) of 0.93, 0.91 and 0.97, respectively, and also between the body measurements (Table 1). Similarly Mukherjee *et al*., (1981) and Singh *et al*., (2004) reported a high significant correlation between body weight with heart girth in brown Bengal does and grey Bengal goats.

Means for BWt and linear body measurements were not significantly different between male and female goats but were significantly different between age groups. As an animal got older, body weight and linear body measurements would also increase (Table 2). Male goats were noted to be heavier in weight, longer in body length, taller in height at withers and larger in heart girth compared to the female goats as was also reported by Khan *et al*., (2006). The results obtained regarding the live weight of Boer goats were lower than those reported previously by Lu and Potcoiba (2002). Factors affecting these measurements are known to be sex, nutrition, type of birth, and environment (Hassan and Ciroma, 1990).

**Table 1.** Correlation coefficients among body weight and body measurements in Boer goats

| | Body length | Height at withers | Heart girth |
|---|---|---|---|
| Body weight | 0.93** | 0.91** | 0.97** |
| Body length | | 0.93** | 0.94** |
| Height at withers | | | 0.95** |

** Correlation significant at p = 0.01 level.

**Table 2.** Means (±SE) for body weight and linear body measurements for sex and age groups of Boer goats

| | | Body weight[1] (kg) | Body length[1] (cm) | Height at withers[1] (cm) | Heart girth[1] (cm) |
|---|---|---|---|---|---|
| Sex | Female | $37.31^{a}$±0.72 | $56.26^{a}$±0.50 | $62.79^{a}$±0.59 | $76.85^{a}$±0.62 |
| | Male | $40.06^{a}$±1.18 | $59.71^{a}$±0.82 | $64.15^{a}$±0.96 | $78.80^{a}$±1.01 |
| Age groups | 4 -12 months | $22.56^{a}$±1.13 | $47.44^{a}$±0.79 | $53.13^{a}$±0.93 | $62.99^{a}$±0.97 |
| | 13 -18 months | $37.15^{b}$±1.27 | $53.57^{b}$±0.90 | $60.16^{b}$±1.05 | $75.96^{b}$±1.10 |
| | 19 - 24 months | $42.60^{c}$±1.27 | $60.18^{c}$±0.90 | $64.30^{b}$±1.05 | $80.77^{c}$±1.10 |
| | 25 - 36 months | $52.44^{d}$±1.73 | $70.75^{d}$±1.21 | $76.30^{c}$±1.42 | $91.58^{d}$±1.49 |

[1]Means with different superscripts between rows within each category of sex and age groups are significantly different at p=0.05.

**Table 3.** Regression coefficients (b) and coefficients of determination ($R^2$) for the different models to predict body weight in Boer goats

| Regression Model[1] | | Coefficients* | | | | |
|---|---|---|---|---|---|---|
| | | $b_0$ | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
| 1-IV model | HG | -43.358 | 1.054 | | | |
| | BL | -32.358 | 1.234 | | | |
| | HAW | -40.286 | 1.245 | | | 0.835 |
| 2-IV model | HAW + BL | -37.746 | 0.796 | 0.483 | | |
| | HG + BL | -42.988 | 0.932 | 0.159 | | |
| | HG + HAW | -42.561 | 1.185 | - 0.175 | | 0.950 |

*cont'd Table 3*

| | | | | | | |
|---|---|---|---|---|---|---|
| 3-IV model | HG + HAW + BL | -41.566 | 1.070 | -0.269 | 0.243 | 0.953 |

[1] Regression models: 1-IV model includes 1 independent variable, 2-IV model includes 2 independent variables and 3-IV model includes 3 independent variables
[*] Significant at p=0.05

Simple linear regression models were developed to include one or more independent variable as the predictor variable to predict BWt. The 1-IV models included one independent variable of BL, HAW or HG and the dependent variable, BW. The 2-IV models included two independent variables and the 3-IV model included all three independent variables (Table 3). Among the 1-IV models, HG as a predictor variable of body weight had the highest $R^2$ value (0.948). Using 2 independent variables in predicting body weight, the inclusion of HG and BL or HG and HAW resulted in equal $R^2$ values (0.948 - 0.950), similar to the model with HG as the predictor variable. The highest $R^2$ value (0.953) was obtained by including all three independent variables of HG, BL and HAW in the model. In terms of $R^2$ value, the best regression models for one independent variable of HG (0.948), the 2 independent variables of HG and HAW (0.950) and the 3 independent variables of HG, BL and HAW (0.953) had almost similar coefficients of determination. This meant that using HG alone was sufficient to predict body weight as indicated by the high $R^2$ value of its regression model. The predictive value of the linear body measurement of animals had been used to determine body weight by Benyi (1997) in goats and Dale and Bunnell (1984) and Attah *et al*. (2004) in sheep and the chest girth was found to be a useful tool in this regard.

## Conclusion

There is a positive and significant relationship between the body weight and linear body measurements (body length, height at withers and heart girth) in Boer goatsand the most practical way to estimate the live weight of goats is by measuring the heart girth of the goats, especially where equipment to definitively quantify the weight of animals are not available and inaccessible. Since heart girth has a high correlation with the body weight, this may be used as a selection criterion to improve body weight indirectly. Earlier reports also indicated that selection based on the body measurements could improve meat production. However, further research is needed to investigate the relationship between body weight with linear body measurements in larger samples of similar and other breeds of goats in other parts of the country.

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# Effect of Sublethal Unionized Ammonia on Mortality Rate of Red Tilapia (*Oreochromis* Spp.) Fingerlings in *Aeromonas hydrophila* Infection

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## Abstract

A study was carried out to investigate the effect of sublethal unionized ammonia concentration on the mortality rate of tilapia fingerlings in *Aeromonas hydrophila* infection. Mono-sex tilapia about 5 cm in length was used in this experiment. Sublethal unionized ammonia concentrations were determined and 1.1, 1.7, 2.2, 3.6 and 4.6 mg/L ammonium chloride was chosen for the experiment. The sublethal effects of unionized ammonia concentration on *A. hydrophila* infection in fish fingerlings were then determined. There were 2 mortalities each at ammonia concentrations of 2.2 and 3.6 mg/L in fingerlings infected with *A. hyrophila*. However, there seemed to be no association between concentration of unionized ammonia and fingerling mortality suggesting that sublethal unionized ammonia concentrations do not cause death in fingerlings exposed to *A. hydrophila* infection.

**Keywords**: Unionized ammonia, Tilapia, *A. hydrophila*, mortality rate

## Introduction

The accumulation of ammonia in aquatic systems is due to the metabolism of protein from uneaten feed and organic matter inside the pond. However, fish themselves, either being fed or starved are the primary source that excrete ammonia as their byproduct (Francis-Floyd *et al.,* 2005). Fishes exposed to sublethal ammonia concentrations displayed histopathologic alterations in the gills, liver and kidney. Gill tissues showed hyperemia, chloride cell proliferation, fusion in secondary lamella, and telangiectasis. Liver tissue revealed cloudy swelling and hydropic degeneration, whereas in kidney tissues, hyperemia and glomerulonephritis were observed (Aysel *et al.,* 2008). *Aeromonas hydrophila* and other aeromonads are among the most common bacteria in freshwater habitats throughout the world. The clinical signs shown by affected fishes varied from septicemia, ascites, erosion,

ulceration, detachment of scale and exophthalmia. The postmortem findings varied from congestion to focal lesions in the liver, spleen, and kidney. This study was to investigate the effect of sublethal concentrations of unionized ammonia on the susceptibility of tilapia fingerling to *A. hydrophila* infection based on mortality rate.

## Materials and Methods

### *Sublethal concentration*

#### *Unionized ammonia*

Five hundred mono-sex red tilapia fingerlings (*Oreochromis* spp.) about 5 cm in length were bought from a farm at Sg. Buah, Bangi, Selangor, Malaysia. The fishes were placed in two separate tanks with aeration for 4 d before experimentation. Ammonium chloride ($NH_4Cl$) was used as a source of ammonia (Xu *et al*., 2005). The presence of ammonia in water was calculated based on the molecular weight. In this experiment, the sublethal concentration of unionized ammonia (UIA), that is where no mortality occurred in the fingerlings, was determined. Ammonium chloride was added to the fish tanks at the rate of 40, 75, 120 and 250 mg/L and left for 4 d. The concentrations of unionized ammonia in the respective tanks were 1.1, 1.7, 2.2, 3.6 and 4.6 mg/L.

#### *Aeromonashydrophila*

Three concentrations ($5x\ 10^4$, $5x\ 10^5$ and $5x\ 10^6$ cfu/mL) of *A. hydrophila* were used as sublethal concentrations.

#### *Effect of Ammonium chloride on A. hydropila infection*

This experiment was carried out in fibre plastic aquariums. The aquarium was filled with a 7 L of water each. Each aquarium housed 14 fingerlings supplied with aerations using compressed air via an air-stone. Sublethal concentrations of ammonium chloride and *A. hydrophila* were added to the respective tanks. One tank each served as the negative control (without $NH_4Cl$ or *A. hydrophila*). The fingerlings were fasted for 24 h prior to experiment and left for 4 days. The aquariums were duplicated. The temperature and pH was measured at the first day of experiment in order to determine the value from the table of fraction of unionized ammonia. The dead fingerlings were recorded and removed from the aquarium each day.

#### *Statistical analysis*

Chi-square test using SPSS 16 was used in order to see the relationships between the different concentrations of unionized ammonia and the mortality rate of tilapia fingerlings in *A. hydrophila* infection.

## Results

### *Effect of sublethal concentration on tilapia fingerlings*

### *Unionized ammonia*

In the determination of sublethal concentration of unionized ammonia, there were 3 mortalities on day one, at 4.6 and one at 1.7 mg/L $NH_4Cl$ concentrations. On the second day, there were three mortalities one each at 1.1, 2.2 and 4.6mg/L$NH_4Cl$. No mortality was observed on day three. On day 4 there were two mortalities, one each at 1.1 and 2.2 mg/L.

### *A. hydrophila*

In the second experiment to determine the sublethal concentration of *A. hydrophila,* the highest number of fingerling that died was in the lowest concentration of bacteria (5 x $10^4$ cfu/mL) which was 3 of 14 fingerlings. One fingerling died in medium concentration (5 x $10^5$ cfu/mL). No fingerling died in high bacteria concentration (5 x $10^6$).

### *Unionized ammonia and A. hydrophila infection*

The third experiment was to investigate the effect of sublethal ammonia concentration on susceptibility of tilapia fingerling to *A. hydrophila* infection. The result showed that there were no deaths at 0 and 1.1 mg/L unionized ammonia. However, at 2.2 and 3.6 mg/L, there were two deaths each.

## Discussion

The result from the first experiment showed that the number of fingerling deaths were not consistent for each concentration of unionized ammonia. Deaths were observed at all concentration of ammonia except at 3.6 mg/L. Therefore, the cause of death remains uncertain. If the fingerlings died because of unionized ammonia, than there should also be more mortalities as 3.6 mg/L $NH_4Cl$. For the sublethal concentration of unionized ammonia determination, the range between 1.1 to 3.5 mg/L was chosen. This range is comparable to one study that showed that the median lethal concentration within 48 h for tilapia fingerlings to be 7.1 mg/L unionized ammonia (El-Sherif *et al*., 2008).

The result for second experiment showed, that the highest number of fingerling that died was in the lowest bacterial concentration. No fingerlings died in high concentration of bacteria. This result was similar to the previous result in which death was observed mainly at low instead of high concentrations of ammonia. There is a possibility that other factors such as stress due to transportation and sudden changes in environment may be the contributing factors to the mortalities of the fingerlings. From observation, the fingerlings that dead was smaller in size than those that lived.

The result for third experiment showed there was no mortality at 0 and 1.1 mg/L $NH_4Cl$. However, at 2.2 and 3.6 mg/L $NH_4Cl$, there were two deaths for each concentration. This shows that even at 3.6 mg/L unionized ammonia very few fingerlings died. Most of the mortalities occurred in first two days of the experiment. Another study conducted by Evans *et al*. (2005) on the susceptibility of *Streptococcus* infection due to ammonia exposure showed that the median lethal concentration ($LC_{50}$) for tilapia was 1.46 mg/L unionized ammonia at 24 and 48 h post-exposure, 1.33 mg/L at 72 h post-exposure, and 0.98 mg/L at 96 h post-exposure. However, 93–100% mortalities were observed within 24 h among fish exposed to 2.0, 3.0, or 4.0 mg/L UIA. Their study showed that even sublethal concentration of ammonia had significant effects on mortality rate in *Streptococcus* infection. We failed to show a similar effect with *A. hydrophila* infection. The result also showed that there was no association between fish fingeling mortality and concentration of unionized ammonia.

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# Serological Prevalence of FeLV and FIV in Cats in Peninsular Malaysia

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## Abstract

Feline Leukemia (FeLV) and Feline Immunodeficiency Virus (FIV) are the two feline retroviruses that were studied in 65 client-owned cats from eight veterinary centres in selected areas throughout Peninsular Malaysia during a 4-week period. Blood samples were collected for serological tests using SensPERT® FeLV Ag/FIV Ab test kits. Five of 65 cats (7.69%) tested positive for the FeLV antigen and 10 cats (21.54%) tested positive for FIV antibodies. Only one cat had a dual infection. Chi square analysis revealed a significant association ($P<0.05$) between the health status and lifestyle of the cat and FeLV-positive status. FeLV infections were more likely to occur in a pedigree, adult male cat while FIV was more likely to infect the adult, intact cat that is aggressive towards other cats.

**Keywords**: Cats, FeLV, FIV, prevalence, risk factors, SensPERT® test kit

## Introduction

Feline Leukaemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) originate from the family of *Retroviridae*. These viruses affect domestic cats worldwide and infections contribute to considerable immunosuppression in cats. FeLV was first discovered in 1964, whereas FIV was isolated later in 1986, from a sick cat that was chronically infected with FeLV (Pedersen *et al*., 1987). A general lack of disease control, their effects of the immune system and the fact that these diseases are easily transmissible, have allowed FeLV and FIV to persist and become one of the more important diseases affecting domestic cats. It is crucial that the prevalence rates and epidemiology FeLV and FIV infections in domestic cats are understood so that control measures for these viral infections can be implemented.

In Malaysia, the prevalence for FIV was first reported 20 years ago (Cheng, 1990) and since then, very few studies have been conducted on the disease. More

recently, a 2009 study on FIV in cats admitted to University Veterinary Hospital, Universiti Putra Malaysia reported a prevalence of 24.7% (Bande *et al*., 2009). This was followed by another report in the following year that showed a 33.2% FIV prevalence. Both these studies were conducted in the state of Selangor, Malaysia. While information is available for FIV, at least for the Selangor, no such information is available for FeLV. Numerous test kits for FeLV and FIV are available in Malaysia, but there is no information on the use of FeLV or FIV vaccines for the control of these diseases. Furthermore, the risk factors, clinical signs and vaccination programmes for the diseases are not known. Thus this study was designed to determine the prevalence of FeLV and FIV, to report on the clinical signs and risk factors associated with these diseases, to determine the use of vaccines in the prevention of these diseases in small animal practices in Peninsula Malaysia

## Materials and Methods

### *Study area and design*

This study was conducted in three different regions of Peninsular Malaysia, the northern, southern and eastern regions. The Northern region consists of Perlis, Kedah, Penang and Perak. The Southern region comprised Negeri Sembilan, Malacca and Johore while the Eastern region comprised of the states of Kelantan, Pahang and Terengganu. A cross-sectional study was used in the serological testing performed on plasma samples from feline patients presented to clinics or hospitals for treatment. Samples were collected during a period of 4 weeks from 20 November until 16 December 2010.

### *Animals*

Sixty-five cats presented to eight veterinary centres for routine vaccination and treatments were the target population in this study. All cats in the study were sampled with the consent from their owners. The owners were given a questionnaire upon participating in the study to obtain relevant information on the environment, health and behavior of the cats participating in the study.

### *Investigation of vaccination practices for FeLV and FIV*

Information was also gathered from all participating veterinary centres involved in this study, regarding the use of vaccines against FeLV and FIV.

### *Risk factors*

Information for study of risk factors for FIV and FeLV was obtained by questionnaire. These factors include gender, age, sexual intact, breed, behavior, multi- or single-cat household, vaccination status, the health status, and other concurrent diseases.

### *Clinical signs*

A thorough physical examination was conducted and findings documented for each cat. Cats were classified into healthy and sick groups. The history of treatment was also obtained in order to relate to the likely risk factors.

### *Samples*

Blood samples were collected from the jugular veins or cephalic veins using 23G needles (B. Braun®, Melsungen AG, Germany) and EDTA tubes (BD®, Franklin, USA). Plasma was obtained by centrifugation of blood samples at 1000 x g at 4°C (HettichZentrifugen, Germany) for 10 min and stored in plain tubes at -20°C (Sanyo Biomedical Freezer, Sanyo Electric Co. Ltd., Japan) until analysis.

### *Serological test*

This serological test (SensPERT®, FeLV Ag/FIV Ab kit, Vetall Laboratories, Korea) based on the principle of immunochromatography, was performed according to the manufacturer's instructions. The test detects the antigen of FeLV (p27) and antibodies against FIV (p24) in the plasma of infected cats. Each test kit has two specimen wells to allow for simultaneous testing for the antigen and antibodies of FeLV and FIV respectively, using the same plasma sample. The sensitivity and specificity of test kit for FeLV antigen detection are 97 and 99% respectively, whereas sensitivity and specificity for FIV antibody detection are 98.5 and 99.7% respectively.

### *Statistical analysis*

The number of cats sampled were calculated according to each risk factor and recorded as a ratio and percentage. Results of cats that were serologically positive for FIV and FeLV were recorded in ratio and percentage. The associated risk factors for FIV or FeLV were recorded and compared using Pearson Chi-square ($\chi^2$) analysis for each sample with 95% confidence interval (95% C.I) using SPSS software 16.0 (SPSS software Inc. Chicago Illinois). Each risk factor calculated using odd ratios instead of relative risk as the study was obtained by the cross-sectional method. Association was considered statistically significant when $P<0.05$ (Zar, 1999).

## Results and Discussion

From this study, FIV infection in cats was more prevalent (21.5%) compared with FeLV infection (7.69%) or dual infections of FeLV and FIV (0.01%). The prevalence of FIV was higher than that of FeLV, and this observation is similar that reported earlier (Cheng, 1990). Although the overall FIV prevalence rate

(21.4%) in this study is higher than FeLV or dual infection prevalence rates, it is still lower compared to the previous report. Previous prevalence rates of FIV, based on samples from cats in Selangor, were 33.1 (personal communication), 24.7 and 27% respectively (Bande *et al*., 2009; Cheng, 1990). The lower prevalence rate may be explained by the differences in geographical areas where these studies were conducted. However, the results from this study may be a true reflection of the prevalence of these diseases in cats in Malaysia.

In this study, sick cats were shown to be significantly associated with FeLV infection (P = 0.016). The clinical signs observed in these FeLV-positive cats were dullness and depression, anorexia, weight loss, gingivitis and stomatitis. This is consistent with the findings of Dunham and Graham (2008) who reported that cats infected with FeLV will develop immunosuppression before dying of other diseases. They suggested that the immunosuppression in the affected cats is believed to be caused by protein p15e.

The lifestyle of cats is significantly associated with FIV infection. Semi-roamers seem to have the highest prevalence. However, in this study, only one of 20 outdoor cats was positive for FIV. This cat was an intact male, which was sick at the point of testing. This finding suggests that intact and sick cats are more likely to be FIV-positive (Pedersen *et al*., 1987). Thus the current recommendation by the American Association of Feline Practitioners (Levy *et al*., 2008) and European Advisory Board on cat Diseases (ABCD) is that neutering should be adopted to serve as a control measure for FIV infection (Hosie*et al*., 2009). This should also serve to reduce roaming and aggressive behaviours more often seen in intact cats.

None of the cats sampled in this study were ever vaccinated against FeLV or FIV. In Malaysia the core vaccines currently recommended for catsfor feline panleukopenia (FPL), feline herpesvirus, and feline calicivirus. The FeLV vaccine is a non-core vaccine recommended based on risk assessment while the FIV vaccine is not recommended due to firstly, the lack of information on the FIV clade type present in Malaysia and secondly, the questionable cross-protection for the different clades rendered by the FIV vaccine.This is surprising because although FeLV vaccination is recommended for high risk cats, only one of eight clinics that participated in this study had FeLV vaccine in stock. None of the participating clinics practiced vaccination against FIV.

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# Field Evaluation of Ivermectin and Mebendazole Treatment against Gastrointestinal Parasites in Stable Horses

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## Abstract

Deworming is one of the routine healthcare in equine management especially for stable horses. Even healthy horses harbour some adult worms and eggs and the incidence of clinical and sub-clinical diseases of horses can be minimized through controlling the gastrointestinal parasites. It was the objective of this study to determine the prevalence and species of gastrointestinal parasites in stable horses in Malaysia. In this study efficacy of ivermectin and mebendazole in reducing faecal egg count (FEC) under field condition was also evaluated. Ninety-four male and female horses of mixed breed, 11.8 ± 4.36 years of age from two stables were selected in this study. Fresh faecal samples were collected at pretreatment period for initial screening and these data were used to determine the prevalence rate. Fifty more than two-year old horses with positive epg from both stables were selected for field evaluation of mebendazole and ivermectin. Commercially available mebendazole and ivermectin paste were administered orallyaccording to manufacturer's recommended dosage, in which the pre-treatment and day 10 post–treatment epg was utilized to determine the percentage of FEC reduction. Of the 94 fecal samples, 51 (54.3%) were positive and 3(3.2%) had fecal egg count of more than 2000 epg. There are significant association ($p<0.05$) between the age groups, sex and breeds. *Strongyloides westeri* and cyathostome were the most prevalence worm cultured. Ivermectin showed 100% reduction while mebendazole only showed 65% reduction in FEC. This study revealed that in these stables, there is low prevalence of gastrointestinal parasites. However nine species of worms were identified. The study also showed that ivermectin had greater efficacy than mebendazole in reducing FEC.

**Keywords**: Gastrointestinal parasites, horses, ivermectin, mebendazole, helminth species, anthelmintics

## Introduction

Horses are susceptible to a variety of gastrointestinal parasites. It is well-recognized that a relationship exists between parasitic infestations of horses and the clinical signs such as spasmodic colic. General control of parasite infestation in horse care involves paddock management and chemical control using anthelmintics, fly repellents or insecticides. Deworming is one of the routine healthcares in equine management especially for stable horses. The control of parasitic helminths in domestic animals rely largely on the use of anthelmintic drugs. There are five main groups of anthelmintics namely; piperazine, benzimidazoles, imidathiazoles, salicylanilides and avermectin.

Horse can harbor approximately 40 different species of parasites, mainly gastrointestinal parasites. Three of the main helminths are the large strongyles, small strongyles and *Strongyloides westeri* (Yazwinski, 2003; Chuah, 1987). The large strongyle, *S. vulgaris*, can cause severe damage to the anterior mesenteric artery and its branches. This will results in aneurysms, emboli and thrombi to body organs. Small strongyles (cyathostomes) will create less harmful reactions. Inflammation (catarrhal, haemorrhagic or fibrinous) of the ventral and dorsal colon may cause intestinal ulcers and possible perforation of the intestinal wall. This study was conducted to determine the prevalence of gastrointestinal parasites in stable horses and to evaluate the efficacy of ivermectin and mebendazole in reducing faecal egg count.

## Materials and Methods

### *Horses*

This study was conducted on horses from two stables in Selangor, Malaysia. The age of the horses in these stables ranged from 2 months to 21 years.

### *Sample*

Initial screening of faecal samples was done by using quantitative analysis.All positive samples (94) were used for faecal culture. Samples from 50 horses aged more than 2 years with positive faecal egg count were selected. These samples used for the faecal egg count reduction test (FECRT).

### *Faecal Egg Count*

The number of strongyle eggs per gram of faeces (epg) was determined by the modified McMaster technique, with the lowest detection level of 100 epg. Two grams of faecal samples were weighed in a 100 mL beaker using triple beam balance. Saturated sodium chloride (NaCl) with specific gravity of 1.2 was added to the beaker until the volume reached 60 mL. The solutions were mixed

thoroughly and filtered through plastic tea sieve into a beaker. The residues in the sieve were discarded. Using a pipette, the filtrates were stirred and just enough of the filtrates were pipetted into the counting chambers of the McMaster slide. The filtrates were stirred again and pipetted into the second counting chamber. The slides were allowed to stand for 10 sec and visualized under light microscopy and eggs counted. The calculation of epg was by the following formula:

$$\text{Ova/g faeces} = \frac{\text{Vol. of NaCl soln (60 mL)}}{2} \text{ X } \frac{\text{No. of ova}}{\text{Wt of faeces}}$$

***Faecal Culture***

Two medium size culture jars was ¼-filled with pooled positive pre-treatment samples and water was added to obtain a moist and crumbly consistency. The feces were loosely compacted, the jar sealed with moisten gauze, and incubated at room temperature (27 - 30°C) in the shade for 7-10 days. The third stage larvae were then collected by means of the Baermann procedure and identified. The gauze was removed and the jar was filled with lukewarm (29- 34°C) distilled water until a meniscus is formed. A petri dish was placed on the meniscus over the jar's mouth. The petri dish and jar was then inverted and filled with more distilled water (5-10 mL) allowed to stand for 30 min. The water containing the migrated larvae was then transferred into a centrifuge tube and allowed to sediment. The supernatant was discarded and the deposit stained with Lugol's iodine and examined for larvae under compound microscope. Larvae identification was made based gross morphology, number and shape of the intestinal cells (Bowman 2003; Zajacand conboy, 2006).

***Treatment with anthelmintic***

All horses were not treated with antihelminthics during the previous five months. Fifty horses were treated with anthelmintic as follows; Stable A (24 horses) treated with ivermectin (Bimectin®) at 0.2 mg/kg body weight; Stable B(26 horses) treated with mebendazole (Telmin Paste®) at 8.8 mg/kg body weight.

***Fecal Egg Count Reduction Test***

The FECRT was performed the 50 horses treated with anthelmintic. The percentage reduction in FEC was determined based on FEC before and after anthelmintic treatments, using the following formula:

$$\%\text{FECRT} = \frac{\text{Pre-treatment FEC} - \text{Post-treatment FEC}}{\text{Pretreament FEC}} \text{ X } 100$$

### *Statistical analysis*

Chi Square test was used to determine the significant association between the prevalence with the breeds, sex and age groups at 95% confidence level. Percentage of FEC reduction calculations were made based on arithmetic means of individual animals and compared with the parametric statistical analysis using independent *t*-test at 95% confidence interval. All statistical analysis was performed using SPSS 16.0 for Windows.

## Results

### *Prevalence of Gastrointestinal Parasites*

The modified McMaster Faecal Egg Count showed that 51 samples (54.3%) were positive. Among the four age groups, horses from age group 6-14 years showed the highest prevalence of gastrointestinal parasitism, while horses aged <2 years showed the lowest prevalence (Figure 1). Between sexes, male horses showed higher faecal egg count than females (Figure 2).

### *Identification of $L_3$ stage larvae*

Faecal culture revealed the prevalence of 9 types of worm species in these stables (Figure 3 and Plates 1 to 5). The species identification of these larvae was made based on observation of the gross morphology of the larvae and the number and shape of the intestinal cells as previously described (Bowman, 2003; Zajac and Conboy, 2006).

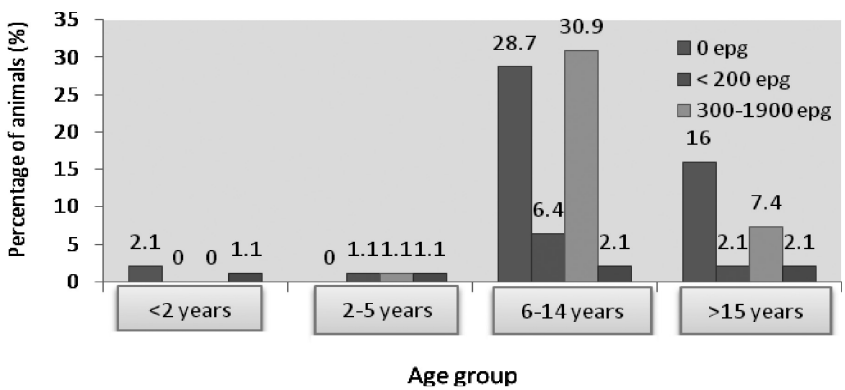


**Figure 1.** Faecal egg count of horses in different age groups

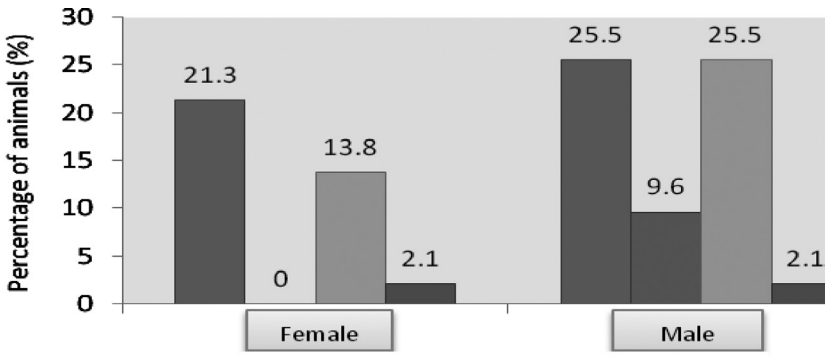


**Figure 2.** Faecal egg count in male and female horses

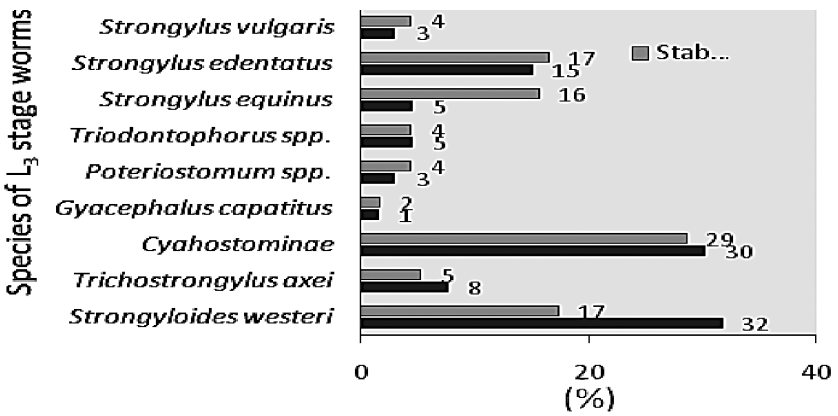


**Figure 3.** Prevalence of infective stage larvae in Stable A and B based on species

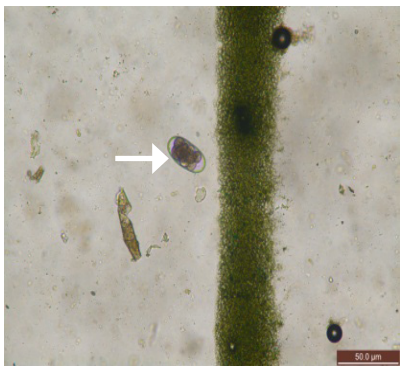

**Plate 1.** Morulated strongyle egg (arrow)

**Plate 2.** Larvated strongyle egg (arrow)

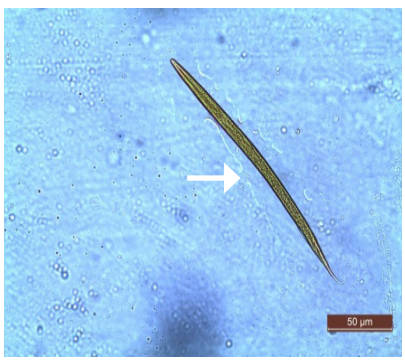

**Plate 3.** Infective third-stage larva *Strongyloides westeri* (arrow)

**Plate 4.** Infective third-stage larva of Cyathostominae (arrow)

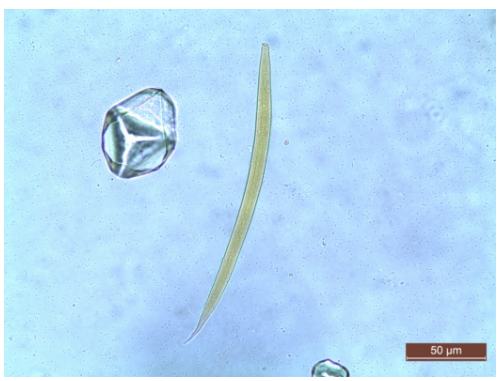

**Plate 5.** Infective third-stage larva of *Trichostrongylus axei* (arrow)

## Faecal Egg Count Reduction Test

The results from FECRT is presented in Table 1.

Horses that received ivermectin had zero epg value when examined 10 days after deworming, suggesting the efficacy of the drug was 100%, mebendazole had a significantly lower efficacy (65%) than ivermectin.

**Table 1.** Effect of anthelmintic treatment on faecal egg count in horses

| Stable | Anthelmentic | n | mean pretreatment FEC (epg) | mean posttreatment FEC (epg) | FECR (%) |
|---|---|---|---|---|---|
| A | ivermectin | 24 | 413 | 0 | 100 |
| B | mebendazole | 26 | 1246 | 512 | 65 |

FEC = Faecal egg count; epg = eggs/g faeces; FECR = Faecal egg count reduction
The FEC and %FECR values represent the mean at day 10 posttreatment

## Discussion

Very few studies on the occurrence of gastrointestinal parasites in horses have been carried out in Malaysia. One study reported the prevalence of gastrointestinal parasites in local indigenous ponies in Kelantan was 43.97 % (Mimi, 1999). The current study in two stables showed prevalence of gastrointestinal parasites was higher in horses that had been deworming during the five month period prior to the study. However, none of these animals showed overt clinical signs, even when the FECs were high. It was also shown that there is significant association (C.I= 95%) between FEC and age groups and sex. There were 4 different breeds of horses sampled from both stables. Both stables in this study practiced deworming program for every 6 months. The management of these stables in term of hygiene, feeding, healthcare and preventive medicines is similar with proper stabling and frequent manure disposal. Young horses are more susceptible to gastrointestinal parasites than older horses and they often show high FEC prior to immunity development. It seems that the immune response is slow to develop and incomplete in most horses, regardless of the age. Thus horses tend to harbour a significant population of intestinal parasites. There was significant association between the prevalence with sex. Physiological status such as gestation and nursing period may also play a role in suppressing immune response towards the worms in mares.

Faecal cultures from each stable revealed that the two most prevalence species of gastrointestinal nematodes in horses were Cyathostominae and *Strongyloides westeri*. Generally, the majority of the gastrointestinal parasitism is dominated by small strongyle (Cyathostominae) over *Strongylus vulgaris, Strongylus equines* or

*Strongylus edentatus*.These findings were similar with other studies (Costa *et al*., 1998; Mimi, 1999;Chuah1987).

There are over 40 species of small strongyles with maturation periods ranging from six to twelve weeks, although it may extend to several years if their development is inhibited or arrested at $L_3$ stage. Consequently, in anthelmintic studies, it is preferably to use horses aged more than two years, so that the efficacy against the slower maturing small strongyles can be also determined. In this study, 100% reduction in FEC in horses at day 10 after treatment with oral ivermectin paste. However, when mebendazole was used, the FEC was only 65 % suggesting that oral ivermectin is more efficacious than mebendazole in two-year or older horses in the control of gastrointestinal parasitism.

## Conclusion

The prevalence rate of gastrointestinal parasites in horses from the two stables surveyed was very low (3.2%). In these stables have Cyathostominae spp. was most prevalence helminth. Ivermectin mebendazole is more effective in reducing FEC in stabled horses.

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# Prevalence of Noninfectious Respiratory Disease in Thoroughbred Racehorses

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## Abstract

The objective of the study was to identify the most common noninfectious respiratory disease in Thoroughbred racehorses and to determine effect on their performance. One hundred and thirty randomly selected records of Thoroughbred racehorses at the Perak Turf Club, Ipoh, Perak, Malaysia diagnosed with noninfectious respiratory disease and with complaints of poor athletic performance accompanied by coughing, exercise intolerance and abnormal respiratory noises were obtained. Among the data recorded and analysed were surgery and racing performance records, which were used to determine the most prevalent noninfectious respiratory disease in the Thoroughbred racehorses. The result showed that the most common noninfectious respiratory disease in Thoroughbred racehorses is exercise-induced pulmonary haemorrhage (EIPH) (49.2%), recurrent laryngeal neuropathy (28.2%), respiratory allergy (10%), epiglottic entrapment (EE) (7.7%), displacement of the soft palate (DDSP) (3.8%) and subepiglottic cysts (0.8%). The study also showed that surgical correction for recurrent laryngeal neuropathy (RLN) (Grade IV and Grade V), epiglottic entrapment, dorsal displacement of soft palate (Persistent) and subepiglottic cysts (SEC)gave good resolution and good prognosis for recovery. Thus surgical treatments of Thoroughbred racehorses with noninfectious respiratory disease can improve their athletic performance.

**Keyword:** Exercise Induced Pulmonary Haemorrhage (EIPH), Recurrent Laryngeal Neuropathy (RLN), Respiratory Allergy, Epiglottic Entrapment (EE), Dorsal Displacement of the Soft Palate (DDSP) and Subepiglottic Cysts (SEC), upper respiratory disease, surgical treatment and racing performance

## Introduction

Respiratory diseases are common in racehorses in training and an important cause of economic loss in the racehorse industry. Respiratory diseases can be categorised into infectious respiratory disease and noninfectious respiratory disease, both of which cause poor performance and this is a serious economic concern to the horse-racing industry worldwide (Rossdale *et al*., 1985). Noninfectious respiratory diseases that usually occur in racehorses in Malaysia are exercise-induced pulmonary haemorrhage (EIPH), recurrent laryngeal neuropathy, respiratory allergy due to environmental factors, epiglottic entrapment (EE), persistent dorsal displacement of the soft palate (DDSP), arytenoid chondritis (AC) and subepiglottic cysts (SEC). Respiratory problems are generally diagnosed by endoscopy, which involves the use of a flexible fiber optic or video endoscope to visualize the pharynx, larynx, trachea and bronchiols. Diagnosis of respiratory disease may be difficult without help of standard flexible endoscope because these diseases have similarities in clinical signs such as coughing, nasal discharge, fever, anorexia, depression, inspiratory and or expiratory noises and poor performance. Thus this study was conducted to determine the common noninfectious respiratory diseases in Thoroughbred racehorses and to evaluate their performance after treatment.

## Materials and Methods

### *Selections of horses and location*

The study involved 130 randomly selected records of Thoroughbred race horses diagnosed with noninfectious respiratory disease. These horses were recorded with a complaint of poor athletic performance accompanied by coughing, exercise intolerance and abnormal respiratory noises. Endoscopic examination was used for diagnosis and to view and determine the abnormalities of the upper respiratory tract.

### *Record keeping*

Individual records of each racehorse with information on identification, signalment, clinical signs, diagnosis of noninfectious respiratory disease, treatment and vaccination status were retrieved. Racing performance records after treatment for each selected horse were accessed for reference from the Singapore Turf Club website.

### *Data Collection*

Data was collected from individual records of horses presented with history of poor performance and diagnosed with noninfectious respiratory disease. Racing performance data was evaluated and categorised in separate evaluation forms. The

data was analysed to determine the prevalence of each noninfectious respiratory disease in Thoroughbred racehorses and to identify the most common noninfectious respiratory disease in this population. This study also assessed the prognosis of the disease condition, performance of horses with respiratory diseases before and after treatment and corrective surgery.

### *Grading*

*Exercise-induced pulmonary hemorrhage*

Grading of exercise-induced pulmonary haemorrhage (EIPH) was made through, tracheobronchoscopic examination of horses 30 to 120 min after racing or strenuous exercise. Tracheobronchoscopic assessment of severity of EIPH using a 0-4 grading scale was according to the method previously described (Hinchcliff *et al*., 2004) as follows: Grade 0 - no blood detected in pharynx, larynx, trachea or main-stem bronchi; Grade 1 - presence of stream of blood in the trachea or main-stem bronchi visible from the tracheal bifurcation. The presence of blood at less than one quarter the length of the trachea (<10% of the trachea surface area); Grade 2 - a long stream of blood (more than half the length of the trachea) or greater than 2 short streams occupying less than one-third of the tracheal circumference; Grade 3 - presence of multiple, distinct streams of blood covering more than one-third of the tracheal circumference without blood pooling at the thoracic inlet; Grade 4 - multiple, coalescing streams of blood covering more than 90% of the trachea surface with pooling of blood at the thoracic inlet.

*Larygeal Function*

The laryngeal function was determined on standing and unsedated horses. The grading system for laryngeal is according to that described earlier (Lane, 1993). There are 5 Grades of recurrent laryngeal neuropathy (RLN) as follows; Grade 1 - normal synchronous movement and full abduction of left and right arytenoids cartilage; Grade 2 - asynchronous abduction of left and right sides, but full abduction achieved and maintained; Grade 3 - slight asymmetry at rest, although full abduction could be achieved by the left arytenoids but not maintained; Grade 4 - obvious asymmetry at rest, with some movement of left arytenoids cartilage and failure to achieve or maintain full abduction; Grade 5 - symmetry left arytenoids cartilage resting on or near midline and without movement of left arytenoids cartilage.

## Results

The prevalence of noninfectious respiratory disease in Thoroughbred racehorses is shown in Table 1. Exercise-induced pulmonary haemorrhage has the highest prevalence, which is followed in order by RLN, RA, EE, DDSP and SEC

**Table 1.** Prevalence of noninfectious respiratory disease in Thoroughbred race horses

| Disease | No. of Cases | Prevalence (%) |
|---|---|---|
| Exercise Induced Pulmonary Haemorrhage | 64 | 49.2 |
| Recurrent Laryngeal Neuropathy | 37 | 28.5 |
| Respiratory Allergy | 13 | 10 |
| Epiglottic Entrapment | 10 | 7.7 |
| Dorsal Displacement of Soft Palate | 5 | 3.8 |
| Subepiglottic Cyst | 1 | 0.8 |
| Total | 130 | 100 |

**Table 2.** Frequency of respiratory disorders in racehorses

| Horse | Frequency n (%) | | | | |
|---|---|---|---|---|---|
| | EIPH | RLN | RA | EE | DDSP |
| Gelding | 56 (87.5%) | 28(96.6) | 13(100) | 10(100) | 5(100) |
| Stallion | 6 (9.4) | 1(3.4) | 0(0) | 0(0) | 0(0) |
| Mare | 2 (3.2) | 0(0) | 0(0) | 0(0) | 0(0) |
| Total | 64 (100) | 29(100) | 13(100) | 10(100) | 5(100) |

The numbers are mean values
EIPH = exercise-induced pulmonary hemorrhage; RLN = recurrent laryngeal neuropathy;
RA = Respiratory allergy; EE = epiglottic entrapment; DDSP = dorsal displacement of soft palate

**Table 3.** Effect of treatment of noninfectious respiratory disease on performance of Thoroughbred racehorses

| Disease | Number of cases | Improved Performance | | Reduced Performance | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| EIPH | 64 | 41 | 64 | 23 | 36 |
| RLN | 37 | 36 | 97.3 | 1 | 27 |
| RA | 13 | 10 | 76.9 | 3 | 23.1 |
| EE | 10 | 8 | 80 | 2 | 20 |
| DDSP | 5 | 4 | 80 | 1 | 20 |
| SEC | 1 | 1 | 100 | 0 | 0 |

The numbers are mean values
EIPH = exercise-induced pulmonary hemorrhage; RLN = recurrent laryngeal neuropathy; RA = Respiratory allergy; EE = epiglottic entrapment; DDSP = dorsal displacement of soft palate;SEC = subepiglottic cyst

The prevalence of EIPH is highest in gelding followed in order by the stallion and mare (Table 2). The severity of EIPH is shown by 64.1% cases with Grades 1, 2 and 3 and 35.9% with grade 4. For RLN, the highest frequency of EIPH was in geldings followed by the stallions. From the analysis, all the recorded data for RLN were either grade 4 (69%) and grade 5 (31%), which requires surgical correction. There is no record for grades 1, 2 and 3 RLN and the performances of horses with these grades were not affected.

The study showed that RA, EE and DDSP were only observed in the geldings.

Horses with EIPH showed good performance after undergoing medical treatment (Table 3). Usually these horses were categorised as grades 1, 2 or 3 after a first episode of EIPH. After treatment and 3 month rest, 31.3% returned to original performance when raced. The severity of the condition worsened with in grade 3 and 4 after a second episode of EIPH. Only 4.7% of these horses had grave prognosis and died immediately upon completion of the race.

Most of the horses with RLN and that underwent corrective surgery returned to racing with good performance. This suggests that corrective surgery is effective in assisting the affected horse to return to the previous level of performance. However, a small percentage of these horses did not return to original performance level after treatment.

The majority of horses affected with respiratory allergy had good prognoses after undergoing medical treatment. The record showed about 23% of these horses produced reduced performance after treatment.

In most horses with DDSP and EE, surgical treatments had provided good prognoses for return to level of performance before they were affected by the diseases.

## Discussion

This study was conducted to determine the most common noninfectious respiratory diseases and the prevalence of each noninfectious respiratory disease in racehorses.

The study suggests that EIPH is the most common noninfectious respiratory disease in racehorses and the frequency of the disorder increased with age. Grade 4 EIPH was the most frequent occurrence. Among 64 horses with EIPH, three died on track after finishing the race.

Treatment and management of horses with EIPH at the first bleeding episode is by resting the horse for 3 months. With second bleeding episode the horse should be rested for 6 months and barred from racing if bleeding occur a third time. The management for EIPH patients include avoiding fast work or racing during bad haze, avoiding racing on hard tracks, placing feed and water at ground level for good lung drainage, dampening dusty feed and bedding, fresh air, providing good ventilation in the stable or open stable with fresh air, antibiotics to prevent secondary bacterial infection, nebulisation therapy, bronchodilators to improve lung airflow, antioxidant, omega 3 and corticosteroids to control the inflammation. Repair of lung tissue may be assisted with glucosamine and anabolic steroids and a reduction in blood pressure with diuretics such as furosemide. The prognosis for EIPH is guarded because of the progressive nature of the disease. Since the etiological factor is unknown, it is very difficult to prevent EIPH in racehorses.

Recurrent laryngeal Neuropathy is the second most common noninfectious respiratory disease in the racehorses at the Perak Turf Club. The condition is associated with abnormal stridor, abnormal vocalization, exercise intolerance and poor athletic performance. It has a high frequency of occurrence among geldings. This disease can be diagnosed by endoscopic examination of the larynx at rest, immediately after cessation of exercise. According to the records, corrective surgeries such as ventriculocordectomy, laryngoplasty and laryngeal prothesis are the current standard surgical treatments. Older horses tolerate and respond better to surgery than young horses (Holcome, 2001). Since the etiological agent is idiopathic, it is hard to prevent this disease. The most commonly used surgical technique to treat RLN is a laryngeal prosthesis. The procedure, significantly improves upper airway flow mechanics in RLN-affected horses and many horses have raced successfully after surgery (Parente, 2004). Most of the horse showed improved performance after corrective treatment.

In Thoroughbred racehorses, the prevalence of RA was low. Specific causes of respiratory allergy is poorly defined, but environmental factors to include a variety

of etiological agents such as dust, noxious gases (ammonia), microorganisms, mite debris, aerosolised allergens and endotoxin from hay and bedding had been implicated. Respiratory allergy usually affects horses above 7-years old, but with no apparent breed or sex predilection. In this study the horses affected by RA were adults and geldings. Systemic corticosteroids are effective for treatment of RA (Couëtil *et al*, 2007). Other drugs may be prescribed such as bronchodilators, mucolytics and antihistamines. Improvement of mucociliary clearance may also help reduce airway obstruction. Prevention strategies such as reduced environmental irritant to airways and improve ventilation in the stable to increase removal of airborne particles and noxious gases is beneficial.

The best method to diagnose DDSP is after strenuous exercise or while the horse is exercising on a treadmill. Only a small percentage of the affected horses in this study showed DDSP. Surgical correction such as staphylectomy may produce good prognosis. The prognoses after surgical correction by sternothyrohyoid myectomy is moderate and the horses may return to racing with good performance (Hinchcliff, 2004). Other treatments that may be instituted include systemic and topical anti-inflammatory medication with glycerin, DMSO and nitrofurazone (Auer and Stick, 2006). The horse need to be stable-rested for 4 weeks after surgical treatment and medical treatment with antibiotic and analgesic.

Epiglottic entrapment seemed to occur in geldings only and that too at low prevalence. The clinical signs that are usually observed in EE are abnormal respiratory noise during exercise. However coughing or dysphagia is rare. Diagnostic confirmation for EE is best made by endoscopic examination. There are several surgical approaches for the treatment of EE to include laryngotomy, transoral or transnasal axial division with hooked bistoury and transendoscopic division with a laser. The prognosis after surgery is usually good.

From the study, SEC is a rare occurrence in Thoroughbred race horses. In fact SEC among noninfectious respiratory diseases is among the lowest in prevalence. The etiological agent for SEC is unknown. Subepiglottic cysts can be treated by surgical removal of the cysts through a laryngotomy incision with the horse in dorsal recumbency under general anesthesia. The prognosis for return to function and resolution of coughing after surgical treatment is good to excellent (Holcome, 2001).

## Conclusion

It can be concluded that the most common noninfectious respiratory disease in Thoroughbred racehorses is EIPH followed in order by RLN, RA, EE DDSP and SEC. The noninfectious respiratory diseases commonly associated with anatomical and physiological abnormalities. Besides history, clinical signs and physical examination, endoscopic examination is the best method to visualize

the morphology and to diagnose noninfectious respiratory diseases. Surgical treatments seem to be effective for the disease and the horses may return to racing with good or improved performance.

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# Detection of Glasser's Disease in Clinical Samples using Polymerase Chain Reaction

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## Abstract

Glasser's Disease is one of the porcine common respiratory problems in our country. Glasser's disease is caused by *Haemophilus parasuis* (HP). The HP has a wide range of antibiotics sensitivity and can be treated during early infection; therefore early diagnosis is very important. In the past, diagnosis was based on history, clinical signs and postmortem lesions, then confirm by bacteria isolation and identification. These traditional microbiology methods are time-consuming and labourious. Polymerase chain reaction (PCR) is one of the methods that provide rapid and accurate diagnosis. The procedure can be completed within 1 to 2 days and only require one trained personal to perform.Currently, there is limited publish articles on PCR test for the diagnosis of Glasser's Disease in Malaysia. For the analysis 2 pairs of primers set for HP were selected; HPS1-forward (5' AGT ATG AGG AAG GGT GGT GT 3') and HPS1-reverse (5' CGT TTC GTC ACC CTC TGT GT 3') and HPS2-forward (5' TAG AAA AAA TCT TTA ATT G 3') and HPS2-reverse (5' CAC CAT AGA AAC TTC TTT TC 3'). Lung tissues, pericardial swabs and thoracic swabs samples were collected from farms in Sepang, Selangor, Malaysia. The PCR test was carried after some modifications and optimisation. Both HP primers able to detect positive clinical lung samples and can be further developed as PCR diagnostic tool in our country.

**Keywords**: Glasser's Disease, Polymerase Chain Reaction

## Introduction

*H. parasuis* infection is costly to the pig industry because of it causes high mortality (Maclnnes *et al*., 2008). The most characteristic clinical signs of the infection are fibrinous polyserositis, polyarthritis and meningitis. The affected pigs are the 4 to 6 week age group in the nursery, and they die at 2 days post-infection with sign

of acute septicaemia (Rapp and Gabrielson, 1992).The problem of *H. parasuis* infection affects many countries including China, Japan, USA, Germany, Spain and Australia. Therefore, there is a need for correct diagnosis of the disease to reduce losses through the application of appropriate control measures (Oliveira and Pijoan, 2004). Early diagnosis of the disease is important because the causative agents are sensitive to a wide range of antibiotics. If treatment is given at early stage, the disease is curable. The conventional method of diagnosis of this disease is isolation of the causative agent, but this has been always difficult time-consuming because of the fastidious nature of the organisms. Polymerase chain reaction offers and advantage over the convention methods because of its rapidity and accuracy in the diagnosis diseases. In this study, the main objective was to determine suitable PCR primers set for the detection of HP in swine samples from farms in Malaysia.

## Materials and Methods

### *Clinical samples*

A total of nine lung samples were collected from postmortem piglets aged from 5 to 10 weeks old. One clinically healthy pig with no abnormal lung lesion was sacrificed as negative control. Lung tissues, pericardial and thoracic swabs samples were collected from farms in Sepang, Selangor, Malaysia. Two grams of lung tissues, which were obtained from naturally infected pigs were macerated with scissors then grinded by using mortar and liquid nitrogen. Then, 25 mg of ground tissues were transferred into 1.5 mL tube with spatula. The tissue samples were process according to manufacturer protocol (i-genome CTB DNA Extraction Mini Kit, Intron Biotechnology).

### *PCR Primers*

Two set of primers were selected for this study from published articles. The first set of primers chosen is HPS1-forward (5' AGT ATG AGG AAG GGT GGT GT 3') and HPS1-reverse (5' CGT TTC GTC ACC CTC TGT GT 3'), by Oliveira *et al*. (2001).This primer set is widely used. The predicted PCR product using this primer set is 821bp. This set primer targets *H. parasuis* species-specific regions 16S small subunit ribosomal RNA gene sequence. The second set of primers is HPS2-forward (5' TAG AAA AAA TCT TTA ATT G 3') and HPS2-reverse (5' CAC CAT AGA AAC TTC TTT TC 3') (Lu *et al*., 2010). These primers target the ompA gene of the outer membrane of HP. The predicted PCR product using this primer is 1104bp in size. This is the latest primers set, which is created for genotyping purpose.

### *PCR Conditions*

The PCR was carried out using the manufacturer commercial kit (i-Taq ™ DNA Polymerase, Intron). For the both set of primers, 25 $\mu$L reaction mixture containing 1 $\mu$L of extracted DNA templates, 0.5 $\mu$L of each 20 $\mu$M of primer, 0.5 $\mu$L of i Polymerase (5 U/$\mu$L), 2.5 $\mu$L 10x PCR buffer, 2.5 $\mu$LdNTP mixture (2.5 mM each) and 17.5 $\mu$L of double distilled water. The PCR was performed for 35 cycles consisting of denaturation for 1 min at 94°C, annealing for 1 min at 59°C, and extension for 1 min at 72°C using a thermal cycle (Swift maxi®, ESCO), lastly final extension for 5 min at 72°C.

### *Electrophoresis and Imaging*

Agarose gel (1.5%) was made by mixing 1.5 g of agarose gel powder (Agarose D-1, Low Electeoendomosis, Pronadisa, CONDA) in a 100 mL bijour bottle with 1% Tris-acetate-EDTA (TAE) to 100 mL. For primers HPS2, 2% agarose gel is used. Agarose gel is placed in the gel holder tank and submerged with 1% TAE buffer. Seven microlitres of 100bp marker (100 bp BLUE extender DNA ladder, Bioron) is loaded into the first well, while for HPS2, 1 kbp marker was used (1k bp ready-to-use ladder, Bioron). Two microliters loading dye (6x DNA loading dye, Fermentas) were mixed well with 8 $\mu$L of PCR product. The PCR products were run for 1 h at 66V. Subsequently, the gel was stained with ethidium bromide (BOP BASIC) solution for 30 min. The gel was placed under a UV gel imaging capturing machine (U: Genius, Syngene) to visualize the desire band size. The images were captured and recorded.

## Results

With the two primers of HPS1-forward and HPS1-reverse, amplification of 16s rRNA *H. parasuis* species-specific gene sequence from tissue sample (Ts)1, 3, 5 and 8 produced a band at 821 bp (Figure 1).Ts 1 and 3 showed more prominent bands while bands of Ts 5 and 7 were slightly degraded.

The HPS2 primers set were designed to amplify o*mpA* gene of *H. parasuis* reference strains with specific fragment at about 1104 bp. The PCR results of HPS2 revealed that only Ts 1 and 3 were positive for *H. parasuis* (Figure 2), with Ts 1 showing prominent band and Ts 3 showing weak band.

For comparison, Ts 1, 3, 5 and 8 were amplified with HPS1 and HPS2, and then loaded into one gel. From the results, HPS2 was only able to detect 50% of the positive samples detected by the HPS1 primer set (Figure 3).

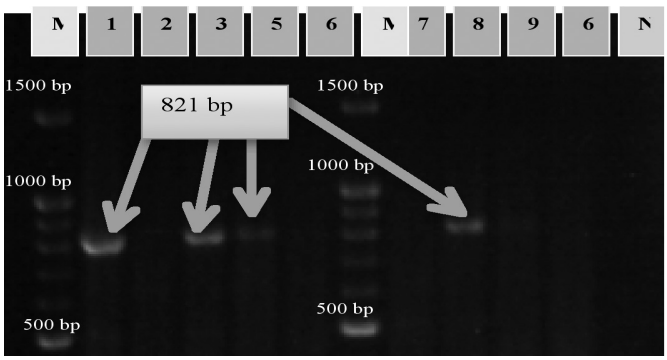


**Figure 1.** The PCR results of HPS1 from all lung tissue samples. M, 100 bp molecular marker. N=negative control with double distilled water.

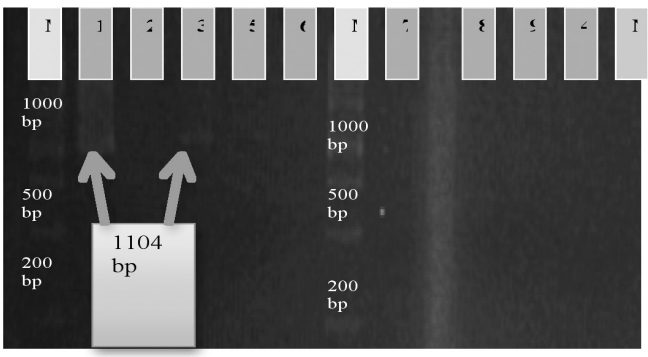


**Figure 2.** The PCR results of HPS2 from all lung tissue samples. M, 100 bp molecular marker. N= negative control with double distilled water.

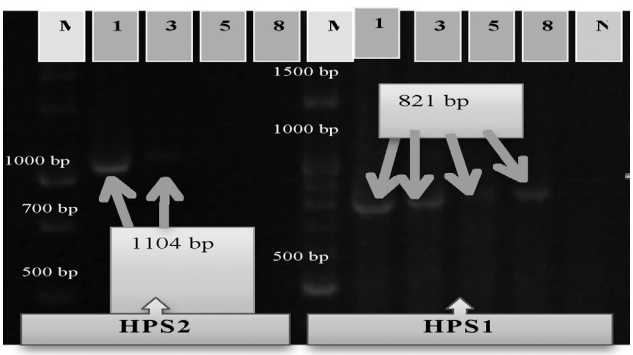


**Figure 3.** Ts 1, 3, 5 & 8 were amplified by HPS2 (lanes 2-5) and HPS1 (lanes 7-10). M for HPS2, 1k bp molecular marker.M for HPS1, 100 bp molecular marker. N= negative control with double distilled water.

## Discussion

According to Oliveira *et al*. (2001), HPS1 could detect a minimum concentration of 1 x 102 cfu/mL of *H. parasuis* and 0.69 pg of pathogen DNA. This indicates that the HPS1 primer set is sensitive in detecting *H. parsuis* in pure culture and clinical samples. Our study suggests that the HPS2 primer setis less sensitive than the HPS1 primer set in the detection of *H. parasuis* samples. This is shown by the fact that HPS1 could detect Ts 5 and 7 while HPS2 could not. From the results, both HPS1 and HPS2 were able to detect H. parasuis in clinical samples and they did not amplify DNA from healthy pig and negative controls.

## Conclusion

The results suggest that HPS1 and HPS2 primers are both suitable to be used as diagnostic tool in the detection of *H. parasuis* in clinical samples of pigs that show signs and lesions of the infection. The HPS1 primers are more sensitive and useful than the HPS2 primers for screening of Glasser's Disease.

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# Fermentation Kinetics of Some Oil Palm By-Products as Ruminant Feeds

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## Abstract

The ruminant industry in Malaysia is still not self-sufficient where smallholder farmers keep the majority of ruminant livestock. Limited pasture and poor quality forages urge them to find alternative feedstuffs which are cheaper, of satisfactory nutritive value and available throughout the year. Oil palm by-products meet these criteria, however their fermentation kinetics in the rumen need to be evaluated. The *in vitro* fermentation kinetics of selected oil palm by-products, namely the oil palm fronds (OPF), palm kernel cake (PKC) and decanter cake (DC) were evaluated using the *in vitro* gas production technique. The by-products were assessed at inclusion levels of 100% (raw 100% by-product), 15% (15% by-product + 85% concentrate feed, w/w) and 30% (30% by-product + 70% concentrate feed, w/w). The *in vitro* fermentation of the oil palm by-products was carried out in 100 mL sealed syringes with 0.25 g of substrate and 25 mL of rumen fluid-buffer mixture (1:4 v/v), which were incubated at 39°C under anaerobic condition for 48 h. Evaluation of the fermentation kinetics was performed based on the following parameters, namely gas production, rumen pH, protozoal population, volatile fatty acid (VFA) and long chain fatty acid (LCFA) profiles compared with concentrate feed which acted as the control. The decanter cake (DC) at inclusion levels of 15% and 30% yielded similar gas production, rumen pH, VFA, and total unsaturated fatty acid profile as the concentrate feed. However, the 15% and 30% DC significantly increased the total C18:1 *trans* fatty acids ($p<0.05$) compared to the concentrate and the other by-products. It is concluded that the decanter cake showed the greatest potential to be included into ruminant livestock feed which should reduce feed costs, although the increase in the unhealthy *trans* fatty acids must be taken into account.

**Keywords:** Fermentation kinetics, *in vitro* gas production, oil palm by-products, rumen fermentation, rumen protozoa

## Introduction

Oil palm (*Elaeis guineensis* Jacq.) is widely cultivated as a source of vegetable oil in Malaysia, Indonesia and Thailand. The extensive oil palm industry provides an abundant supply of oil palm by-products such as oil palm fronds (OPF), trunks, empty fruit bunches, palm oil mill effluent, palm kernel cake (PKC), decanter cake (DC), and palm press fibre (PPF). Oil palm by-products have the potential to be included as concentrate substitutes in compound feed formulation for ruminants. The major constraint in the Malaysian ruminant industry where most of the ruminant livestock is owned by smallholder farmers is the lack of supply of quality feed throughout the year, especially during peak cropping periods. The aim of this study was to investigate the suitability of OPF, PKC, and DC as ruminant feeds by measuring the *in vitro* fermentation kinetics and their effects on rumen parameters.

## Materials and Methods

An evaluation of the *in vitro* fermentation kinetics of oil palm fronds (OPF), palm kernel cake (PKC), and decanter cake (DC) and concentrate feed as the control was carried out. Each substrate was evaluated as raw (100% by-product), 15% (15% by-product + 85% concentrate feed, w/w) and 30% (30% by-product + 70% concentrate feed, w/w) compared to the control (100% concentrated fed). The *in vitro* fermentations were performed simultaneously in triplicate using a single source of rumen fluid inoculum. Another triplicate with rumen fluid inoculum containing no substrate was incubated in the batch as blanks (negative control). Pooled rumen content were collected from four rumen-fistulated Kacang crossbred goats (45.0±2.0 kg BWt; 11-12 months old) under the same feeding regime (fed twice daily at 09:00 and 17:00 h. with 70 % commercial goat concentrate and 30 % oil palm frond, w/w). Twenty-five millilitres of rumen fluid and bicarbonate-phosphate buffer mixture (1:4 v/v) were introduced along with 0.25g of substrate into a 100 mL air-tight plastic syringe and incubated at 39˚C for 48 hours under anaerobic conditions. The *in vitro* gas production of each feedstuff was measured according to the method of Fievez *et al*. (2005). The rumen liquor pH was measured immediately after the incubation period with a Mettler-Toledo pH meter (Mettler-Toledo Ltd., England). Protozoal counts in the rumen liquor were carried out using a haemocytometer, following the improved Neubauer ruling and identification procedure outlined by Towne *et al*. (1990) and Hungate (1966). The volatile fatty acid profile and the long chain fatty acid profile of the rumen liquor were determined using a 5890 Hewlett-Packard Gas-Liquid Chromatograph (Hewlett-Packard, Avondale, PA) and an Agilent 7890N Gas-Liquid Chromatograph, respectively. All data were analysed by the least-squares means method using the GLM procedures of SAS®. Significantly different means were then further differentiated using the least significant difference (LSD) comparison procedures.

All statistical tests were conducted at 95 % confidence level. Data were analyzed using the model $Yij=\mu+Ti+\varepsilon ij$ where Yij is the observation from treatment i, j, the replication; μ, the overall mean; Ti, the mean of treatment and εij, the residual effect.

## Results and Discussion

### *Gas production*

Significant differences ($p<0.05$) in cumulative gas production were observed between the concentrate (43.00±1.22 mL) and the raw OPF (12.17±0.60 mL), raw PKC (19.83±1.33), raw DC (34.50±1.57 mL), 30% OPF (33.67±2.08 mL) and 30% PKC (36.60±2.25 mL). The 15% OPF, 15% PKC, 15% DC, and 30% DC had similar gas production as the concentrate ($p>0.05$). The results showed that inclusion of the cheaper oil palm by-products as substitutes for the expensive concentrate is highly possible and applicable, without affecting the quality and digestibility of the feed. The DC would be the by-product of choice compared to the PKC since the former can be included up to 30% proportion of the finished feed without affecting significantly the digestibility.

### *Volatile fatty acid (VFA) production*

There is a significant correlation between gas production and VFA production where fermentation end products such as the VFA influence gas production. Differences in total gas production could also be explained by the differences in the total and indiviual VFA produced. The production of acetic and propionic acid for all the treatments was similar. Unlike the PKC and OPF, the inclusion of DC increased ($P<0.05$) the production of butyric acid, which confirmed an earlier report of Hasliza *et al*. (2007) where they fed sheep with different inclusion levels of OPF.

### *Rumen pH*

The high rumen pH, which ranged between 7.01 to 7.20 for all treatments, suggested that the rumen environment was conducive for fermentation by cellulolytic bacteria. There was no significant difference in pH between the by-products and the concentrate (7.09±0.02) except for the raw OPF which yielded a significantly higher rumen pH (7.20±0.01) than the concentrate, and the highest pH among all the treatments. This result was consistent with that of Khamseekhiew *et al*. (2002) who suggested that the high rumen pH was caused by low production of volatile fatty acids due to the low digestibility of OPF. The high fibre fraction of the oil palm by-products could also aid in buffering, thus delaying any decrease in pH (Feng *et al*., 1993).

***Rumen protozoa population***

Two major protozoa, namely the entodinium and holotrichs, were identified. The *in vitro* fermentations of all by-products produced a significantly higher entodinium population than the concentrate, with raw DC showing the highest population. The holotrich population resulting from fermentation of the concentrate was not significantly different with those for either 30% OPF, raw PKC, 30% PKC, and 15% DC. The raw OPF (0.39±0.10 x$10^6$/mL), raw DC (0.53±0.08), 15% OPF (0.40±0.04), 15% PKC (0.37±0.07), and 30% DC (0.41±0.04) showed a significantly higher ($p<0.05$) holotrich population than the concentrate (0.16±0.06). Generally, the results demonstrated that the inclusion of the oil palm by-products significantly increased the protozoal population in the rumen environment. This suggests that the concentrate feed may have a defaunation effect against the rumen protozoa. This result confirmed the earlier findings of Ebrahimi (2009) who reported a significant increase in the the rumen protozoal population in goats fed with increased inclusion levels of OPF.

***Saturated and unsaturated fatty acids***

All the raw by-products at 15% inclusion levels yielded significantly higher total saturated acids (SFA) than the concentrate. The 30% inclusion of PKC yielded a significantly higher SFA but the 30% inclusion of OPF and DC and the concentrate yielded similar amounts of SFA. At 15% inclusion levels, only the OPF (11.47±0.47%) and PKC (13.66±0.26%) yielded significantly lower unsaturated fatty acids (UFA) than the concentrate (16.16±0.48%) while the levels of UFA for the 15% DC and concentrate were similar. At 30% inclusion only the PKC (13.56±0.06 %) yielded significantly lower UFA than the concentrate.

The decanter cake which yielded significantly higher UFA and lower SFA may increase the UFA:SFA ratio in the rumen content resulting in an increased unsaturated fatty content in the ruminant products, such as milk and meat. Hasliza *et al*. (2007) also showed that the inclusion of 30% OPF (w/w/ DM) in complete feed increased the availability of UFA in the rumen. The 30% OPF in this study also produced UFA:SFA ratios similar to the decanter cake. However, the poor nutritive value and digestibility of OPF would compromise its value as a feed ingredient.

***Trans fatty acids***

Both the 15% DC and 30% DC yielded significantly higher total *trans* fatty acids than the other treatments. The levels of total *trans* fatty acids were lowest for the 30% OPF, followed by raw OPF, 15% OPF, raw PKC and 15% PKC, which all had similar production of *trans* fatty acids. The levels of *trans* fatty acids

in the concentrate, 15% OPF, 30% PKC, and raw DC were similar. The results demonstrated that the DC produced an increased production of the unhealthy total *trans* fatty acids which could increase their deposition and content in the ruminant tissues. It has been hypothesized that an increased human consumption of *trans* fatty acids may increase risks of coronary heart diseases, colon cancer, breast cancer, and prostate cancer (Mensink and Katan, 1990; Stender and Dyerberg, 2004)

## Conclusion

Based on the similar gas production, rumen pH and VFA production with the concentrate, and the increased UFA, decreased SFA and hence UFA:SFA ratios compared to the concentrate, the decanter cake at inclusion levels of 15% and 30% showed the greatest potential to be included into ruminant livestock feed which should also reduce feed costs, although the increase in the unhealthy *trans* fatty acids must be taken into account. However, PKC and OPF could also serve as alternative feed supplements to improve ruminal fermentation end products such as an increased protozoal population and modification of rumen pH.

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# Molecular Study of *Babesia* in Canine Blood and Comparison between Conventional and Molecular Diagnostic Methods

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## Abstract

A molecular study was conducted to detect the presence and determine the prevalence of *Babesia* species in stray and pet dogs in Kuala Lumpur using the Polymerase Chain Reaction (PCR) method. Seventy dogs, 35 from pet dogs presented to clinics around Kuala Lumpur and 35 from stray dogs from the Dewan Bandaraya Kuala Lumpur (DBKL) dog pound, were included in this study. Thin blood films were made, stained with Giemsa and examined under a light microscope for the detection of *Babesia* organisms. Two out of 70 dogs (2.8%) were positive for canine *Babesia*. One was identified as *Babesiacanis* positive and the other *Babesiagibsoni* positive. Genus-specific screening PCR was performed on DNA extracted from all 70 samples followed by *Babesia canis*-specific and *Babesia gibsoni*-specific PCR. Nine out of 70 dogs (12.8%) were positive following genus-specific screening PCR but of the 9, only one was positive for *Babesia canis* and one for *Babesia gibsoni*. The two positive samples were the same as those detected using light microscopy. Both of the positive samples were from the stray group. Haematological abnormalities in the two *Babesia* positive dogs included anemia and thrombocytopaenia. The prevalence rate of canine babesiosis was 5.8% for the stray group and 0% for the pet group. The overall prevalence of canine babesiosis in Kuala Lumpur was found to be 2.85%.This is the first molecular study of canine *Babesia* in Malaysia.

**Keywords**: Canine babesiosis, *Babesia canis, Babesia gibsoni*, dogs, thin blood films, polymerase chain reaction

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# Haematological and Blood Biochemistry Profiles of Adult Black and Red Tilapia in Different Habitats

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## Abstract

The objective of this study was to establishthe standard blood values in Tilapia species as reference. This study was conducted on 40 blood samples collected from adult Black and Red Tilapia from three different habitats; cultured fresh water, wild fresh water and brackish water. Blood samples were collected from 20 cultured adult Black and Red Tilapia (10 for each species), ten adult Black Tilapia from brackish water and the remaining 10 were adult Black Tilapia from the monsoon waterway in front of the Aquatic Unit of Faculty of Veterinary Medicine, University Putra Malaysia (UPM).Blood samples were collected from lateral caudal vein located at the lateral line of fish using 22 Gauge needles, 3 mL syringe and lithium heparinised tubes. Blood smear was done to examine leukocyte (WBC) morphology and determine differential count. Haematological analysis was done using an automated haematology analyser. Total manual count for erythrocytes (RBC) and WBC werealso performed to compare results obtained the haematology analyser. Serum were analysed using an automated chemistry analyser for biochemistry parameters. Results showed that blood samples collected from Tilapia in the brackish water habitat had the highest reading in most of the haematological and serum biochemical parameters compared to the other two habitats. Most of the parameters showed significant differences between the habitats ($p<0.05$). For the haematological results, the automated machine showed comparable result to the manual total RBC count,while neither the total WBC count nor WBC differential count was comparable between the two methods. The total WBC count from the machine was higher than the manual count as total number of platelet was also counted as WBC by the machine. The manual WBC differential result was used to calculate the corrected number of the total WBC. Meanwhile, the morphology of the WBC of Tilapia species from different habitats showed no difference. For serum biochemical profiles, most of the parameters showed significant differences between the habitats including total protein, albumin, globulin, creatinine, alkaline

phosphatase, aspartate aminotransferase, sodium, potassium, chloride, phosphorus, glucose, cholesterol, triglyceride, uric acid, low density lipoprotein, lactate and lactate dehydrogenase.

**Keywords**: Tilapia, haematological, biochemical profiles, habitats

6th Proceedings of the Seminar in Veterinary Sciences, 11 – 14 January 2011:113

# Bacterial Analysis of Australian Jade Perch Frys

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## Abstract

Fifty-two Jade Perch frys from Australia were sampled for bacterial analysis to determine if any bacteria of pathogenic significance could be cultured. The frys were supplied in two groups: the first batch comprised of 32 frys obtained directly from the hatchery in Queensland, Australia and the second batch comprised of 20 frys from the same source that had been at a farm in the Klang Valley for one week. The kidneys of the fish and accompanying water were sampled for bacterial growth on Tryptic Soya agar (TSA) and Blood agar. Bacteria were identified using conventional biochemical tests and DNA sequencing. Seven known species of bacteria were identified through conventional and sequencing methods. Three of these are known bacterial pathogens of fish, namely *Edwardsiella tarda*, *Vibrio spp*. and *Photobacterium damsela*. Four of the identified bacteria namely *Pleisiomonas shigelloides, Vibrio spp*., *Acinetobacter spp*., and *Pseudomonas aeruginosa* are of public health significance. In addition, two relatively unknown species of bacteria, *Aquitalea magnusonii* and *Hydrogenophaga spp*., were successfully identified using the sequencing method.

**Keywords:** Jade Perch, bacteria, aquaculture, DNA sequencing

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# Immunoregulatory Response following Benzo-A-Pyrene Instillation in Embryonated Chicken Eggs

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## Abstract

Benzo[a]pyrene ($C_{20}H_{12}$,BaP), is a five-ring polycyclic aromatic hydrocarbon compoundthat is found in high concentrations during biomass-based air pollution. Although being reported as mutagenic, carcinogenic and immunosuppressive in chickens, its *in ovo* effect has not been documented. Thus, this study was conducted to assess the immunosuppresive action and related pathological changes of lymphoid organs of embryonated chicken eggs and hatchling following intra-allantoic instillation of BaP. The embryonated eggs were divided randomly into two groups, namely control and treatment groups. The treatment group was inoculated with 0.81 mg/mL BaP via the intra-allantoic route, while the control group was similarly inoculated with tricaprylin. Candling was daily and embryos that died during the experiment were removed while allantoic fluid and yolk were harvested. The embryos that survived until hatching were bled via the intra-cardiac route and subsequently sacrificed on day 3 post-hatching via cervical dislocation and immediately necropsied. This study showed that embryonated chicken eggs which were exposed to BaP produced higher antibody titer against ND. Likewise, histologic appearance of the lymphoid organs showed evidence of hyperplasia as suggested by increase in cellular density of the tissues. This study showed that BaP has the potential to cross the allantoic barrier and adversely affect the embryo development.

**Keywords:** Benzo-a-pyrene (BaP), immunoregulatory, allantoic route, embryonated chicken eggs.

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# Erythrocyte Glutathione Peroxidase Activity for Assessment of Health Status of the Timorensis Deer (*Cervustimorensis*)

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## Abstract

Erythrocyte glutathione peroxidase activity analysis was carried out on 40 Timorensis deer (*Cervus timorensis*) of which 30 were born in Taman Pertanian Universiti (TPU), Universiti Putra Malaysia (UPM) and the remaining 10 born in Lenggong, Perak and relocated at TPU in July 2009. The haematological and serum biochemical analyses and serological disease screening on melioidosis, brucellosis, Johne's disease and caseous lymphadenitis (CLA) were done to evaluate the health status of these deer. Comparisons of erythrocyte glutathione peroxidase activity were made between different TPU-born and Lenggong-born deer, age groups and sexes of these deer by using a manual DTNB direct method. The analyses and screening showed that the deer were clinically healthy and disease-free. There were no significant ($p>0.05$) difference in glutathione peroxidase activity different TPU-born and Lenggong-born deer or age groups and sexes of these deer. Evaluation of erythrocyte glutathione peroxidase activity plays an important role in disease correlations and can be used inassessment of health status of the Timorensis deer.

**Keywords:** Timorensis deer, erythrocyte glutathione peroxidase, manual DTNB direct method

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# Stress Level in Red Tilapia Hybrid (*Oreochromis*Sp.) Treated with Chemical and Nonchemical Anesthesia

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## Abstract

Red Tilapia hybrid, *Oreochromis* sp., is one of the most reared food fish due to its good growth and high tolerence to adverse condition. Aquatic animals can be easily stressed by physical disturbances such as handling and anesthesia. Stress is the response in the form of a suite of neuroendocrine events that is activated by a perceived threat and whose purpose is to protect any physiological imbalance or to reestablish homeostasis. In aquaculture, anesthetic protocol can be commonly divided into two, i.e. chemical and non-chemical anesthesia, which are the MS-222 and hypothermia, respectively. In this study, Red Tilapia hybrid (n=45) were exposed to chemical and non-chemical anesthesia using MS-222 and hypothermic method, respectively. Their stress level was determined by measuring the plasma glucose level and hematology. Non-chemical method which is the hypothermia showed the highest glucose level followed by MS-222 and control groups. This showed that the non-chemical anesthesia was more stressful than the chemical anesthesia. However, there were no significant observation in haematology.

**Keywords:** Tilapia, MS-222, hypothermia, stress response, blood glucose, hematology

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# Body Weight and Body Conformation of Cyprus Shami and Boer Goats in Malaysia

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## Abstract

Thirty Boer and 30 Cyprus Shami goats were measured for their body weights, height at withers, body length and heart girth. Each breed was divided into three age groups: group 1 (6-12 months old), group 2 (12-18 months old) and group 3 (more than 18 months old). Means for linear body measurements according to age groups were compared between Shami and Boer goats. There was a highly significant differences ($p<0.005$) between Boer and Cyprus Shami goats in term of each body measurement and body weight. In the present study, to predict the body weight of Cyprus Shami goat, the combination of three parameters of linear body conformation can be used.

**Keywords:** Boer and Cyprus Shami goats, body measurements, body weights

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# An Investigation on Antibiotic Resistance of *E. coli* in the Red Jungle Fowl from a Farm in Sepang

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## Abstract

This study was carried out to isolate and identify the normal bacterial flora in the cloaca of the Red Jungle fowl and to determine the level of antibiotic resistance in the *E. coli* obtained from the cloacal swabs of these birds. This study was done in a farm in Sepang, which produces Red Jungle fowl high crosses. Fifteen cloacal swabs were taken from individual birds of a one-year old female flock. The most prevalent normal flora bacteria in the cloaca of these birds were *Escherichia coli* and *Staphylococcus aureus* spp., both were present in 73% of the samples. Other bacteria isolated include *Klebsiella spp., Chromobacterium spp., Achromobacter spp., Staphylococcus pseudintermidius, Staphylococcus intermidius, Staphylococcus hyicus, Corynebacterium urealyticum, Corynebacterium phocae,* and *Enterococcus faecalis*, which were present in 7to 33% of the samples. The *E. coli* isolates from the Red Jungle fowl exhibit complete (100%) multiple resistance to antibiotics used in the farm, which were erythromycin, norfloxacin, and tetracycline; and penicillin G, which was never used in the farm. Although 15% of the isolates were sensitive to cephalixine, 23% were resistant. The occurrence of antibiotics resistance towards drugs that was never used in the Red Jungle fowl suggests that the antibiotic resistance may be acquired through other means other than exposure to the drug.

**Keywords:** Red Jungle fowl, normal flora, *E. coli*, antibiotics resistance

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# Blood Profile of Rusa Deer (*Cervus timorensis*)

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## Abstract

The objective of the study was to establish the reference range for blood and coagulation parameters in normal, healthy male and female rusa deer (*Cervus timorensis*) of different ages. The study was conducted at Taman Pertanian Universiti, Universiti Putra Malaysia. The sample population comprised of 40 rusa deer, which was divided into 4 groups of 13 young (≤1-year-old) males, 5 young females, 13 adult males and 9 adult females. Jugular venous blood samples were collected to determine concentrations of blood and coagulation parameters. The data obtained were normally distributed. However, the analytical results revealed that significant ($p<0.05$) higher values were observed in erythrocyte count, $Ca^{++}$ concentration and prothrombin time in the adult male than female rusa deer. The total protein concentration was significantly ($p<0.05$) higher in adult female than adult male rusa deer. No significant difference ($p>0.05$) in blood or coagulation parameters was observed between sex in the young deer. Between age group, adult deer had significantly ($p<0.05$) higher mean cell volume, plasma protein and globulin concentrations than young rusa deer. Thus, it is necessary to take into account the age and sex when using blood reference values for the diagnosis of diseases in the rusa deer.

**Keywords**: Rusa deer, blood, coagulation, parameter, sex, age

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# Effect of Local versus Imported Rodent Diet on Body Weight and Blood Parameters of Sprague-Dawley Rats

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## Abstract

This study was conducted to determine the effect of short-term (28 day) feeding of a local and two imported rodent diet on the body weight and blood profile of the Sprague-Dawley rats. Thirty female Sprague-Dawley rats aged between five to six weeks were placed in pairs in the polyethylene cages. Water and feed were given *ad libitum*. The rats were assigned to three groups where one group was fed the local diet; the second fed a United States of America diet and the third an Australian diet. The rats were weighed on days 0, 7, 14, 21 and 28 and the feed excess weighed every two days. From this study, it can be concluded that in short-term feeding, the body weight of Sprague-Dawley rat were affected by the type of diet. However, there was no significant difference between blood parameters of rats fed local and imported diet. This also holds true for the liver enzymes. However, there was a significant ($p>0.05$) difference in blood urea nitrogen (BUN) concentrations between these rats. The Australian diet produced less effect on BUN concentrations.

**Keywords:** Sprague-Dawley rat, rodent diet, acute toxicity, body weight, blood parameter

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# Mastitis in the Dairy Herd at Taman Pertanian Universiti Putra Malaysia

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## Abstract

This study investigated mastitis in the dairy herd at Taman Pertanian Universiti Putra Malaysia (TPU). Questionnaire was used to describe the farm performance and management. Nineteen lactating cows and 150 quarters were tested by clinical examination, the California Mastitis Test (CMT) and bacteriological culture to estimate the incidence and prevalence of mastitis. Relationship between subclinical mastitis and some risk factors were evaluated. Prevalence of the aetiologic agents and their antibiotic sensitivities were also determined. Some management deficiencies were identified. No clinical mastitis observed. The incidence risk of subclinical mastitis based on CMT was 8 cases in 100 cows and 6 cases in 100 quarters, in a 2-week period. The period prevalence of subclinical mastitis by CMT was 68% (cows) and 48% (quarters), and from culture, 100% cows and 91% quarters. *Staphylococcus hyicus* (68%) was predominant followed by *Staphylococcus aureus* (32%). Prevalence of subclinical mastitis by CMT or culture was significantly associated with positive CMT, position of quarters, and stage of lactation. Antibiotics sensitivity and resistance of the isolates were identified. In conclusion, the prevalence of mastitis in the dairy herd at TPU was high. Hence, the results of this study would be useful for the prevention and control programme for mastitis in this herd.

**Keywords:** Mastitis, cows, CMT, culture, bacteria, Universiti Putra Malaysia

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# Influence of Age, Reproductive Status and Vulvar Conformation on Canine Vaginal Microflora

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## Abstract

The influence of age, reproductive status and vulvar conformation on canine vaginal microflora of dogs reared in a tropical environment was successfully determined. Vaginal swab samples were obtained from 15 intact and 15 spayed bitches from a shelter. The dogs were grouped according to age and vaginal cytological examination was conducted to determine the oestrous cycle stage of the bitch. Physical examination and images of the vulvar conformation were captured and classed into three categories (I, II and II) based on the position, size and percentage of occlusion. The effect of vulvar conformation on bacterial load was determined. Canine vaginal microflora isolated in this study is similar to that reported in temperate climates. Coagulase-positive *Staphylococcus* was the most common bacteria isolated from 86.7% of the bitches. All isolated bacteria were normal opportunistic microflora of the vagina. Bitches less than one-year old had a higher bacteria load, which was 50% higher than bitches above one-year old. This finding may be attributed to the differences in immunity maturity and physiological responses of the dogs. Spayed bitches have higher bacterial load compared to intact bitches in anestrus and this may be associated with the partially occluded vulvae which occurred in 87% of these bitches. Category III, which included bitches with >50% vulvar occlusion by skin folds had a higher load of bacteria (60%) compared to category I where the vulva was not occluded (33.3%).

**Keywords:** Vaginal microflora, intact bitches, spayed bitches, bacterial load, vulvar conformation

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# Semen Evaluation in River Terrapin (*Batagur affinis*)

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## Abstract

The *Batagur affinis* or the southern river terrapin is one of the rarest chelonians in the world, found in the rivers of Malaysia. Over years the population of this species has decreased dramatically. Currently there are no data on the reproductive parameters and performance, to include semen collection and evaluation method, for these species. Therefore, this study documented the first-ever semen collection and evaluation in the freshwater turtle, the *Batagur affinis*. This study was conducted in December 2010 on 15 male river terrapins. Before electroejaculation, the *Batagur affinis* were sedated with Ketamine (5 mg/kg) IM and then restrained on a wooden stool. An electrical rectal probe was inserted into the cloaca and stimulated with 1-4 volts electrical stimuli in 5-6 cycles followed by manual stimulation. Semen samples were collected immediately after electrical stimuli and/or after manual stimulation. The results of semen evaluation showed that the average semen volume was 3.3 mL (range 0.85-7.45 mL). The seminal fluid was watery, clear, viscous and slimy to touch. An average sperm motility of 4% (range 0-24%) and average concentration of 2.3 million/mL were observed. The sperms had slightly curved narrow heads and the live sperm percentage was 59%. This study forms the basis for the development of a protocol for semen cryopreservation and artificial insemination in chelonians. The method should contribute to preservation of one of the world's most critically endangered chelonians.

**Keywords:** River terrapin (*Batagur affinis*), semen evaluation, electroejaculation

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# Comparison between Two Staining Techniques using Cellular Reactions of Trombiculid Mites Lesion

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## Abstract

The larval stage of trombiculid mites are parasitic and cause skin lesions in many species of vertebrates including man. The nymphs and adults are found on the ground and are not parasitic. In Malaysia, poultry kept under free range system are commonly infested with trombiculid mites. The clinical sign shown include hyperkeratosis and swelling of the epidermis. Sixteen Red Jungle fowls with typical lesions of trombiculid mites were brought in for postmortem in this study. The gross lesions observed were hyperkeratosis and edematous swelling of the skin. The lesions can be classified into two, which are active and regressing lesions. Skin samples were taken from each bird for histopathology study. Two fixatives were used which were; 10% Normal Buffered Formalin and Helly's fluid and stained with two different stains, namely Hematoxylin and Eosin (H&E) and Giemsa stains. The comparison was done based on the anatomical changes in skin tissue sections and the visibility of cellular reaction between these two fixatives and stains. Eight hundred and thirty-one lesions were found on all ventral body areas of the 16 birds examined. The number of lesions per bird ranged from 19 to 119. The larvae were orange in colour, oval-like shape, with their head burrowed into the lesions. A total of 4731 trombiculid larvae were counted in the birds. The mite number ranged from 50 to 635. The average number of lesions per bird was 51 and the average number of mites per bird was 295. Histopathologically, there was hyperkeratosis of the epidermal layer with the stylostome burrowed within anuclear keratin layer of the skin which can be seen under low magnification. Numerous inflammatory cells infiltrated in the epidermis surrounding the stylostome. The refractility of eosinophils granules was clearer in Helly's stained with H&E as compared to 10% formalin with similar stains under 100 x magnification. However, there was no significant difference in cell count for scoring cellular reaction in both fixatives and stains. In conclusion, there was a minor difference in appearance of

cellular reaction in trombiculid mite lesions using two different fixatives and stains. However, there was no significant difference in the cell numbers for 10% formalin and Helly's-fixed tissues.

**Keywords:** Red Jungle fowl, ectoparasite, trombiculidiasis, mites, formalin, Helley's fluid

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# Number and Distribution of Gastrin Cells in Response to Different Diets in the Pylorus of Goats

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## Abstract

Gastrin is one of the important hormones in gastrointestinal endocrine system. Its function is to stimulate gastric acid secretion during food digestion. Gastrin is synthesised and stored in gastrin cells (G cells) located in the pylorus of the mammalian stomach. Lesser amounts are produced throughout the small and large intestine. Twenty-four Katjang-Cross goats, aged 6 mo were used in this study. The goats were divided into control and treatment groups. The control group (n=8) was fed with a diet consisting of 60% Guinea grass and 40% concentrates which included corn, soya bean meal, calcium, mineral and vitamin with crude protein of 12.8%. The first treatment group (n=8) was fed with 10% Guinea grass and 90% palm kernel cake together with calcium, mineral and vitamin with crude protein of 15.2%. The second treatment group (n=8) was fed with same diet as the goats in the first treatment group but with addition of molybdenum (40 ppm) and sulphur (100 ppm) with crude protein of 15.2% (same as treatment 1). The diets were fed to the goats for 120 d and the goats were slaughtered on the 121st day. The stomachs were removed from the carcasses of the goats and the histological slides of all four parts of the abomasum were prepared. The numbers and distribution of the gastrin cells were counted and compared between control goats and two treatment goats. There were significant differences in the means of gastrin cells between the control goats and treatment goats. Both treatment goats fed with higher protein diet has high number of gastrin cells as compared to the control goats. As for distribution of gastrin cells, positive immunoreactive gastrin cells were found only at the pyloric region of the abomasum. No gastrin cells were found in the cardia, fundus and body of abomasums. There were no specific patterns of distribution of gastrin cells in the pyloric.

**Keywords**: Goat, gastrin cell, protein diet

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# Prevalence of West Nile Virus Antibody in Captive Bird Populations in Selected Areas in Selangor, Malaysia

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## Abstract

West Nile Virus (WNV) infection is a zoonotic emerging disease caused by RNA virus genus of *Flavivirus*. WNV virus is maintain in the environment via cyclic transmission with mosquito particularly *Culex* spp. served as a vector, birds as amplifying host and human or mammals as dead-end host. Currently, no studies have been carried out to determine the prevalence of WNV antibody in captive bird populations in Malaysia. This study was conducted because there are many risk factors that can contribute to the presence of WNV in Malaysia such as high biodiversity including migratory birds, presence of vector and importation of the birds among pet owners and zoological collections. This cross-sectional study was conducted in four selected areas in Selangor which were National Zoo, Sunway's Wildlife Park, Tanjung Karang and Faculty of Veterinary Medicine, UPM. Sixty-eight serum samples from 17 different species of captive birds were collected via venipuncture at the wing vein. Then, these sera were tested using ID Screen® West Nile Competition (Competitive ELISA) against anti-protein E antibody. Three samples showed seroconversion and each positive sample was from different species and different places. The overall prevalence in this study was 4.41%. The prevalence based on locations were National Zoo (5.88%), Sunway's Wildlife Park (6.17%) and, Tanjung Karang (9.09%). Thus, this preliminary study confirmed the exposure to WNV among captive bird populations in selected areas in Selangor.

**Keywords**: WNV, competitive ELISA, antibody, seroconversion, seropositive, prevalence

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# Effect of Garlic on Serum Cholesterol Level in Rats on High Fat Diets

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## Abstract

Garlic has been reported to possess the effect of lowering serum cholesterol level in blood thus may reduced the occurrence of cardiovascular disease but scientific reports are lacking. Twenty-four Sprague-Dawley rats of 16-weeks old were divided into four groups (n = 6). Hypercholesterolemia was induced by feeding 20% butter as high fat diet for two weeks and the treatment diet for three weeks. Group A was fed normal diet, group B fed normal diet + butter, group C fed normal diet + butter + garlic and group D was fed normal diet + butter + dexamethasone. The serum cholesterol concentration, average body weight and organ weight were determined for five weeks. The results showed a significant ($p<0.05$) decrease in the serum total cholesterol concentration for groups C and D. The average body weight was significantly ($p<0.05$) different between the initial body weight and final body weight. The organ weight showed a significant ($p<0.05$) decrease in mean blotted dry liver weight in groups C and D. These observations indicate that consumption of raw garlic has beneficial effect in lowering serum total cholesterol concentration and promote weight control.

**Keywords:** Garlic, hypercholesterolemia, total cholesterol, butter

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# Identification of *Vibrio* Species isolated from Marine Fish using Polymerase Chain Reaction

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## Abstract

*Vibrio* species are found in marine and estuarine environments. Vibriosis can cause more than 50% mortality in fish culture facilities once an outbreak is in progress. The objectives of this study were to subculture and identify *Vibrio* spp. that were isolated previously from marine fish; to develop a technique for simultaneous identification of several *Vibrio* spp. (*V. alginolyticus, V. parahaemolyticus, V. fluvialis* and *V. vulnificus*) using polymerase chain reaction (PCR); and to compare the rapid identification kit and PCRtechniques commonly used for the identification of the *Vibrio* spp. In this study, 20 isolates from four *Vibrio* species, which consisted of five eachof V. *alginolyticus, V. parahaemolyticus, V. fluvialis* and *V. vulnificus* isolates were provided by National Fish Health Research Centre (NaFisH). The species of *Vibrio* were identified using an identification kit, API 20E system. These organisms were isolated from various marine fish such as Asian Seabass (*Latescalcarifer*), Grouper (*Epinepheluscoioides*), Silver Pomfret (*Pampusargenteus*) and Red Snapper (*Lutjanuscampechanus*). The isolates were previously stored at -80˚C and subcultured onto TSA+. The pure cultures were then transferred to TSB+. These isolates were subjected to DNA extraction. Once the DNA is ready, PCR was used to optimise the products with the designated primers. All the PCR products were electrophoresed through 1% agarose gel for 1 h. The designated primers in this study were found suitable for the detection of *V. alginolyticus,V. parahaemolyticus,V. fluvialis* and *V. vulnificus*.Using the API 20E system, 15% (3/20) isolatesof *Vibrio* spp. were negative, indicating the the PCR technique is still required to confirm the result obtained by the use of the API 20E system.

**Keywords:** *Vibrio* species, Vibriosis, marine fish, PCR

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# Anatomical Structure of the Limb of White-nest Swiftlet (*Aerodramus fuciphagus)* and White-headed Munia (*Lonchura maja)*

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## Abstract

This study was conducted with the aim to examine the anatomical structures of the limb of white-nest swiftlet (*Aerodramus fuciphagus*) to determine the reason why the birds are not able to walk, stand and perch while standing. In addition, an attempt was made to compare the results with the white-headed munia (*Lonchura maja*), which has similar body weight and appearance. Four left limbs from each species were examined macroscopically using stereomicroscope. The bone and muscles of both species were measured and compared. The limb muscles of white-nest swiftlet were twice smaller than the white-headed munia. The tibial bone was approximately similar in length, but the tarsometatarsal bone of the white-nest swiftlet was shorter than the white-headed munia. The digits of the white-nest swiftlet were also shorter than the white-headed munia. The tibial bones for both species were taken for histological examination and the results revealed no significance difference between the two species. Four groups of muscles namely the biceps femoris, semimembranous, semitendinosus and gastrocnemius from each bird were also taken for histological examination. The muscle sections were stained with H&E and Masson's Trichome. Histologically, the white-nest swiftlet had smaller sized limb muscles compared to the white-headed munia. Similarly, the muscle bundles of the white-nest swiftlet were also less than the white-headed munia. In conclusion, the short tarsometatarsal bone and digits, and small limb muscles could be the reason why the white-nest swiftlets are not able to use their limbs for walking, standing and perching.

**Keywords:** White-nest swiftlet, white-headed munia, bone, muscles

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# Morphological and Meat Quality of Breast Muscle of Wild Red Jungle Fowl and Malaysian Indigenous Chicken

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## Abstract

The aims of this study were to define the morphological structure, to evaluate the collagen distribution and to determine the meat quality traits of breast muscle in wild Red Jungle fowl and Malaysian indigenous chickens. The Red Jungle fowl is the ancestor of the domestic fowl. The Malaysian indigenous or *Gallus gallus Domesticus*, commonly known as village chicken are crossbreed of the RJF with mixed exotic domestic breeds. Seven samples of breast muscle (*Pectoralis major*) from adult RJF (n=7) and Malaysian indigenous chickens (n=7) were used in this study. The wild RJF were captured from the secondary forests in Peninsular Malaysia, while the Malaysian indigenous chicken were collected from Jenderam Hilir, Sepang, Selangor. The parameters of meat quality evaluated were moisture and ash content, crude protein and fatty acids profile, pH and colour measurement, percentage of cooking loss and shear force value. For the morphological characteristics, the mean diameter of muscle fiber, cross-sectional area of muscle bundle and total number of muscle fiber were evaluated. The results revealed there were significant ($p<0.05$) differences in the morphology and collagen distribution of the breast muscle between the wild RJF and Malaysian indigenous chickens. The muscle bundles area and diameter of muscle fibers of Malaysian indigenous chickens were larger compared to those of the wild RJF. However, the total number of muscle fibers was less in Malaysian indigenous chickens as compared to wild RJF. The findings for the meat quality traits revealed wild RJF had higher protein and ash content, low shear force value and higher pH value than the Malaysian indigenous chickens. Thus, based on the findings of this study, the RJF fowl had better meat quality than the Malaysia indigenous chickens.

**Keywords:** Red Jungle fowl, Malaysian indigenous **c**hicken, breast muscle, morphology, collagen distribution, meat quality

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# Histopathological Changes in Chickens infected with *Pasteurella multocida* and Ducks infected with *Riemerella anatipestifer*

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## Abstract

A description and evaluation of histopathological changes in organs of chickens infected with three different serotypes of *Pasteurella multocida* and ducks infected with *Riemerella anatipestifer* were carried out. Seventy-five 4-week old chicken and 25 ducks were selected for the study. The chickens were divided into 3 groups of 25 chickens each while the ducks were not divided. At the start of the experiment, all chickens of group 1 were infected intramuscular with 0.5 mL inoculums containing $10^9$ cfu/mL of live *Pasteurella multocida* A:1. Chickens of groups 2 and 3 were similarly infected with *Pasteurella multocida* A:3 and A:1, 3 respectively while all ducks were infected with *Pasteurella multocida* A:1 followed by *Riemerella anatipestifer.* All animals were observed for clinical signs and the survivors killed on day8 post-infection. The lungs, liver and kidneys were collected during postmortem and histology examinations, and the lesions were described and severity objectively scored. Intramuscular inoculation of chickens with *Pasteurella multocida* A:1, A:3 and A:1,3 killed most chickens at the rate of 92, 96 and 86%, respectively. The gross lesions consisted of generalised congestion of internal organs with occasional haemorrhages. Histology revealed severe pulmonary, hepatic and renal congestions with occasional haemorrhages and focal hepatitis. Based on rate of mortality and histology lesion scoring, *Pasteurella multocida* A:3 was found to be most virulent while *Pasteurella multocida* A:1,3 the least virulent of the three serotypes. Infection by *Pasteurella multocida* A:1 followed by *Riemerella anatipestifer*in ducks resulted in similar lesions with 94% mortalityrate.

**Keywords:** Histopathology, *Pasteurella multocida, Riemerella anatipestifer*, chickens, ducks

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# Parasites of the White-Breasted Waterhen (*Amaurornis phoenicurus*)

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## Abstract

A study was carried out to determine the species and prevalence of ecto- and endoparasites in one of the most common waterbird, the White-Breasted Waterhen (*Amaurornis phoenicurus*). Twenty-two White-Breasted waterhen were caught in the state of Selangor, Malaysia. They were of mixed ages and gender. The feathers and skin were examined thoroughly for ectoparasites and the gastrointestinal tracts, other internal organs (crop, proventriculus, kidney, heart, lungs, liver, brain and trachea), and faeces were examined for endoparasites. The Giemsa stained blood smears were examined microscopically for haemoparasites. The species of ectoparasites found in the study includes, the feather mites, *Grallobia spp*. detected in 100% of the birds. Other species of feather mites found, *Megninia spp*. were detected in 77.3% of the birds. The lice, *Lipeurus spp*. were found in 18.2% of the birds. Another species of lice which has yet to be identified were detected in 4.6% of the birds. Only one species of endoparasite, *Capillaria spp*. was found in 22.7% of the birds. One species of blood parasite was found in this study which is the *Plasmodium spp*., detected in 31.8% of the birds.

**Keywords:** Ectoparasite, endoparasite, haemoparasite, White-Breasted Waterhen (*Amaurornis phoenicurus*)

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# Identification and Confirmation by Koch's Postulate the Cause of Red Leg Syndrome in Captive Bullfrog (*Rana catesbeiana*)

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## Abstract

American bullfrog (*Rana catesbeiana*) is currently raised world-wide for a variety of reasons. Frog farming in Malaysia was established more than 20 years ago to supply frog meat for the local non-Muslim community as well as for exportation. However, frog farming still remains as a minor aquatic industry in this country. Red leg syndrome is a common disease among the frog population and has been observed to be highly fatal. The present study was conducted to investigate the cause of red leg syndrome which commonly occurrs in a farm in the state of Penang. Nineteen species of bacteria were isolated from 28 frogs showing typical clinical signs of red leg syndrome. To confirm Koch's postulate, the 19 pure isolates were inoculated in frogs. However, an attempt to reisolate the bacteria from the internal organs failed to show any bacterial growth. On the other hands, only four species of bacteria were reisolated from the frogs that did not show clinical signs. The current study provides baseline information on the causative agent of red leg syndrome and a reference to further studies for treatment, prevention and control to improve the industry.

**Keywords:** American bullfrog (*Rana catesbeiana*), red leg syndrome, Koch's postulate, bacteria

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# Seroprevalence of *Helicobacter hepaticus* in Mice from Laboratary Animal Facilities in Klang Valley, Malaysia

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## Abstract

Helicobacter is a genus of Gram-negativebacteria possessing a characteristic helix shape. They were initially considered to be members of the Campylobacter genus, but since 1989 they have been grouped in their own genus. *Helicobacter hepaticus*is an enterohepatic Helicobacter species (EHS) belonging to the family *Helicobacteriaceae* of the order *Campylobacterales* of Epsilon-proteobacteria division. It is a Gram-negative, microaerophilic, urease-positive, spiral rod. *H. hepaticus* colonises the colon and invades the liver of mice causing chronic severe active hepatitis and proliferative typhlocolitis. It can also induce hepatocellular carcinomas in certain breeds. The bacterium has been associated with inflammatory bowel disease in immunocompromised mice. Certain strains of mice will develop a proliferative, inflammatory typhlitis and/or colitis that may result in rectal prolapse. Detection of *H. hepaticus* in laboratory mice is therefore important because of its effect on research animals ultimately complicating the research findings. Currently PCR, culture, serology test or histologic examination of silver-stained liver sections is used to diagnose *H. hepaticus* infection. Most mice colonised with helicobacters remain asymptomatic for long periods of time. This study was conducted to investigate the serological prevalence of *Helicobacter hepaticus* in mice in 5 laboratory facilities in the Klang Valley. Fifty sera were collected and examined for presence of *H. hepaticus* antibodies by commercial ELISA test kit. Result showed two facilities had positive sera towards *H. hepaticus* while the rest were negative. Four of fifty sera were positive while other serum samples were negative.

**Keywords:** *Helicobacter hepaticus*, enterohepatic, typhlocolitis

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# Detection of *Salmonella* in Chicken Meat and Chicken using Conventional and Rapid Culture Methods

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## Abstract

A study on the detection of *Salmonella* in chicken meats and chickens using conventional and rapid culture methods was conducted over a four weeks period in Selangor area of Malaysia. Thirty chicken meats of various parts included wing, breast and thigh were sampled from three markets and 75 cloacal swabs were sampled from three farms located near UPM. Two isolation methods used in this study were conventional culture method and rapid culture method for both food and cloacal swab samples. Of the thirty chicken meats sampled, three (10%) were positive for *Salmonella*. The occurrence of *Salmonella* in chicken meat maybe due to contamination during handling, processing, supply, transportation and the processing areas. Of the 75 cloacal swabs sampled, four (5.3%) were positive for *Salmonella*. The occurrence of *Salmonella* in chicken may due to poor management and stress. From the two isolation methods used, rapid culture method isolated 10% of *Salmonella* in chicken meat and none from cloacal swabs. On the other hand, the conventional method isolated 5.3% of *Salmonella* from cloacal swabs and none from chicken meat. The presence of *Salmonella* in chickens and chicken meat is of public health importance.

**Keywords:** *Salmonella*, chicken meat, chicken, rapid culture method, conventional culture method

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# Effect of Splash Block using Lidocaine in Dogs Undergoing Ovariohysterectomy

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## Abstract

Twelve dogs undergoing ovariohysterectomy were randomly assigned to receive either 8 mg/kg of lidocaine 1% or an equal volume of NaCl 0.9% as the splash block. Following celiotomy and prior to manipulation of ovarian pedicles, lidocaine or 0.9% NaCl was instilled onto the mesovarium by using a dog urinary catheter. Pulse rates, respiratory rates, systemic arterial pressures and oxihemoglobin saturation levels were measured throughout the surgery at pre-determined time points. Ketamine-diazepam supplemental boluses (0.05 mL/kg, intravenously) were administered when there were movements, vocalization, increased in palpebral reflexes and jaw tones. There was no difference between the lidocaine-treated and the control group in the cardiopulmonary parameters. All animals recovered and were returned to their owners without complications. Only 2 dogs in the lidocaine group, compared to 5 dogs in the control group required supplementary dose of ketamine-diazepam to complete surgery. The use of 8 mg/kg lidocaine 1% as splash block in addition to the routine anesthetic protocol was safe and did not cause suppression to the cardiopulmonary parameters. It significantly reduced the need for supplementary dose of ketamine-diazepam.

**Keywords:** Local anesthesia, lidocaine, splash block, ovariohysterectomi

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# Coprological Diagnosis of Gastrointestinal Parasites in Captive Primates in Peninsular Malaysia

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## Abstract

The present study was undertaken to determine the prevalence of gastrointestinal parasites in captive primate populations in three Zoos in Peninsular Malaysia. A total of 52 faecal samples were collected from the enclosures of five species of local primates comprising Orang Utans (*Pongo pygmaeus*), White-Handed Gibbons (*Hylobates lar*), Siamangs (*Symphalangus syndactylus*), Stump-tail Macaques (*Macaca arctoides*) and Slow Loris (*Nycticebus coucang*). The samples were subjected to Formal-Ether sedimentation, Ziehl-Neelsen and Giemsa staining for microscopy detection of helminth ova and protozoan cysts. PCR with species-specific primers were used to detect *Cryptosporidium*. A total of 46 (88%) faecal samples were positive for various parasites by microscopy. The most common parasite harboured by the captive primates was *Entamoeba* (65.4%), followed by Strongyles (40.4%), *Strongyloides* (15.4%) and *Cryptosporidium* (9.6%). *Balantidium* and *Trichuris* showed relatively low infection rates (1.9%). PCR assay had a higher sensitivity (15.4%) for the detection of *Cryptosporidium* compared to conventional microscopy and Ziehl-Neelsen staining (9.6%). The high rate of infection with *Entamoeba* and *Cryptosporidium*, and the presence of *Balantidium* in the captive primates are of concern as they pose a potential zoonotic risk to animal handlers, keepers and the public.

**Keywords:** Gastrointestinal parasites, primates, PCR

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# Carcass Composition of Organic, Broiler (*Gallus domesticus*) and Malaysian Indigenous (*Gallus gallusdomesticus*) Chickens

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## Abstract

There is an increasing awareness of consumers towards organic food such as organic vegetables or organic chickens. Most of them believe that organic chickens are healthier because of their low amount of fat content. In this study, carcass composition was done to differentiate the carcass of organic, broiler and Malaysian indigenous chickens. Forty adult chickens consisting of 20 broiler chickens, 10 Malaysian indigenous chickens and 10 organic chickens were used in this study. All chickens were slaughtered by cutting the cervical veins and arteries, trachea and oesophagus and then frozen at 0°C for 24 h. The carcasses were then divided into 2 halves; forequarter and hindquarter. Forequarter was separated into breast, wings and ribs whereas the hindquarter was not separated. The muscle, bone, fat and skin of each part were separated, weighed and recorded. Broiler chicken had the highest reading in each part. Malaysian indigenous chicken had the lowest amount of fat and skin compared to broiler and organic chickens. There were no significant differences between organic and Malaysian indigenous chicken.

**Keywords:** Organic, Malaysian indigenous chicken, broilers, carcass composition

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# Effect of Transportation Stress on Physical and Blood Parameters of Thoroughbred Racehorses under Malaysian Conditions

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## Abstract

Twenty Thoroughbred racehorses from Perak Turf Club, which were registered to be competing in a race at Selangor Turf Club in December, 2010 were selected to study the effects of transportation stress on physical and blood parameters under Malaysian conditions. These horses travelled from Perak to Selangor Turf Club at the same time on the same day. The travelling distance was 220 km. Blood samples were taken from each horse at pre- and immediately post-transportation upon arrival at Selangor turf club. Physical examination was carried out and recorded for both pre- and post- transportation. Blood samples were evaluated for both haematological and biochemical components, which were the erythrocyte and leukocyte counts, packed cell volume, segemented neutrophil and lymphocyte counts, total protein, creatine kinase, glucose, lactate as well as cortisol. In this study, significant changes in most blood parameters and physical parameters indicates that travelling horses or road transportation induced some physiological responses in horses. Physiological parameters such as the rectal temperature, heart rate, respiratory rate and skin recoil differ as compared from pre- and post-transportation. These indicate changes of physical response due to effect of transportation stress. Blood parameters such as lactate, glucose, cortisol, leukocyte count, plasma protein, haemoglobin, and erythrocyte counts increased significantly. Ambient temperature and relative humidity were recorded throughout the travelling process. However, there was no significant change in both climatic factors in the horse float.

**Keywords:**Thoroughbred racehorses, transportation, stress

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# Antibacterial Activities of Sea Cucumber (*Holothuroidea*) in Poultry

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## Abstract

The antibacterial activities of sea cucumber (*Holothuroidea*) were studied. Three commercialized sea cucumber products and one dried sea cucumber were tested for antibacterial activities against six clinical isolates of common pathogenic bacteria causing diseases in poultry using Disc Diffusion Method (Kirby-Bauer Method). Only one commercial product; "Gamat Gel" produced inhibition zones towards 5 tested bacteria namely *Escherichia coli, Salmonella sp., Pasteurella multocida, Staphylococcus aureus, and Streptococcus sp*. in the initial susceptibility test. The biggest inhibition zones were produced against *Pasteurella multocida*, while smallest inhibition zones were produced against *Streptococcus sp*. No inhibition zone was produced against *Pseudomonas aeruginosa*. The determination of Minimal Inhibitory Concentration (MIC) of the Gamat Gel was carried out. It was found that the MIC value for all test bacteria were more than 1/12**.**

**Keywords:** Antibacterial activities, sea cucumber (*Holothuroidea*), pathogenic bacteria, poultry, disc diffusion method (Kirby-Bauer Method)

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# *Trans* Fatty Acids and Conjugated Linoleic Acids in Milk, Yogurt and Cultured Milk Drink

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## Abstract

Biohydrogenation of dietary unsaturated fatty acids by the rumen microbes to saturated fatty acids yields intermediate products comprising of *trans* fatty acid (TFA) and conjugated linoleic acids (CLA). *Trans* fatty acids have been shown to be detrimental to human health whereas CLA has positive effects on human health. In view of the potential health effects of these fatty acids, the aims of this study were to determine the TFA and CLA levels of selected dairy products (milk, yogurt and cultured milk drink) in locally produced and imported dairy products accessible to the general Malaysian population. The subsequent objective is then to estimate the daily intake of TFA and CLA from these dairy products among local population. Fresh milk samples were obtained from the UPM dairy unit located within a 3 km distance from the analytical laboratory and commercial samples were purchased from local supermarkets. The samples consist of 21 milk samples, of which 12 are locally produced; 42 yoghurt samples, of which 18 are local products and 11 cultured milk samples of which 5 are local products. All samples were then subjected to total fatty acids extraction and their fatty acid composition determined using gas liquid chromatography. Results showed that local dairy products have less polyunsaturated fatty acids compared to imported products ($P<0.05$). However, among locally produced dairy products, yogurt contained the highest levels of TFA and CLA. This disparity in results could be attributed to the fact that both TFA and CLA contents in milk were under the influence of not only farm and animal factors, but could also be result of specific manufacturing processes. Findings of this study showed that continuous efforts have to be made to control the levels of TFA in our local dairy products, while enhancing the content of CLA in dairy products. The consumer should also be educated and be aware of the health benefits or detriments of CLA and TFA, respectively.

**Keywords:** Milk, yogurt, cultured milk drink, *trans* fatty acids, conjugated linoleic acids

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# Effects of Conditioning Regimes on Blood Parameters of Endurance Horses under Malaysian Condition

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## Abstract

Endurance conditioning program will increase fitness level in horses. Twenty-six endurance horses, which were registered to be compete in endurance race were selected to evaluate the soundness and blood parameters of the horses in training prior to the competition. The Fitness levels of these endurance horses from establishments practicing different conditioning regime were evaluated after a 6-weeks of conditioning period. Standardized exercise test was carried out for all horses prior and after conditioning. Three sets of blood samples were taken from each horse, i.e. preride, immediate postride and 30 min after ride. All horses were trained at a distance of 35 km, on the same track and at the same time each day. Heart rates were monitored and blood samples were obtained throughout the exercise test, blood samples were then processed and analyzed for biochemistry components, i.e. electrolytes concentration like Ca, P, Na, K, Cl, and muscle enzymes including aspartate aminotransferase, creatine kinase and lactate. Paired sample t-test were performed to evaluate the effects of different conditioning program on these physiological variables. In this study, by the significant changes in most blood parameters, it indicated that different conditioning regimes induced improvements of physiological responses in horses to variable degree. The minimal release of muscle enzymes and little loss of water and electrolytes were reflected by the changes in blood parameters. Although elevations in serum muscle enzymes and lactate were significant, this is believed to be a normal physiological responses of horses towards training without noticeable muscle injuries and/or metabolic acidosis.

**Keywords:** Endurance horse, conditioning regime, biochemistry, performance

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# Occurrence of *Campylobacter* and *Salmonella* Spp. in Ostrich

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## Abstract

*Campylobacter* spp. and *Salmonella* spp. are important pathogens in veterinary medicine and public health. In Malaysia, there has been limited research done in ostriches due to the small-scale farming and small number of animals. Nevertheless, ostrich farming has a huge potential. Some of the disease agents affecting ostriches, such as *Campylobacter* spp. and *Salmonella* spp. can also cause disease in humans. As such, infected ostrich may serve as potential public health risk. Hence, this study was undertaken to determine the occurrence of *Campylobacter* spp. and *Salmonella* spp. in ostrich and this is the first report documenting the occurrence of these two bacteria in ostrich in Malaysia.Samples were collected from three ostrich show farms, with 8 ostriches from Farm A, 11 from Farm B and 12 from farm C. Samples from the cloaca and skin swabs were obtained from each of the ostrich and examined for *Campylobacter* spp. and *Salmonella* spp. All samples were cultured for *Campylobacter* spp. via direct plating on CCDA. One out of 62 samples (1.6%) was positive for *Campylobacter* spp. and it was isolated from the cloacal swab. Pre-enrichment, enrichment and direct plating on XLT4 and Salmonella Chromogenic agar were done for isolation of *Salmonella* spp. from the ostriches. Two samples, one from the skin swab and another one from the cloacal swab, from two different birds were positive for *Salmonella* spp. The study showed the occurrence of *Campylobacter* spp. and *Salmonella* spp. in ostriches. The ostrich is of public health significance as they are used in recreational activities and for human consumption.

**Keywords:** Ostrich, occurrence, *Campylobacter* spp., *Salmonella* spp.

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