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Content

BIRD SPECIES COMPOSITION AND FEEDING GUILD DETERMINATION AT AYER HITAM FOREST RESERVE, PUCHONG, SELANGOR

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Introduction

A study on bird species composition and feeding guild determination was conducted at Ayer Hitam Forest Reserve (AHFR), Puchong, Selangor. The forest is located at Puchong area, about 25 km from Universiti Putra Malaysia (UPM) and 45 km southwest of the Kuala Lumpur. A previous study conducted by Zakaria and Rahim (1999) recorded 160 species of bird which belonged to 38 families. The objectives of this study were to identify the bird species in this area by using morphology of their head, bill, feather, color, tail, feet and special appearance and the feeding guild of the species identified by their bills and feet topography.

Materials and Methods

The study was conducted at four compartments (C12, C13, C14 and C15) of AHFR area. Mist-netting method was used to capture the birds in the study area. Nets were opened just before sunrise about 6.00 to 6.30 am and closed one hour before sunset, at about 6.00 to 6.30 pm. The nets were checked every two or three hours.

Results

In this study a total of 158 birds were captured. The captured birds belonged to 26 families and consisting of 56 species of birds (Table 1).

All birds were evaluated for feeding guild characteristics. The bird composition comprised of insectivorous (six families), carnivores (five families), frugivores (two families), nectarivores (two families) and combination of above feeding guilds (11 families) (Table 2). The feeding guilds were identified based on their bill and foot morphology (Table 2).

Discussion

During the four weeks of study period, 55 species of birds were recorded from the 158-individual captured. In a previous study, Zakaria and Rahim (1999), bulbul was the commonly found species. The most abundance species identified in this study was Collared Scoop Owls. This might be due to the abundance of prey in the forest such as rats, squirrels and small size birds (Sures, 2005). One of the factors determining species

Table 1: Number of Species According to Families

FAMILY	COMMON NAME	SCIENTIFIC NAME	LOCAL NAME
ACCIPITRIDAE	Japanese Sparrow hawk	*Accipiter gularis*	Lang Sewah
ALCEDINIDAE	Blue Eared Kingfisher	*Alcedo meninting*	Pekaka Bintik
	Blacked Backed Kingfisher	*Ceyx erithacus*	Pekaka rimba
	White Throated Kingfisher	*Halycon smyrensis*	Pekaka belukar
	Ruddy Kingfisher	*Halcyon coromoda*	Pekaka Belacan
CAMPHEPAGIDAE	Pied Triller	*Lalaga nigra*	-
CAPRIMULGIDAE	Large Tail Nightjar	*Caprimulgus macrurus*	Tukang Kubur
COLUMBIDAE	Spotted dove	*Streptopedia chinensis*	Terkukur
	Zebra dove	*Geopelia striata*	Merbuk
	Green winged Pigeon	*Chalcophas indica*	Punai Tanah
CUCULIDAE	Rusty Breasted Cuckoo	*Cacumantis sepulcralis*	Sewah
	Chestnut Breasted Cuckoo	*Clamator coromandus*	Sewah
CHLOROPSEIDAE	Green Iora	*Aegithina viridissina*	Kunyit Bakau
	Common Iora	*Aegithina tiphia*	Kunyit Kacat
DICAEDAE	Crimson Breasted Flowerpecker	*Prionochilus percussus*	Sepah Puteri Pelangi
HIRUNDIDAE	Pacific Swallow	*Hirundo tabitta*	Sualo batu
	Barn Swallow	*Hirundo rustica*	Sualo Api
PICIDAE	Rufous Piculet	*Sasia abnormis*	Belatuk Kecil
	Rufous Woodpecker	*Micropternus brachyurus*	Belatuk Biji Nangka
	Rufous Woodpecker	*Picus mintalis*	Belatuk Ranting
	Banded yellownape	*Picus miniacens*	Belatuk Merah
	Buff-necked Woodpecker	*Meiglyptes tukki*	Belatuk Tuki-tuki
DICRURIDAE	Bronzed Drongo	*Dicrurus aeneus*	Cecawi keladi
	Crow-Billed Drongo	*Dicrurus annectans*	Cecawi sawai
	Greater Racquet- tailed Drongo	*Dicrurus rennifer*	Cecawi Anting-anting
MOTACILLIDAE	Richard's Pipit	*Anthus novaeseelandiae*	Pipit Tanah
MUSCICAPIDAE	Asian Brown Flycatcher	*Muscicapa latirostris*	Sambar Asia
	Ferruginous Flycatcher	*Muscicapa ferru*ginea	Sambar Rimba
	Narcissuss Flycatcher	*Ficedula narcissini*	Sambar Bunga
NECTARINIIDAE	Purple napped Sunbird	*Hypogramma hypogrammicum*	Kelicap Rimba
	Little Spiderhunter	*Arachnothera longirostra*	Kelicap Jantung
FALCONIDAE	Black-Thighed Falconet	*Microbierax fringillarius*	FalkoRajawali
TYTONIDAE	Oriental Bay Owl	*Phodilus Badius*	Hantu Jampok Pantai
STRIGIDAE	Collared Scops-Owl	*Otus bakkamonea*	Hantu Reban
	Reddish Scops-Owl	*Otus rufescens*	Hantu Merah
	Brown Boobook	*Ninox scatulata*	Hantu Bertemak
	Mountain Scop Owl	*Otus spilocepalus*	Hantu Gunung
	Oriental Scop Owl	*Otus sunia*	HantuKuang Kuik
TIMALIIDAE	Chestnut- winged Babbler	*Stachyris erythroptera*	Rimba Merah
	Black- throated Babbler	*Stachyris nigricollis*	Rimba Bertam
	Short -tailed Babbler	*Trichastoma malaccense*	Rimba Ekor Pendek
	Black Capped Babbler	*Pellorneum capistratum*	Rimba Ekor Hitam
MEROPIDAE	Blue- tailed Bee-eater	*Merops philippinus*	Berek-berek Carik Dada
PLOCEIDAE	White headed Munia	*Monticola gularis*	Pipit Uban
ORIOLIDAE	Black- napped oriole	*Oriolus chinensis*	Dendang
STURNIDAE	Asian Glossy Starling	*Aplonis panayensis*	Perling Mata Merah
	Purple -backed Starling	*Sturnus sturninus*	Perling Belakang Unggu
PYCNONOTIDAE	Yellow-Vented Bulbul	*Pycnonotus goaivier*	Merbah Kapor
	Cream Vented Bulbul	*Pycnonotus simplex*	Merbah Mata Putih
LANIIDAE	Brown Shrike	*Lanius cristatus*	Tirjup Tanah
	Tiger Shrike	*Lanius tigrinus*	TirjupRimau
PITTIDAE	Hooded Pitta	*Pitta sordida*	Pacat Gembala Pelanduk

diversity is the pattern of resource availability (Zulhadzelan, 1998). Lowland tropical forest environment with relatively stable temperature and high humidity support extremely diverse terrestrial organisms, as many of the bird species exist at low density and are expected to be susceptible to any form of disturbance that alters the pattern of resource availability (Karr, 1976). According to Daud (1999), AHFR is still full with edible and potential edible fruits

trees for the frugivores, nectarivores and the omnivores groups. The situation also the same for the insectivores group because of the presence of migratory species such as Flycatcher which typical insect dependent species. Tropical forest such as AHFR is suitable for insect growth (Zulhadzelan, 1998). The availability of fruits in the forest are not only as a food source for birds, but also for small mammals such as rats and squirrels which provide food source for carnivore birds (Sures, 2005). Based on this short-term study, the results will provide veterinarian some familiarity with wild bird species and their diet for future medical management program.

Table 2: Numbers of family identified according to their feeding guild

Feeding Guild	Number of families
Carnivores	5
Insectivores	6
Frugivores	2
Nectarivores	2
Insectivores/Frugivores	3
Insectivores/ Carnivores	1
Insectivores/Omnivores	1
Frugivores/Omnivores	2
Insectivores/Nectarivores/Frugivores	1
Insectivores/Frugivores/Omnivores	3

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EFFECTS OF ANAESTHESIA AND SURGERY ON BLOOD PARAMETERS IN SHEEP

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Changes in blood parameters following ketamine-xylazine-halothane anaesthesia and stifle arthrotomy were determined in 6 male sheep. Packed cell volume, erythrocyte, leucocyte, haemoglobin, total protein and albumin decreased during anaesthesia and increased towards baseline values during sternal recovery. Leucocytosis peaked at 12 hours post-surgery, then decreased and normalized at 60 hours post-surgery. Increase in fibrinogen was detectable at 12 hours post-surgery and it continued to increase even at 60 hours post-surgery. Blood glucose was markedly elevated during anaesthesia and sternal recovery. Elevation in creatine kinase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were detectable at 6 hours post-surgery. Creatine kinase declined very quickly while other enzymes still continue to increase at 12 to 36 hours post-surgery and remained higher than baseline value at 60 hours post surgery. Significant changes over time were not detected in platelet, alkaline phosphatase, amylase, total bilirubin, direct bilirubin, calcium, cholesterol, creatinine, gamma-glutamyltransferase, phosphate, urea, triglyceride, sodium, potassium, chloride and lactate. It is concluded that anaesthesia and surgery can affect blood parameters in sheep.

Keywords: Sheep, anaesthesia, surgery, blood parameters

Introduction

Blood profile is a very useful tool in disease diagnosis. It is an indicator to systemic changes or alterations. Since anaesthesia and surgery can have a direct effect on the body system, it may affect blood parameters. Thus, interpretation of blood parameters in the peri-operative period should take into consideration the effects that anaesthesia and surgery can have on blood profiles. At the Faculty of Veterinary Medicine in Universiti Putra Malaysia, there are increasing numbers of sheep being used as animal models in researches that involve anaesthesia and surgery. However, there are no report documenting changes in the blood profile of sheep following anaesthesia and surgery in the tropical environment. This study is aimed to determine the effect of xylazine-ketamine-halothane anaesthesia and arthrotomy on blood profile of sheep used as research model in a typical tropical environment.

Materials and Methods

Animals, anaesthesia and surgery
This study utilized six healthy male sheep, 6 months of age with body weight 16.3 ± 2.9 kg. They were housed in pairs, fed *ad libitum* with pellet feed and fasted for 18 hours prior to anaesthesia and surgery. Anaesthetic protocol consisted of premedication with xylazine 0.1 mg/kg, IV, and induction with ketamine 5-10 mg/kg, IV, to effect. The sheep were intubated

and then maintained under halothane with 100% oxygen and controlled ventilation. Stifle arthrotomy was performed on the right hind limb to implant a piece of bioengineered tissue for another unrelated study. Joint capsule was sutured using polyglactin 3-0 and skin was sutured using polyglactin 3-0 or polyamide 2-0. Normal saline was administered at 10 ml/kg/hour, IV, during the surgery. The surgical procedure lasted for 84.8 ± 23.4 min while general anaesthesia lasted for 95.5 ± 21.3 min. The sheep received meloxicam 0.2 mg/kg subcutaneously at the end of surgery.

Blood sampling

Blood samples were collected before anaesthesia, after induction, during sternal recovery, and at 6-, 12-, 36-, and 60-hours post-surgery. At each time point, 5 ml of blood were collected from the jugular vein using 5 ml syringes with a 21 G needle. One millilitre of the blood was transferred into a plastic blood tube containing ethylenediaminetetraacetic acid for determination of packed cell volume (PCV), plasma protein (PP), complete blood cell and differential leucocyte (WBC) counts. Two millilitres of blood was mixed with sodium citrate for coagulation tests; another 2 mL was transferred into a plain plastic tube for blood chemistry. All samples were processed and analyzed within 24 hours except serum samples for blood chemistry, which were stored at -18°C until analyzed.

Blood analysis

Packed cell volume was determined using the microhaematocrit method, where blood was centrifuged in standard plain haematocrit tube at 12,000 rpm for 5 min (Haematokrit 20, Hettick zentrifugen, Germane) and read on a Hawsley Micro-haematocrit Reader. Plasma protein concentration was measured using a refractometer (Atago T2-NE, Atago CO. LTD, Japan). Total erythrocyte (RBC), WBC, platelet counts and haemoglobin concentration (Hb) were determined using an automated cell counter (ABC Vet, Horibar-ABX, France). Differential WBC count was determined by examining blood smear stained with Wright Stain using a light microscope under 100x magnifications. The battlement method was used.

Plasma harvested from the citrated blood were analyzed for prothrombin time (PT), kaolin-activated partial thromboplastin time (APTT) and plasma fibrinogen concentration using a coagulation analyzer (ST art 4 by Junior instrument for Diagnostica Stago, France) as described in the manual.

Blood samples in plain tubes were left for at least 5 min to clot and centrifuged at 2140 x g (Sigma 2K-15, Sigma, Germane) for 5 min to collect the serum for biochemistry analysis (Hitachi 902, Hitachi LTD, Japan). The standard biochemical test kits from Roche Diagnostica were used. The parameters analyzed were albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), amylase (Amyl), total bilirubin (TBIL), direct bilirubin (DBIL), calcium (Ca), cholesterol (Chol), creatine kinase (CK), creatinine (Crea), gamma-glutamyltransferase (GGT), glucose (Glu), phosphate (Phos), urea, triglyceride (TG), total protein (TP), sodium (Na), potassium (K), chloride (Cl), lactate (Lact) and lactate dehydrogenase (LDH).

Results and Discussion

Packed cell volume, RBC, WBC, Hb, TP and Alb concentration over time showed almost similar pattern (Table 1). They decreased during anaesthesia and started to increase after surgery. The values peaked at 12 to 36 hours postsurgery and returned towards baseline

values at 60 hours postsurgery. The decrease in these parameters was most likely due to hemodilution from fluid infusion during anaesthesia. The increased values for 12 to 36 postsurgery could be due to dehydration following reduced fluid and food intake in the immediate postoperative recovery. In addition, sequestration of RBC into the spleen during anaesthesia, and subsequent release of RBC into the circulation following excitement and stress from postoperative recovery may explain the changes in PCV, RBC and Hb (Schalm *et al*., 1975).

There was a significant increase in neutrophils at 12 hours post-surgery. Neutrophilia is typical in inflammation. Neutrophils are the first line of defense and may represent the major leucocytes several days during inflammation (Male, 2001). In this study, neutrophilia was a normal response to tissue injury induced by the surgical procedures.

Fibrinogen concentration started to increase significantly at 12 hours postsurgery and continued to increase until 60 hours postsurgery, the last sampling point in this study. The increase in fibrinogen following surgery is expected, as fibrinogen is a fibrin precursor involved in haemostatic plug formation following endothelial injury (Dodds, 1989). There was no significant change in platelet number, PT and APTT over time.

Blood glucose increased significantly during anaesthesia, and decreased after surgery. The increase is likely explained by xylazine's action on the α_2-adrenoreceptor of the pancreatic β cells, causing inhibition of insulin production, and lead to hyperglycemia (Felberg and Symonds, 1980).

Creatine kinase, LDH, ALT and AST started to increase at 6 hours postsurgery. Gamma-glutamyltransferase tended to increase but not significant. The increase in these enzymes is explained by muscle injury following surgery in this study (Thrall, 2004). However, the possibility of anaesthesia contributing to the elevation of LDH, ALT and AST as a result of hepatocytes injury cannot be ruled out. Determination of sorbital dehydrogenase would better quantify the extent of hepatocytes injury in this study; however, we were limited by the availability of the reagent.

Creatine kinase started to decrease at 12 hours postsurgery while the other enzymes continued to increase and peaked at 36 hours before decreasing. The earlier decline of CK is explained by its short half-life of approximately 4 hours in ruminants, while half-lives of AST, ALT and LDH are estimated to be around 50 hours (Thrall, 2004). Therefore, analysis of blood after 12 hours following acute muscle injury may miss the CK elevation, and may lead to misinterpretation of the elevation of LDH, ALT or AST being attributable to hepatocyte injuries.

Table 1: Effects of anaesthesia and surgery on blood parameters in sheep (n=6)

Blood parameter	Baseline	Anaesthesia	Post-Surgery				
	pre-anaesthesia	post-induction	sternal recovery	6 hours	12 hours	36 hours	60 hours
PCV (L/L)	0.27±0.02^{bcde}	0.24±0.01^{a}	0.26±0.03^{ab}	0.27±0.03^{c}	0.30±0.4^{de}	0.31±0.04^{e}	0.28±0.03^{bcd}
TP (g/L)	64.33±5.29^{ae}	60.10±4.87^{bd}	59.92±4.45^{bc}	63.23±4.65^{ad}	67.07±5.96^{e}	68.02±3.63^{e}	64.25±3.90^{abce}
Albumin (g/L)	29.47±3.21^{ac}	27.35±2.71^{bd}	27.53±2.19^{bc}	28.92±2.19^{ad}	30.57±2.93^{a}	30.82±2.94^{a}	28.83±2.91^{ab}
RBC (x10^{12}/L)	8.24±0.64^{bcd}	7.27±0.72^{a}	7.76±0.88^{b}	8.24±0.92^{c}	9.17±1.39^{d}	9.00±1.31^{cd}	8.36±0.91^{bc}
Hb (g/L)	85.00±5.02^{bd}	73.50±8.41^{a}	80.00±9.17^{b}	82.17±10.23^{b}	94.00±13.58^{d}	91.33±13.34^{cd}	85.33±9.56^{bc}
WBC (x10^{9}/L)	14.05±4.10^{b}	11.30±3.11^{a}	10.78±3.55^{ad}	15.23±5.27^{bc}	22.17±5.62^{e}	18.35±3.66^{c}	15.75±2.80^{bcd}
Neutrophil (x10^{9}/L)	6.34±2.2^{a}	6.13±2.43^{a}	4.87±2.53^{a}	15.66±10.67^{ab}	17.31±6.03^{b}	11.08±8.5^{a}	6.52±2.76^{a}
Platelet (x10^{9}/L)	222.67±97.99	253.83±89.55	198.67±70.79	193.67±93.56	246.67±74.90	227.50±60.44	268.50±57.85
Fibrinogen (g/L)	2.11±1.05^{ab}	1.85±0.53^{a}	1.96±0.36^{a}	2.03±0.79^{a}	3.60±0.85^{b}	5.59±0.40^{c}	6.51±0.48^{d}
PT (sec.)	21.87±2.13	22.80±2.06	23.30±0.94	23.23±3.80	25.23±1.91	24.53±2.07	23.23±1.30
APTT (sec.)	25.05±5.62	23.12±4.51	24.82±3.72	27.22±15.08	26.80±7.47	27.12±4.61	26.88±4.72
Glucose (mmol/L)	3.77±0.77^{a}	8.55±1.32^{c}	5.65±1.43^{b}	4.08±1.19^{a}	5.12±2.58^{abc}	3.42±0.48^{a}	3.60±0.43^{a}
CK (U/L)	158.00±49.72^{ac}	149.00±68.04^{c}	264.00±149.60^{abd}	867.00±546.67^{b}	273.00±53.39^{d}	161.50±75.40^{ac}	142.50±63.86^{ac}
LDH (U/L)	784.42±191.43^{a}	794.72±177.85^{a}	833.23±182.55^{a}	968.22±189.71^{b}	1074.50±221.67^{c}	1063.25±247.60^{bc}	1008.87±180.72^{b}
ALT (U/L)	11.30±3.91^{a}	10.33±5.13^{a}	10.55±3.22^{ac}	13.82±3.83^{b}	15.77±5.65^{b}	16.95±6.98^{b}	14.50±5.23^{bc}
AST (U/L)	83.37±16.83^{a}	82.12±23.69^{a}	84.17±20.78^{a}	105.10±26.37^{bd}	127.28±36.05^{ce}	137.25±51.42^{bc}	117.35±36.59^{de}
GGT (U/L)	48.67±9.71	46.17±9.77	46.50±9.97	48.83±9.89	51.33±8.85	53.17±8.52	53.83±8.40
Urea (mmol/L)	6.17±2.91	6.88±2.66	6.68±2.47	6.83±2.02	5.40±1.39	5.90±1.83	7.08±2.11
Creatinine (μmol/L)	106.17±18.54	108.83±23.27	104.83±25.73	128.00±43.58	122.83±28.32	128.33±42.61	117.50±28.79

*PCV (packed cell volume); TP (total protein); RBC (total erythrocytes); Hb (haemoglobin concentration); WBC (total leucocytes);
PT (prothrombin time); APTT (activated partial thromboplastin time); CK (creatine kinase); LDH (lactate dehydrogenase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); GGT (gamma glutamyltransferase)
Data are expressed as mean ± standard deviation. Within each parameter, values with same superscript are not different ($p > 0.05$).

Significant changes over time was not detected in ALP, Amyl, TBIL, DBIL, Ca, Chol, Crea, Phos, urea, TG, Na, K, Cl and Lact.

In conclusion, anaesthesia and surgery can affect blood parameters in sheep. Therefore, interpretation of blood parameters in the perioperative period should take into consideration the confounding effects of anaesthesia and surgery.

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GROSS AND HISTOLOGICAL EVALUATION OF FRESH CHICKEN CARCASS: COMPARISON BETWEEN SLAUGHTERED AND CERVICAL DISLOCATED METHODS

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The study was conducted to evaluate the gross and histological appearances of fresh carcasses from slaughtered and cervical dislocated chickens. In this study, 5 adult broiler chickens with homogenous weight were slaughtered and 5 cervical dislocated. The chickens were left aside at room temperature for 30 min prior to gross examination and sampling for histological examination. The gross appearance of muscle and internal organs was evaluated and compared. The internal organs were fixed with 10% formalin for 72 h before undergoing the process dehydration, clearing, impregnation, embedding s into paraffin wax, sectioning and staining with Haematoxylin and Eosin. The sections were examined under light microscope. The results revealed that the carcasses killed by slaughtering method had pallor appearance, while almost all organs of chickens killed by cervical dislocation method appeared reddish. Histologically, there was little difference in distribution or composition of erythrocytes in the visceral organs, except in the lungs, kidneys and liver where those of the slaughtered chickens killed contained fewer erythrocytes compared to the chickens killed by cervical dislocation.

Keywords*:* chicken, slaughtering, cervical dislocation, gross, histology

Introduction

There are various methods of killing chickens. It can be done by slaughtering, cervical dislocation, decapitation, electrical stunning, gas mixtures (90% argon in air or a mixture of 30% carbon dioxide and 60% argon in air), and conventional electrical waterbath stunning systems [120 mA per bird (50 Hz, alternating current, AC) for 4 seconds]. One study showed that decapitation following stunning did not result in consistent carcass quality defects compared to conventional killing (McNeal *et al.* 2003). The same study also showed that there was no differences in 24 h lightness value, yellowness, cook yield, tenderness, or ultimate pH between conventionally killed and decapitated birds. However, blood loss and breast meat redness were inconsistent.

One method of killing of poultry is by dislocation of the neck vertebrae from the cranium to damage the lower brain region, causing rapid unconsciousness. In order to be humane, dislocation must cause severance of the brain from the spinal cord and carotid arteries. This is best achieved using a stretching motion rather than by crushing the vertebrae. The neck is then extended and dislocated by thrusting downward and backward.

Islamic slaughtering method is when the animal is killed by completely severing the windpipe, the gullet, and the jugular vein with using a sharp object (e.g., knife) to inflict a

precise cut. Only one side of the neck (ventral aspect) is cut, so the birds bleed slowly. The spinal cord should not be cut (as when the head is cut off), because the feathers "set" and are hard to pick. The esophagus should also not be cut to prevent microbial contamination from leakage (Sams, 2001). This is to be done quickly and precisely to avoid undue suffering to the animal.

Being a country in which the majority of the people is Muslim, it is necessary to guarantee the consumers are supplied with completely healthy meat, processed in accordance with the highest standard of cleanness and purity while abiding to the requirements of Islam.

Materials and Methods

Animals

The study was carried out on 10 broiler chickens of 6 to 7 weeks old and weighing between 2.0 to 2.2kg. The broiler chickens were randomly selected from the farms, randomly divided into 2 groups of 5 chickens each. The chickens were deprived of food for 12 hours with water provided *ad libitum* prior to euthanasia. This is to avoid spillage of the intestinal contents into the abdominal cavity. The first group of chicken was slaughtered by cutting with a knife, the trachea completely, the common carotid arteries and the jugular veins. The second group was killed by cervical dislocation (un-slaughtered). The neck was extended and dislocated using a sharp downward and backward thrust. All chickens were left aside at room temperature for 30 min before the carcasses were opened and examined.

Gross examination

For each carcass, the skin was removed to expose the muscle. The appearance of muscle was examined and photographs were taken. The abdominal cavity was then exposed by removing the pectoral muscle and the internal structures and organs were examined grossly. The brain, crop, gizzard, duodenum, heart, spleen, liver, pectoral muscle, kidneys, and lungs were removed from the body and comparisons made between the two groups. The heart and spleen of chickens of both groups were dissected and weighed.

Microscopic examination

The samples were fixed in 10% formalin for 72 h before going through the process of dehydration, clearing, and impregnation and then embedding into paraffin wax. The processed samples were chilled on a cold-plate and they were sectioned to 5 µm thickness with a microtome (Machine brand: Shandon Southern Duplex Processor). The sections were floated on the warm bath tub before placing onto the slide. The slide with the sectioned samples was placed on a hotplate to deparaffinize before staining with Haematoxyllin and Eosin (appendix 2). The sections were then examined under light microscope (Leica DM LB2, Germany) to identify, compare and to evaluate the presence of RBC. Eighty slides were examined for each sample under magnification of 20x.

Scoring method

Scoring method was done based on erythrocyte distribution under light microscope (Leica DM LB2, Germany).

Results

Gross Observation

The carcass of chicken killed by slaughtering method showed slightly whitish appearance as compared to the carcass of chicken killed by cervical dislocation (CD) which appeared reddish. Besides that, the liver of the chickens killed by slaughtering method showed that it has undergone discoloration and became pallor as compared to the chickens killed by cervical dislocation method that appeared reddish and enlarged.

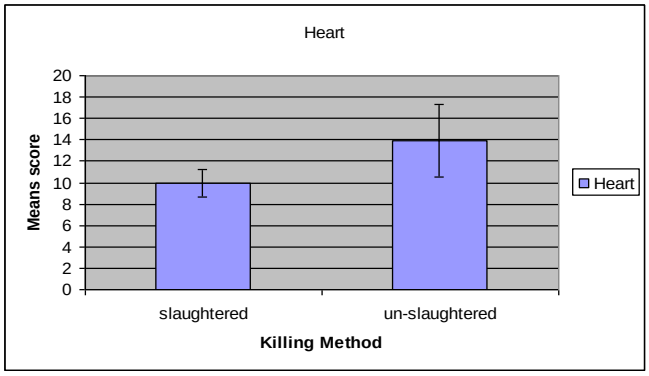

Figure 1: Mean weight of heart between slaughtered and cervical dislocated chickens.

Other organs that show significance changes in its gross appearance were lungs, kidneys, heart, spleen, and duodenum. Organs of the chickens that were killed by cervical dislocation method have shown severe congestion. It can be visibly seen by comparing the color of the organs with the chickens killed by slaughtering method where the organs appear whitish, pale in color.

The heart and the spleen for each chicken were weighed prior to sampling and the result was compared between the 2 methods. The results revealed that the heart and spleen of the chickens killed by slaughtering method had less weight compared to the cervical dislocation method.

Figure 2: Mean weight of spleen between slaughtered and cervical dislocated chickens.

Histological Observation

The histological appearances of lung tissue, liver, kidney and spleen of chicken killed by slaughtering method showed less distribution of erythrocytes as compared to the chickens killed by cervical dislocation (CD) method where the distribution of erythrocytes were widely

distributed and looks compact. The distribution of erythrocytes was scored and was analyzed in the form of mean scores.

Table 1: The distribution of erythrocytes in tissue samples

Organs	**Erythrocyte number (cells/mm^2)**	
	Slaughter	Cervical Dislocation
Brains	1.0 ± 0.0	1.6 ± 0.9
Kidney	2.2 ± 0.5	2.6 ± 0.9
Lungs	3.2* ± 0.8	4.8 ± 0.4
Spleen	2.4* ± 0.6	3.2 ± 0.5
Liver	2.4* ± 0.6	3.2 ± 0.5
Gizzard	1.0* ± 0.0	1.6 ± 0.6
Heart	1.4* ± 0.6	2.0 ± 0.0
Duodenum	2.0* ± 0.7	3.4 ± 0.6
Breast Muscle	1.0 ± 0.0	1.2 ± 0.5

All values are expressed as mean ± std. dev.
For each row means with different superscripts are significantly different ($p<0.05$)

Discussion

The macroscopic comparison and evaluation from this study revealed that there was a significant change in gross appearance of the organs in chickens killed by 2 different killing methods. In slaughtering method, the organ such as lungs, liver, kidney, duodenum and pectoral muscle appeared pallor as compared to the chicken killed by cervical dislocation method where all the organs appeared reddish in color. Chickens killed by slaughtering method appeared pallor in all of the organs. This was because slaughtering method results in rapid gush of blood draining most of it from the chicken's body. Compared to the other method, the organs appeared reddish because the blood had retained in the organs a full volume. This also explained why some organs such as heart and spleen in the chickens killed by cervical dislocation method tend to be heavier than those of the slaughtered chickens. In this experiment, only heart and spleen were weight. This was because, heart is an organ that carries more blood compared to other organs whereas the spleen is the organ that stored blood. It also use as the production of lymphocytes and as the destruction of worn-out erythrocytes.

The microscopic comparison and evaluation in this study revealed that there was a significant finding in erythrocytes distribution within the tissue of some organs. The most significant organ where the distribution of erythrocytes was intense and can be easily seen under microscope (X200) is the lung followed by the liver, kidney and spleen. The blood retention can only be seen in the chickens killed by cervical dislocation method. In slaughtering method, the distribution of erythrocytes was less intense. The main factors that lead to this kind of different were due to the composition of blood in the chicken itself. Chickens that killed by cervical dislocation method causes the spinal cord to become severely damaged and causes the nerves fibers to the heart might be damaged as well leading to cardiac arrest thus resulting in stagnation of the blood in the blood vessels (Aisha El-Awady, 2003). That is why

with this method the organs appeared reddish in color macroscopically and have wide distribution of erythrocytes microscopically.

Conclusion

In conclusion, chickens killed by slaughtering method give more benefits to consumers as compared to the chickens killed by cervical dislocation method. This is because in slaughtering method, it results in the rapid gush of blood draining most of it from the animal's body. The blood must be drained completely before head is removed. This purifies the meat by removing most of the blood that acts as a medium for microorganisms. This microorganism will produce waste and harmful substance that can cause chemical changes in the meat making the meat less healthy and less nutritious. The blood has to be removed to ensure the meat remains fresh longer as compared to other methods of killing.

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BLOOD PROFILE OF THE ADULT MALE ISLAND 'VARIABLE' FLYING FOXES (*PTEROPUS HYPOMELANUS*)

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This study was conducted to establish the baseline haematological values of the island flying fox, *pteropus hypomelanus*. Fifteen blood samples were obtained form 15 adult male bats captured a Tekek, Pulau Tioman, Pahang. The haematological parameters of these wild bats were similar to those of the Little red flying foxes, *Pteropus scapulatus*, except of haemoglobin concentration and PCV, which were lower. The leucocytes counts were within the range reported for other species of bat.

Keywords: island flying fox, haematological parameters

Introduction

The island flying foxes, *Pteropus hypomelanus*, are commonly encountered in the lowland areas in mixed colonies with *Pteropus vampyrus.* They are commonly found in agriculture areas but are absent in primary forests (Heideman and Heaney, 1989; Utzurrum 1984). The 'Variable' flying foxes are one of two megabats that are present in Malaysia. This species habituates the coastal islands of Peninsular Malaysia.

Haematological studies of Chiroptera have been limited to cell counts (Krutzch and Wimsatt, 1963) and a few other selected parameters (Lewis, 1977). Establishing the baseline hematological values is critical for veterinary medicine and care in all species of animals for appropriate clinical application and disease management. However, this information is often lacking in wild animals generally and bats specifically. There is no information on the haematological parameters of the bats in Malaysia; therefore, the objective of the study is to establish the bats' baseline values.

Materials and Methods

A total of 15 blood samples were obtained from 15 adult male bats captured at Kampung Tekek, Pulau Tioman, Pahang. The bats were captured using the mist-net method at dawn and sunset when the flying foxes emerged and returned to their roost. Bats were placed in individual cloth bags tied with a drawstring. Following physical examination, the bats were anaesthetised using ketamine and xylazine intravenously at the dose of 0.1 ml per bat. The bats were then placed in a ventral position and 1 ml of blood was drawn from the cephalic vein into the EDTA tubes. The blood was kept chilled until analysed at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, UPM. Blood smears from each bat were made as soon as the blood was collected. The dried smears were stained with the Wrights' stain and kept in a box until further analysis. The PCV and the blood plasma protein were measured as soon as blood samples were taken.

Results and Discussion

The mean body weight of the captured male bats was 0.5 ± 0.1 kg. The morphology of the erythrocytes of *P. hypomelanus* is similar to other mammals. The cells are biconcave and anucleated resulting in the central pallor. The neutrophils have lobulated nuclei condensed with chromatin that stained blue to purple. The eosinophils have round pinkish granules with nuclei that are less lobulated than the neutrophils. The monocytes are larger than the lymphocytes and have a variable shaped nucleus. The lymphocytes appeared larger than the erythrocytes and have condensed nuclei. The nuclei were round but sometimes oval or slightly indented. The platelets were small, round to oval anucleated cell fragments from cylinders of megakaryocytes cytoplasm. The cytoplasm appeared light blue with many reddish-purple granules.

The mean haematological parameters of the flying foxes are presented in Table 1.

Table 1. Haematological values of the island flying foxes, *pteropus hypomelanus*

Parameters	**Mean ± SD**
Erythrocytes ($x10^{12}$/l)	9.42 ± 0.93
Hemoglobin (g/l)	137.07 ± 13.9
PCV (l/l)	0.42 ± 0.04),
MCV (fl)	46.53 ± 1.68
MCHC (g/l)	313 ± 6.34
MCH (pg)	16.21 ± 6.29
Total Leucocytes ($x10^{9}$/l)	8.71 ± 3.45
Neutrophils ($x10^{9}$/l)	59.5 ± 4.9
Lymphocytes ($x10^{9}$/l)	24.7 ± 9.2
Monocytes ($x10^{9}$/l)	7.4 ± 3.9
Eosinophils ($x10^{9}$/l)	7.8 ± 6.3
Basophils ($x10^{9}$/l)	1.1 ± 1.0
Platelets ($x10^{9}$/l)	550.73 ± 109.84
Plasma protein (g/l)	5.77 ± 0.61

The haematological parameters of these wild bats were similar to those of the Little red flying foxes, *Pteropus scapulatus*, except of haemoglobin concentration and PCV, which were lower. The leucocytes counts were within the range reported for other species of bat.

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COMPARISON OF A-MODE AND B-MODE ULTRASOUND SCANNERS IN PREGNANCY DIAGNOSIS IN GOATS.

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The time taken to make a pregnancy diagnosis and the specificity and sensitivity of two types of ultrasound scanners (A-mode and B-mode) in the hands of two different operators (beginner and expert) was evaluated in this study. Ten does of unknown pregnancy were used in this study. The pregnancy status of the does was assessed using both A-mode and B-mode scanners. Their status using both scanners was checked against their progesterone assay. In this study, we noted that the B-mode scanner was more sensitive at detecting pregnancy in the hands of the expert operator (100%) as against 60% using the A-mode scanner. A diagnosis was arrived at much earlier (68 ± 16 sec) using a B-mode scanner by the expert operator. This was different with the beginner operator; he was unable to optimize both scanners in making a diagnosis (40% for the B-mode scanner and 0% for the A-mode scanner) and a diagnosis was arrived at significantly later than the expert operator (106 ± 69 sec).

Keywords: ultrasound. Pregnancy diagnosis, goats

Introduction

Ultrasound has been available to the medical community since the early 1970s and demonstrated to be a technological breakthrough in the diagnostics of modern assisted reproduction. Since the introduction of the first publication on the use of A-mode ultrasound for the pregnancy detection in sheep over 30 years ago and the uses of the 2-dimensional ultrasound imaging in the equine gynecology over 20 years ago, applications of ultrasonography in the clinical management of animal reproduction has dramatically influenced our understanding of reproduction in farm animals. When artificial insemination became an increasingly accepted practice in animal breeding, the economic priorities, in particular preventing losses and maximizing profits, saw a need for a quick, easy and accurate diagnostic tool. Ultrasonography was the answer, and its usage in many livestock species is well documented.

The A-mode ultrasound (amplitude depth) scanner for pregnancy diagnosis work is based on the detection of foetal mass within a fluid-filled uterus. The ultrasonic waves is emitted from the hand-held transducer, placed externally against the skin of the abdomen, and directed toward the uterus. The reflected ultrasound waves are converted into electrical impulses in the form of audible or visual signal. The unit can detect the fluid-filled organ up to a depth of 20 cm. In the gravid uterus, the unit with oscilloscope display will display ultrasonic reflections as peaks or blips on the screen (Dawson, 1999). The A-mode ultrasound is an easy, fast and simple technique for pregnancy diagnosis but its limitation are in its inability to determine the foetal number and viability of the foetus (Karen and Kovacs, 2001)

The development of real-time (B-mode) or dynamic imaging ultrasonography in late 1970s made this powerful technology adaptable for the study of the internal reproductive apparatus in domestic animals via transrectal route. With ultrasound scanner, an operator may visualize organs previously accessible only with tactile sense (Garcia, 2001). Real-time, B-mode ultrasound displays the returning echoes from a tissue as dots. The intensity of the dot is proportional to the amplitude of the signal and its position is relative to the distance between the probe and the reflective tissue. The image is two-dimensional, created by rapid succession of B-mode traces so that as the ultrasound moves, the image changes, depicting motion in real-time changes. When an electrical field is applied to the crystals in the probe, they change shape and vibrate like cymbals creating waves of sound. The ultrasound probes direct these high frequencies, low intensity sound waves toward the tissues. Different proportions of the sound waves emitted are reflected back to the probe, depending on the density of the tissue. The returning sound waves produce pressure on the crystals, generating electrical change, which is converted to visual image on screen.

Fluids, such as blood or follicular fluid do not reflect sound waves and no image (black) appear on the screen. Bone is the densest tissue and reflects sound waves almost completely depicting white image. Other tissues reflect varying proportion of sound waves and produce image of various shades of gray. The differences of sound waves reflected from various tissue or different angle which sound waves strike tissue surface may cause echo. (Broaddus and Albert, 2005)

In this study we compared (i) the time (in seconds) required before a definitive diagnosis was arrived at and (ii) specificity and sensitivity of the A-mode and B-mode scanners in detection of pregnancy in the hands of two different operators. The first operator was knowledgeable and skilled and the second knowledgeable but had no previous experience on usage of ultrasound for pregnancy diagnosis.

Materials and Methods

This study was conducted at Universiti Putra Malaysia Goat Farm. Ten does of unknown pregnancy status were used in this study. Two operators, one skilled (expert) and the other unskilled (beginner) in the use of the ultrasound for pregnancy diagnosis, evaluated both an A-mode and B-mode scanner ultrasound scanner to diagnose pregnancy.

The operators
The beginner operator was a final year student with the knowledge on principle of ultrasonography, but had no previous experience using it for pregnancy diagnosis in does. The expert operator was a highly trained veterinarian in animal reproduction and has used both mode of ultrasound in pregnancy diagnosis previously.

Samples
Blood plasma was collected to determine progesterone level and this was used as a control confirmatory test of pregnancy (constantly high progesterone level >5 ng/ml throughout the 30 days sampling).

Parameters
The time (seconds) required to make a definitive diagnosis is the time just prior to insertion of probe to the time a diagnosis made was recorded. The specificity and sensitivity of the A-

mode and B-mode scanners in detection of pregnancy in the hands of the operators, was done by comparing progesterone blood plasma level and the examination done using A-mode or B-mode ultrasound scanners. In the analysis for specificity and sensitivity, the results were arranged as follows: true-positive diagnosis (a), false-positive diagnosis (b), true-negative diagnosis (c) and false-negative diagnosis (d). From the values, the sensitivity (a/(a+d) x 100), the specificity (c/(c+b) x 100), the positive predictive value (a/(a+b) and negative predictive value (c/(c+d) x 100) were calculated.

Data recording and analysis

Data was recorded on by an independent individual and the findings of both beginner and expert operators were not revealed until the whole ultrasound session was completed. The data for timing of the scanners by operators was analyzed by T-test at 95% confidence level using SPSS 13.0 computer software.

Results

Table 1 indicates that the accuracy of the A-mode ultrasound in the hands of the beginner operator have a sensitivity of 0% and specificity of 100% with the positive predictive value of 0% and the negative predictive value of 50%. In the hands of the expert operator, the sensitivity was 60% and specificity was 100% with the positive predictive value of 60% and negative predictive value of 71.4%. On the other hand, the accuracy of the B-mode ultrasound in the hands of beginner operator had a sensitivity of 40% and specificity of 100% with the positive predictive value of 40% and negative predictive value of 62.4%. The B-mode ultrasound in the hands of the expert operator gave a sensitivity of 100% and specificity of 100% with the positive predictive value of 100% and negative predictive value of 100%.

Table 1: Sensitivity, specificity and predictive values of A-mode and B-mode ultrasound scanner by different operator in pregnancy diagnosis in does.

Operator	Ultrasound Scanner	a	b	c	d	Se	Sp	+PV	-PV
Beginner	A-mode	0	0	5	5	0%	100%	0%	50%
	B-mode	2	0	5	3	40%	100%	40%	62.4%
Expert	A-mode	3	0	5	2	60%	100%	60 %	71.4%
	B-mode	5	0	5	0	100%	100%	100%	100%

Se=sensitivity; Sp=specificity; +PV=positive predictive value; –PV=negative predictive value

There was a significant difference (at $p<0.05$) in the time taken to diagnose pregnancy using both A-mode and B-mode scanners by both of the operators. The mean time taken by the beginner operator to make a diagnosis using the A-mode scanner was 68 ± 16 sec in comparison to the expert at 53 ± 10 sec. The mean time taken using a B-mode scanner was 106 ± 69 sec by the former, and 41 ± 32 sec by the latter. Results are summarized in Table 2.

Table 2: Time taken to arrive at a diagnosis by both operators using both scanners

Ultrasound Scanner	Operator	N	Mean	Std. Deviation	Std. Error Mean
Bmodetime	Beginner	10	106	69	21
	Expert	10	41	32	10
Amodetime	Beginner	10	68	16	5
	Expert	10	53	10	3

The significantly faster time taken by the expert operator (at $P<0.05$) with both scanners may illustrate that experience can shorten the time required in making diagnosis. The longer time taken by the beginner operator for both scanners can be attributed to a lack of knowledge and familiarization of the reproductive tract. This may imply a lack of confidence and hence hesitation to make a diagnosis in a shorter time.

It should be noted that with the expert operator, the average time needed to make a diagnosis using a B-mode scanner (41 ± 32 sec) was faster than using the A-mode (53 ± 10 sec). This is because the expert operator was capable of locating and identifying reproductive structures and making the diagnosis quicker. Although the A-mode scanner theoretically should be faster and easier to use, the expert operator takes a longer time to make diagnosis compared to B-mode scanner. This could be caused by the fact that A-mode ultrasound requires very precise placement of transducer toward a fluid-filled uterus.

The beginner operator on the other hand arrived at a diagnosis using the A-mode (68 ± 16 sec) in a shorter time than when using the B-mode scanner (106 ± 69 sec). It is possible that in this case, the beginner operator takes a longer time in identifying and interpreting the ultrasound image. With the A-mode scanner, he makes a decision of negative diagnosis only because he did not see the positive spike indicating pregnancy, while being confident that he has placed the probe in the right location. It is also possible that with the A-mode scanner, the tendency to give-up and make a negative diagnosis is higher because of the failure of seeing spikes on the Pregtest Panel.

This study indicated that both scanners had a specificity of 100%. Both scanners are more sensitive when used by the expert operator than by the beginner. It can be implied that getting the most of the scanners would require a working knowledge of reproductive structures and locations as the B-mode scanner would require the ability to interpret ultrasound images and the A-mode scanner requires a correct placement of probe in order not to miss the spike expected in a pregnant animal.

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A RETROSPECTIVE STUDY OF SUPERFICIAL DIGITAL FLEXOR TENDINITIS IN THOROUGHBRED RACEHORSES IN MALAYSIA

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A total of 273 cases of superficial digital flexor (SDF) tendinitis were diagnosed from a total of 447 cases presented with lameness to the turf veterinarian, for which a soft tissue injury distal to the carpus and tarsus was implicated as the cause. This represents 62.3% of all soft tissue injury. The majority (96.3%) of SDFT injury was in the forelimbs, with the right forelimb one and a half times more likely to sustain and injury compared to the left. In 9.7% of these, a bilateral injury was noted. The mid-metarcarpal region or a point 15cm DACB was the most frequent site for SDF tendinitis. At least 50% of the horses in this study, that suffered SDFT injury, did not continue to have an active racing career and were eventually deleted. The majority (42.2%) of core lesion (maximum injury zone) were centrally located in the SDFT. In this study it was also noted that 57.6% of the cases, had percentage of cross-sectional area lesion (% CSAL) ranging between 5% and 26.4%; implying that in most of these cases, the horse will be out of active racing for up to three months at least.

Keywords: Superficial digital flexor tendonitis, equine lameness

Introduction

The superficial digital flexor tendon (SDF tendon) originates from the medial epicondyle of humerus, runs the caudal aspect of the distal radius and along the palmar side of the 3rd metacarpal bone before it inserts into the proximal part of middle phalanx (P2) and the distal part of proximal phalanx (P1) of the forelimb**.** Trauma or injury to the SDF tendon causes inflammation, termed tendinitis. The injury may manifest itself as a curved, bowed-like soft tissue swelling on the palmar side of the metacarpal or metatarsal region, commonly referred to as "bowed tendon". (Smith and Webbon, 1999; Smith and Schramme, 2003; Pasquini *et al*, 1995).

It is one of the most common and potentially career-ending orthopedic injuries in athletic horses (Smith and Webbon, 1999). The injury has been attributed to excessive strain on the tendon from high racing speed (Jorgensen and Genovese, 2003). Studies have shown that the risk of injury from straining forces is higher during the weight-bearing phase of stride when 60% of the horse's weight is loaded in the forelimb. The risk factors for this injury are the high-speed demand on the horses during a race, inadequate training of young racehorses and at the first 3 to 4 races, muscle fatigue and loss of coordination at the end of the race, bad conformation (e.g. angulations of fetlock), incorrect shoeing which causes more strain on the SDF tendon, and working on ground surfaces that are either too soft or too hard (Smith and Webbon, 1999; Jorgensen and Genovese, 2003; Pasquini *et al*, 1995).

The advent of ultrasonography has made it possible to objectively assess the severity of tendon injuries, enable better prognosis and monitoring progress of tendon healing (Reef, 1998; Tucker and Rantenen, 1999; Smith and Webbon, 1999; Jorgensen and Genovese, 2003; Spurlock *et al*, 1989). This study was aimed at identify the type of soft tissue injury common to the equine atheletes in the racing circuit of Malaysia, their common sites and the extent to which injuries can result in lameness in horses.

Materials and Methods

Horses and scope

Thoroughbred racehorses that had raced at the three turf clubs in Malaysia between 2001 and 2005 were the subjects of this study. Ultrasonography records of horses diagnosed with soft tissue injuries in the metacarpal/metatarsal and pastern region of limbs were reviewed. Diagnosis of SDF tendon injuries was made based on review on existing ultrasonograph previously done by the attending veterinarian. Ultrasonographic findings was evaluated to identify limb(s) affected, location of lesion and location on the cross-sectional area of injury, the percent of cross-sectional area (%CSA) involved resulting in lameness. Location of injury was designated by measuring the distance from the accessory carpal bone (forelimb) or tuber calcanei (hindlimb). The identity, sex, age, diagnoses, and comments made by the veterinarian, location and extent of the lesion and their treatment regimes were recorded and subsequently analysed.

Results and Discussion

Four hundred forty-seven cases of lameness attributed to a soft tissue injury were seen at the three turf clubs between 2001 and 2005. It was noted in this study that the majority (96.2%) of soft tissue injury were localized mainly to the SDF tendon and suspensory ligament (SL) in the metacarpal or metatarsal region (3.8%) involved structures in the region of pastern. The type of soft tissue injuries seen are shown in Tables 1 and 2.

The majority (62.3%) of soft tissue injuries of metacarpal or metatarsal region was SDF tendinitis followed by SL desmitis (36.2%). The DDF tendinitis, ALDDFT and annular ligament desmitis are less common at 0.7%, 0.5%, and 0.2% respectively. Approximately half (42.9%) of the horses with SDF tendinitis did not return to race during the period of the study.

Digital flexor tendinitis (both superficial digital and deep digital) in the region of the pastern, and to a lesser extent ODSLD and MCLD were diagnosed during the study.

Off the 268 cases, 50.7% of the SDF tendon injuries occurred in the left forelimb in comparison to 35.8% in the right forelimb. A bilateral limb involvement was seen in 9.7% of the cases. 96.3% of SDF tendon injuries diagnosed were in the forelimb and only 4% were found in hindlimbs. Results are summarized in Table 4.3. Subsequent soft tissue injuries to the contralateral limbs were noted in 15 cases, and this can occur anywhere between 1 and 30 months after the initial injury (Table 4).

Majority (48.1%) of maximum injury zone occurred in the central region, within the cross-sectional area of the SDF tendon and to a lesser extent were core lesions in the palmar border (22.1%), medial border (12.0%), lateral border (4.3%) and dorsal border (2.1%). Also noted,

were a small number of cases (3.4%) where the core lesions were in 2 different locations within the CSA of the SDF tendon. The results are summarized in Table 5.

Table 1. Diagnosis of soft tissue injuries of metacarpal/tarsal regions of horses in turf clubs in Malaysia

Soft Tissue Injuries	Selangor Turf Club	Penang Turf Club	Perak Turf Club	Total (%)
Superficial digital flexor tendinitis (SDF tendinitis)	74	104	90	268 (62.3)
Both branch of suspensory ligament desmitis (Both branch SLD)	10	13	39	62 (14.4)
Lateral branch of suspensory ligament desmitis (Lateral Branch SLD)	17	5	15	37 (8.6)
Medial branch of suspensory ligament desmitis (Medial Branch SLD)	11	5	13	29 (6.7)
Suspensory ligament desmitis (SLD) (Proximal SLD + both branches SLD)	0	6	12	18 (4.2)
Proximal supensory ligament desmitis (Proximal SLD)	1	0	9	10 (2.3)
Deep Digital Flexor Tendinitis (DDF tendonitis)	2	1	0	3 (0.7)
Annular Ligament Desmitis	2	0	0	2 (0.5)
Check Ligament Desmitis (ALDDFT)	1	0	0	1 (0.7)
Total	**118**	**134**	**178**	**430 (100)**

Table 2. Diagnosis of soft tissue injuries in pastern regions of the horses

Soft tissue injury	Total
Superficial digital flexor tendonitis (SDF tendinitis)	5
Deep Digital Flexor Tendonitis (DDF tendinitis)	7
Oblique Distal Sesamoiden Ligament Desmitis (ODSLD)	4
Medial Collateral Ligament Desmitis (MCLD)	1
Total	**17**

Table 3. SDFT and limbs affected with tendinitis in turf clubs in Malaysia

Leg affected	Selangor Turf Club	Penang Turf Club	Perak Turf Club	Total
Left forelimb	37	52	47	136
Right forelimb	28	34	34	96
Bilateral forelimb	7	12	7	26
Left hindlimb	1	4	2	7
Unknown	1	1	0	2
Right hindlimb	0	1	0	1
Total	**74**	**104**	**90**	**268**

Maximum injury zone lesions were noted to localize at a point ranging from 5 to 35 cm distal to the accessory carpal bone (DACB). The mean and median values were 18.2 cm and 17.5 cm DACB respectively. The majority (18.3%) of lesions occurred at 15 cm, followed by 20 cm (11.6%) and 25 cm DACB (10.4%).

Thirty-eight percent of the cases reported were classified mild, 20% were moderate and 42% severe. The results are summarized in Table 6.

Table 4. Interval of time versus leg of horse affected with tendinitis

Time Interval (months)	Leg affected with SDF tendinitis				Total
	Left-fore to Rightfore	Right-fore to Leftfore	Left-fore to Bilateral	Right-fore to Bilateral	
1		1		1	2
3			1		1
4	2	1			3
5			1		1
6				1	1
7	2				2
8	1				1
12	1			1	2
14	1				1
30	1				1
Total	**8**	**2**	**2**	**3**	**15**

Table 5. Maximun injury zone in the limb of horses within a cross-sectional area at presentation.

Location of CL within the cross-sectional area (CSA) or maximum injury zone at the initial presentation	Diagnosis of SDF tendinitis	
	Number of cases	%
Central region	113	48.1
Palmar border	52	22.1
Medial border	28	12.0
Lateral border	10	4.3
Lateral and Medial border	9	3.8
Palmar and Medial border	8	3.4
No CL	8	3.4
Dorsal border	5	2.1
Palmar and Lateral border	1	0.4
Palmar and Dorsal border	1	0.4
Total	**268**	**100.0**

Table 6. Severity of superficial digital tendonitis in horses

Severity of lesion	Diagnosis of SDF tendinitis	
	Number of cases	%
Mild (0-15%)	48	38.4
Moderate (16-25%)	25	20.0
Severe (>25%)	52	41.6
Total	**125**	**100**

Lameness was observed even when percent cross-sectional area lesions (%CSAL) were at 1.5%. In the present study, the mean %CSAL was 26.4 ± 19.78% with the majority (57.6%) of the cases in the lower range (%CSAL between 5% and 26.4%).

Conclusion

This study concluded that SDF tendon injuries are the most common soft tissue injuries in the metacarpal/metatarsal region and pastern region of the equine limb. The results suggested that age and gender of horses are not the related risk for SDF tendonitis. This may be attributed to the phenomenon within the industry, where actively racing population rose from ages of 2 to 3 and decreased from ages 4 to 8 and the majority (80%) of racing horses being gelding. The left forelimb was the most common site of injury. The most frequent breaking-point of the tendon was at the midmetarcarpal or metatarsal region or 15 cm DACB. Core lesion mostly occurred in the central region within the cross-sectional area of tendon. The majority of the cases were severe tendinitis. Severity of injury was highly related to the %CSAL identified during the first presentation of SDF tendinitis.

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EFFECTS OF SUPPLEMENTING MOKUSAKU (WOOD VINEGAR SOLUTION) TO LAYERS RATION

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The effect of Mokusaku supplementation on egg quality, eggshell thickness, and egg production was studied. The quality of the eggs was also determined by a taste panel. In this study, 24 Hisex Brown layers aged 20-weeks were equally divided into control and Mokusaku supplementary groups. The results showed that Mokusaku-treated chickens had lower egg production and thinner eggshells. The taste panel preferred eggs from Mokusaku-treated chickens. This study suggests that Mokusaku is not a necessary supplement for layers.

Keywords: Mokusaku, Hisex Brown, egg

Introduction

Mokusaku is from the wood of broad-leaved trees, such as *Quercus serrata*, *Castanea crenata* and *Prunus jamasakura*. It is known as wood vinegar. Mokusaku is able to eliminate odours (Kutlu *et al*, 2000) and has been used as a supplement in livestock to increase body weight of poultry. The objectives of the current work are to study the effect of Mokusaku supplementation on egg quality, eggshell thickness, and egg production.

Materials and method

Twenty-four layers (20-weeks old Hisex Brown) were equally divided into control and Mokusaku supplementary group. The birds were given water *ad libitum* and commercial feed but the treatment group received 0.5% Mokusaku solution. The birds were weighed weekly and faeces were collected daily. Eggs were collected and the weights were recorded. The eggs were later broken and the albumen thickness were measured using Haugh method. Taste quality scores of the eggs were also taken by serving hard-boiled eggs to the taste panels. Faecal moisture levels were recorded using Moisture Analyser and the ammonia was measured using an ammonia gas detector. Finally, data was analyzed using SPSS software version 11.5.0.

Results and Discussion

The treatment group had a significantly lower egg production rate per day compared to the control and treatment group had a thinner eggshell. However, a taste panel verdict on the hard-boiled eggs preferred the Mokusaku eggs. Results on the faecal ammonia gas level, live weight, egg haugh quality and foecal moisture showed no significant difference.

The lower egg production in the treatment group could be due to the loss of microvilli in the small intestines that causes malabsorption of nutrients and ion (Samanya & Yamauchi, 2002), mainly protein. Higher dietary protein supplement increased egg production (Keshavarz &

Nakajima, 1995). However, Mokusaku that is a wood vinegar reduces the protein content and thus reduces the production.

Reduced eggshell thickness could be attributed to the mineral intake form the supplement. Fatty acids and minerals combination form indigestible mineral soaps that could result in lower energy yield and reduces mineral digestion and this reduces the eggshell thickness.

In conclusion, egg production and quality depend on the diet. Mokusaku is a feed additive, which resulted in lower egg production and shell thickness. Therefore, Mokusaku is an unnecessary supplement for layers.

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HAEMATOLOGY AND SERUM BIOCHEMISTRY PARAMETERS OF ESTUARINE CROCODILES (*Crocodylus porosus*) IN MALACCA ZOO

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This study was undertaken to compile reference haematology and clinical biochemistry values for estuarine crocodile. Blood samples were taken from the dorsal caudal vein of 11 captive adult crocodiles in Malacca Zoo. Basic haematology and serum biochemical values were analyzed from 6 males and 5 females. Blood morphology and differences between sexes were also determined. The present study revealed that values of Hb (117.9 ± 23.2), MCV (258.5 ± 80.0), MCHC (527.8 ± 134.1), MCH (131.8 ± 39.0), lymphocytes (0.9 ± 0.6), monocytes (1.0 ± 0.7), eosinophils (0.4 ± 0.8), basophils (1.8 ± 1.6) showed differences when compared to those values reported by previous studies. The serum biochemistry values were; calcium (4.2 ± 0.9), ALP (148.0 ± 49.2), AST (53.6 ± 27.8), total protein (80.5 ± 8.6), globulin (60.1 ± 8.1), chlorine (94.7 ± 7.8 mmol/L), cholesterol (4.3 ± 1.3 mmol/L) and triglyceride (3.5 ± 5.0). These values differ somewhat with those reported by other researchers. Comparison between sexes revealed significant differences in values of creatinine and ALT.

Keywords: estuarine crocodile, haematology, serum biochemistry

Introduction

Crocodilians are members in the class Reptilia and order Crocodylia. The estuarine crocodile belongs to the family Crocodylidae, subfamily Crocodylinae, genus *Crocodylus* and species *Crocodylus porosus. C. porosus* is characterised by its heavy set of jaws, presence of a pair of ridges along the centre of the snout from the eye orbits, presence of four large nuchals with 2 small scales beside or behind the large nuchals and 19 to 21 single crest caudal whorls. However, it has no hump on snout and postoccipitals. Clinicopathological evaluation is one way of monitoring health status of animals in general, including crocodiles. Haematological values for *Crocodylus porosus* have been reported before on yearlings by Millan *et al*. (1997) and on 2- to 4-year-olds by Canfield (1985). However, no reports have been published on our local *Crocodylus porosus*. Thus, this project was initiated to obtain haematological and biochemistry data of this species of crocodiles reared in captivity.

Materials and Methods

Animals
Eleven crocodiles comprising 6 males and 5 females were chosen from Malacca Zoo. They were fed raw chickens once a week. During sampling all crocodiles were physically restrained. Their Body weights were measured using an EC2000 Indicator® (Tru-Test [99]Weighing Systems). The snout to tail length was recorded via a simple measuring tape based on the snout-tail length, these crocodiles were estimated to be more than 5-year. Blood samples were collected from the dorsal caudal vein using 10 ml sterile syringes and 18G

spinal needles and transferred into lithium heparin and plain vacutainer tubes (Vacutainer®: Becton Dickinson).

Haematology and Biochemical Parameters
All samples were transported within 24 hours to be analyzed at the Haematology & Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Fresh blood smears from each sample were prepared and stained with Wright's Stain (Sigma®) to study the morphology and conduct differential counts of leucocytes.

Pack cell volume (PCV) was determined using a microhaematocrit centrifuge. Haemoglobin concentration was measured by the standard cyanmethaemoglobin method. Total cell counts were obtained using direct manual haemocytometer method (Mohd Halmi and Ragavan, 1999). Samples in plain tubes were centrifuged at 1500 x *g* to extract the serum and placed in serum transport tubes. They were sealed with parafilm and stored at -20 °C pending serum biochemical analysis.

Biochemistry parameters of sodium, potassium, chloride, calcium, inorganic phosphate, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein (TP), albumin (Alb), globulin, A/G ratio, triglyceride and uric acid were estimated from the serum samples using a Chemistry Analyser (Hitachi 902).

Results and Discussion

The haematological and serum biochemistry for estuarine crocodiles are presented in Tables 1 and 2 respectively. The blood cell morphology is shown in Figure 1. The haematological and serum biochemistry values obtained in the present study were compared with earlier reports on *C. porosus* yearlings (Millan et. al., 1997) and on older (2- to 4-year-old) crocodiles (Canfield, 1985) and are shown in Table 3 and 4.

Comparison of the haematological values obtained in this study with those of Canfield (1985) and Millan *et al*. (1997) showed that there are differences in some parameters analyzed. The following parameters: Hb (117.9 ± 23.2 g/L), MCV (258.5 ± 80.0 fL), MCHC (527.8 ± 134.1 g/L), MCH (131.8 ± 39.0), monocytes (1.0 ± 0.7 $\times 10^9$/L), eosinophils (0.4 ± 0.8 $\times 10^9$/L) and basophils (1.8 ± 1.6 $\times 10^9$/L) are found to be higher than those reference ranges reported by Canfield (1985) and Millan *et al*. (1997).

However, the lymphocyte (0.9 ± 0.6 $\times 10^9$/L) concentration in the present study was lower than that of the reference range published by Millan et. al. (1997) but was within the reference range stated by Canfield (1985).

For serum biochemistry, most of the values obtained were within the reference range published by Millan *et al*. (1997) except for globulin (60.1 ± 8.1 g/L), total protein (80.5 ± 8.6 g/L), calcium (4.2 ± 0.9 mmol/L) and ALP (148.0 ± 49.2 U/L) which was higher. However, the concentrations of chlorine (94.7 ± 7.8 mmol/L), cholesterol (4.3 ± 1.3 mmol/L) and triglyceride (3.5 ± 5.0 mmol/L) are slightly lower.

Table 1. Haematological values of estuarine crocodiles

Parameter	**> 5 yrs[a]**	**Yearlings[b]**	**2 - 4 yrs[c]**
PCV (L/L)	0.23 ± 0.06	0.17 - 0.41	0.20-0.22
Hb (g/L)	117.9 ± 23.2	47 - 122	62 – 77
RBC ($\times 10^{12}$/L)	1.0 ± 0.3	0.6 - 1.3	0.9-1.0
MCV (fL)	258.5 ± 80.0	240 - 311	-
MCHC (g/L)	527.8 ± 134.1	261 - 319	-
MCH (pg)	131.8 ± 39.0	72 - 92	-
TWBC ($\times 10^9$/L)	8.7 ± 4.5	6.4 - 25.7	1.8-11.5
Heterophils ($\times 10^9$/L)	4.5 ± 2.1	0.8 - 7.4	0.7-5.8
Lymphocytes ($\times 10^9$/L)	0.9 ± 0.6	4.5 - 21.6	0.2-3.6
Monocytes ($\times 10^9$/L)	1.0 ± 0.7	0.0 - 1.2	0.0 - 1.2
Eosinophils ($\times 10^9$/L)	0.4 ± 0.8	0.0 - 0.7	0.0 - 0.3
Basophils ($\times 10^9$/L)	1.8 ± 1.6	0.0 - 0.4	0.0 – 2.0

[c] Present study; [b] Adapted from Millan et al (1997); [c]Adapted from Canfield (1985);

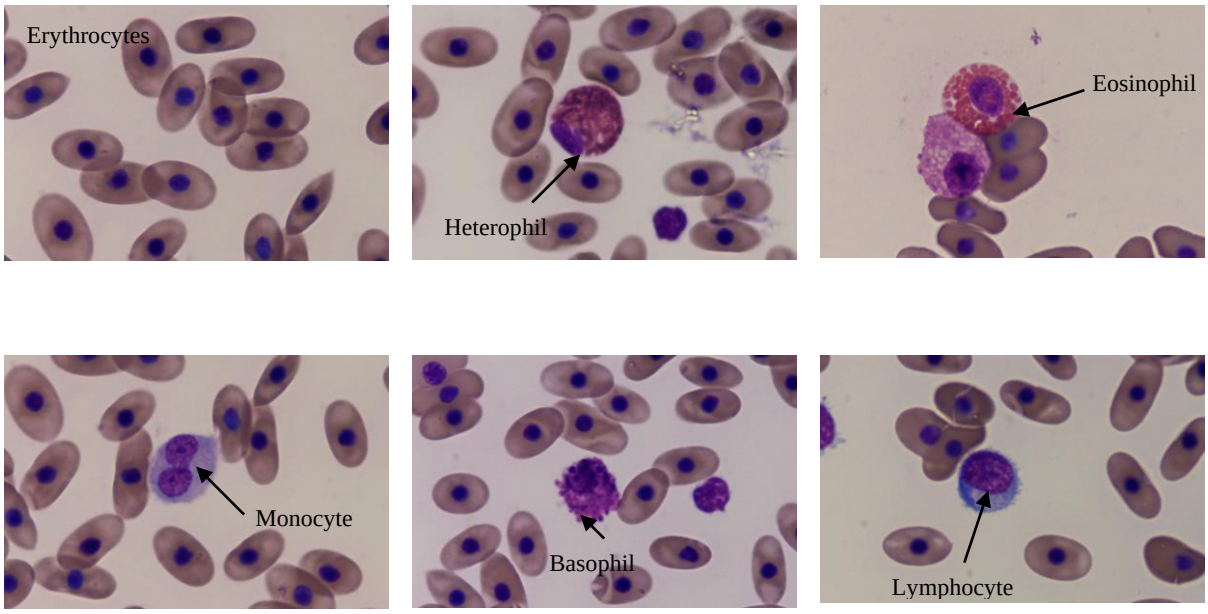
Table 4. Comparison of biochemical values of *C. porosus* between different age groups.

Parameter	**> than 5 years[a]**	**Yearlings[b]**
Sodium (mmol/L)	151.0 ± 6.6	143 - 161
Potassium (mmol/L)	5.4 ± 0.5	3.8 - 7.2
Chloride (mmol/L)	94.7 ± 7.8	88 – 127
Creatinine (mmol/L)	42.1 ± 9.3	20 - 51
Glucose (mmol/L)	6.5 ± 1.7	4.5 - 12.1
ALT (U/L)	28.2 ± 11.4	11 - 51
AST (U/L)	53.6 ± 27.8	23 - 157
ALP (U/L)	148.0 ± 49.2	31 - 180
Total protein (g/L)	80.5 ± 8.6	41 - 70
Albumin (g/L)	20.5 ± 1.6	14 - 23
Globulin (g/L)	60.1 ± 8.1	27 - 50
A/G ratio	0.4 ± 0.1	0.3 - 0.7
Calcium (mmol/L)	4.2 ± 0.9	2.41 - 3.45
Phosphorus (mmol/L)	2.1 ± 0.4	1.2 - 2.9
Cholesterol (mmol/L)	4.3 ± 1.3	1.1 - 7.2
Uric acid (mmol/L)	300.6 ± 170.2	167 - 988
Triglycerides (mmol/L)	3.5 ± 5.1	0.1 - 8.8

[a] Present study; [bAdapted] from Millan *et al* (1997)

In this study, haematology parameters compared between male and female *C. porosus* revealed no significant difference. However, for serum biochemistry, there are significant differences in creatinine and ALT. The number of samples used in the present study was very limited (n=11) and thus, meaningful reference ranges were not established for both haematology and serum biochemical analytes. There are many other factors which can influence these parameters such as environment, reproductive status, nutritional status and most of all stress involved in immobilizing the animal during sampling. Cellular responses of reptilian blood are less predictable than those of endothermic animals whose cellular microenvironment may be more consistent (Mader, 2002).

Figure 1. Blood cells of estuarine crocodiles



Both haematology and serum biochemistry values compiled in this study may provide some valuable guidance for future study of adult *C. porosus*. In order to develop values that can be considered as reference values for adult *C. porosus*, further studies can be made by using larger sample size of clinically healthy animals with better quality control of sampling and processing.

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HAEMOBARTONELLA FELIS IN FLEAS AND CATS

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Haemobartonellosis is an increasingly common infection of cats. In this study on 30 stray and owned cats, 46.7% were positive for *H. felis*. Fleas recovered from these cats were *Ctenocephalides felis felis* (70%) and *Ctenocephalides felis orientis* (17%). Two cats harbouring fleas carried *H. felis*. Although more owned cats (52.6%) had *H. felis* compared to stray cats (36.4%) this difference was not significant. Male cats (56.2%) had higher infection rate compared to female (35.7%). The records obtained from the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM from the period of 2001 to 2005 showed that the overall *H. felis* prevalence during that period was 27%. The infection was detected in all ages of cats from 2 months to 14 years. *H. felis* infection caused a significant decrease in PCV, and increase in icterus index and in plasma alanine aminotransferase concentrations.

Keywords: *H. felis*, cats, fleas

Introduction

Haemobartonellosis or also known as feline infectious anemia (FIA) is a common disease of cats in Malaysia (Lim, 1998). It is caused by the rickettsia, *H. felis*, now reclassified as *Mycoplasma*, hence renamed *Mycoplasma haemofelis* (Brooks, 2004).

In cats with clinical acute haemobartonellosis, there is intermittent fever with progressive anaemia, which corresponds to the level of organisms in the circulating blood. The infection is most common in young cats, if not diagnosed and treated, results in an extended illness with anaemia and perhaps even death (Amstutz, 1998).

The disease is probably transmitted by oral transfer in small amounts of infected whole blood into susceptible cats. Intrauterine transmission can also occur and infection can be transmitted iatrogenically via blood transfusion. However, the natural mode of transmission is believed to be via blood sucking arthropods such as fleas and probably also via bite wounds (Amstutz, 1998). There is no documented reports of *H. felis* in fleas of cats.

This study was undertaken to determine the prevalence of *H. felis* in cat over the period of 5 years from 2001 to 2005.

Materials and Methods

A total of 30 stray and owned cats from Sri Serdang (16), Shah Alam (2), Kota Bharu (6), Gombak (3) and Subang Jaya (3) were used in this project. Ear-tip smears were made from each cat and the presence of *H. felis* was determined using 10% Giemsa staining method and observed under microscope (x 100, oil immersion).

Fleas found on each cat were collected. To collect the flea, "Frontline" (Merial) spray was first applied to the cat. After 10 to 20 minutes, the paralysed fleas were collected.

The data from the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM from 2001 to 2005 of cat cases positive for *H. felis* were collected. The clinical signs were also recorded. The packed cell volume, icterus index and plasma alanine aminotransferase values for the *H. felis*-positive cats were obtained from the Haemaology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, UPM and tabulated.

Results

The 30 ear-tip smears showed that 10 (52.6%) of the 19 owned cats were positive for *H. felis*, while 4 (36.4%) of the 11 stray cats were positive. This difference was not significant ($p<0.05$). The overall prevalence of *H. felis* in the present study was 46.7%.

The prevalence of *H. felis* was higher in male than female cats. The prevalence of *H. felis* infection was 56.3% and 35.7% in male and female cats respectively. This difference between gender was not significant ($P<0.05$).

There were two species of fleas found from the 30 owned and stray cats, identified as *Ctenocephalides felis felis* and *Ctenocephalides felis orientis.* The study also showed that 70% of the cats harboured *Ctenocephalides felis felis,* 17% had *Ctenocephalides felis orientis* and the remaining 13% had no flea. There were only two cases positive for *H. felis* and these cats had fleas that also carried *H. felis.*

The survey from past records of cases positive for *H. felis* from 2001 to 2005 showed the positive cases ranged from 56 to 112 cases. This represents 18.5% to 31.5% of the total feline blood samples submitted to the Parasitology Lab over that period. *H. felis* was detected in all ages of cats ranging from 2 months to 14 years. The data also revealed that cats tested positive for *H. felis* had low PCV, high icterus index and high ALT ($P < 0.05$).

Discussion

The results of the present study showed that the overall prevalence of *H. felis* in the stray and owned cats was 46.7%. It is slightly lower compared to that showed in a survey by Lim (1998). The prevalence of *H. felis* infection from that study over four weeks was 56.1%. This is higher compared to the prevalence of *H. felis* in Glasgow (23.2%) (Nash and Bobade, 1986) and in Wake County (4.9%), North California (Grindem *et al*, 1990).

In our study, the prevalence of *H. felis* was higher in the owned cats compared to the stray cats. However, this difference was not significant. This maybe due to the stray cats developing a certain degree of resistance towards flea infestation and hence did not easily acquire *H. felis* infection. It is easier for the owned cats to be reinfested due to the presence of fleas in the relative confined environment especially in the cages and houses. Fleas can survive such environments for long periods. In the case of the stray cats, their environment is not confined, thus the chance to get flea infestation is lesser compared to the owned cats.

The prevalence of *H. felis* was the same in the male and female cats ($P < 0.05$). Brooks (2004) suggested male are more likely to be infected than female cats, although our study did

not conclusive show this to be true. This may be attributed to the low number of cats used in this study.

Two of the flea smears that were positive for *H. felis* were from owned cats. The reason why there were only two cases positive for *H. felis* from 30 cases was probably absence of engorged fleas in the negative cases. The organism is detected only on the surface of erythrocytes (Cowgill and Latimer, 2001).

Fleas from the cats were also collected for identification. There was only one flea species infestation for each cat. Out of thirty fleas, 21 were *Ctenocephalides felis felis* and 5 were *Ctenocephalides felis orientis*.

The retrospective study showed that the prevalence of positive cases of *H. felis* during the period of 2001 to 2005 ranged from 56 to 112 cases. This represented 18.5% to 31.5% of the total feline blood samples submitted to the Parasitology Laboratory over that period.

The blood parameters of cats tested positive for *H. felis* had packed cell volumes lower than the reference range. This is because the organism can cause destruction of erythrocytes. The icterus index and ALT of the cats tested positive for *H. felis* increased. The increase in icterus index is the result of haemolysis from the infection. *H. felis* do not cause liver damage, thus it is not clear why the plasma ALT in the infected cats increased in this study.

Conclusion

There was no significant difference in prevalence of *H. felis* infection between gender and management of the cats. The present study showed that *H. felis* is present in the fleas of cats. The retrospective study showed that the prevalence of *H. felis* over the period of 2001 to 2005 was from 56 to 112 cases representing 18.5% to 31.5% of the total feline cases referred to the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM. The cats tested positive for *H. felis* showed lower PCV, higher icterus index and plasma ALT than the reference range.

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HIP STATUS OF LARGE/GIANT BREED DOGS IMPORTED INTO MALAYSIA FROM 1999 TO 2005

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The objectives of this study were to document the number of large/giant dogs which undergone hip screen and that their hip status prior to importation into Malaysia. This was a retrospective study of records of the Malaysia Kennel Association (MKA). The results showed that very low percentage of dogs were screened prior to importation into Malaysia. Only 70% of the hips were considered suitable for breeding.

Key words: hip dysplasia, Malaysia, dogs, screening, importation

Introduction

Canine hip dysplasia (CHD) is a common development disease of the coxofemoral joint of dogs (Cook *et al*., 1996; Lust, 1985; Corley, 1985). This disease is genetically inheritable and is passed from sire or dam to offspring. Dogs with hip dysplasia are born with normal hips. When the dog grows older, the joint become abnormal resulting in pain and immobility. This also leads to secondary degenerative changes of the joints. The clinical sign varies according to severity of the malformation. The only tool currently used to examine the occurrence of this disease is hip radiography to access two basic pathological changes; evidences of laxity (looseness/poor fit) and remodeling of the joint due to osteoarthritis changes.

Malaysia imports dogs for various purposes. However, there is no data on the hip status of the imported dogs. Thus, the objectives of this study were to determine the number of dogs imported into Malaysia that had undergone hip evaluation and to determine the hip status of these dogs.

Materials and methods

This is a retrospective study where information was gathered from the registration of imported large and giant breed dogs under Malaysian Kennel Association (MKA). The data collected were from 1st January 1999 to 14th December 2005. Relevant information such as breed, age of importation, country of origin, schemes of hip screening and the hip status were noted for each imported dog.

Results and discussion

One thousand six hundred twenty-five large and giant breed dogs were imported into Malaysia during the period of the study. The five main breeds were German Shepherd Dog (GSD) (467), Golden Retriever (258), Rottweiler (241), Labrador retriever (41) and St. Bernard (27) (Table 1).

Table 1: Age group of imported dogs predisposed to canine hip dysplasia

Breeds	**Age**			**Total importation**
	< 1year	> 1 year	Unknown	
GSD	174	206	87	467
Rottweiler	43	58	140	241
Golden R.	47	34	177	258
Labrador R.	20	8	13	41
St. Bernard	8	0	19	27
Total	**292**	**306**	**436**	**1034**

Only GSD and Rottweiler undergone hip screen prior to importation. The percentages of adult GSD and Rottweiler that undergone hip screen prior to importation were 43% and 6.6% respectively. Only 65% of the GSDs and 69% of the Rottweilers screened were considered suitable for breeding.

A low percentage of dogs were screened prior to importation to Malaysia because most were imported at ages under 1 year. Many countries will only allow screening for hip dysplasia when the dog reaches 12 months of age. Cost of hip screening can also be a contributing factor to the low percentage of hip screening performed. Amongst all, the main factor that leads to low percentage of hip screen prior to importation was the lack of mandatory requirement for the procedure in Malaysia.

The majority of the dogs screened had normal hips. However, there were still about 30% of dogs with hips prone to the development of canine hip dysplasia and considered not suitable for breeding which had been imported into Malaysia.

As a conclusion, this study shows that there is only a very small percentage of dogs imported into Malaysia that had gone through hip screen. Even for those dogs that had undergone hip screen, there were still a high percentage of dogs with hips not suitable for breeding. The authors would like to recommend a mandatory hip screen for adult large and giant breed dogs prior to importation and the screening should be done at the age of one year.

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PATHOGENICITY OF *MYCOPLASMA GALLISEPTICUM* IN SPECIFIC PATHOGEN FREE EMBRYONATED EGGS

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This study was undertaken to determine the pathogenicity of *Mycoplasma gallisepticum* (MG) infection in specific pathogen free chicken embryos and to determine the appropriate viscera organs for sampling in hatched chickens for detection and isolation of MG. Seventy-eight embryonated eggs were grouped into three, according to reference strain, field isolate and uninoculated groups. The embryonated eggs were each inoculated with 0.2 ml "pleuropneumonia-like organism (PPLO)" broth containing 6.2 x 10^5 CCU/ml reference strain or field isolate, via yolk sac, at day 6 of incubation. *Mycoplasma gallisepticum* embryos and control uninoculated embryonated eggs were examined at necropsy on days 7, 10, 13 postinoculation. Postmortem findings of inoculated embryos were dwarfing, curled toes, head oedema, slightly enlarged and pale liver, and slightly enlarged and pale spleen. Reference MG infection group showed more significant gross lesions compared to the group inoculated with field isolate. Histopathological results revealed mild to moderately high inflammatory cells such as neutrophil and lymphocyte infiltrations in the viscera organs (lungs, trachea, bursa, spleen, kidneys, yolk, chorioallantoic membrane, liver, gizzard, and heart). The reference MG infection group showed more significant histopathological lesions compared to the group inoculated with field isolate. Viscera organs such as lungs, trachea, spleens, livers, chorioallantoic membranes, and yolks were shown to be the most probable organs to be sampled for MG, based on the findings of histopathological lesions.

Key words: *Mycoplasma gallisepticum*, pathogenicity, specific pathogen free, embryonated eggs

Introduction

Many mycoplasma serotypes have been isolated from poultry, but only four are commonly recognized as pathogenic species. They are *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis* (MM), and *Mycoplasma iowae* (MI). Most of the other nonpathogenic mycoplasmas are considered to be of questionable pathogenicity (Fabriant, 1969; Jordan, 1979).

Mycoplasma gallisepticum infection commonly causes diseases known as chronic respiratory disease in chickens and infectious sinusitis in turkeys. Although MG is considered the primary cause of chronic respiratory disease, other organisms frequently cause complications. Newcastle disease (ND) or infectious bronchitis (IB) may precipitate outbreak of MG infection. *Escherichia coli* had been found to be a frequent complicating organism.

The transmission of MG infection is found to occur both in vertical and horizontal modes. Vertical transmission (in ova) of MG is known to occur in eggs laid by naturally infected

hens. Increasing the population density increased the rate of the horizontal infection of MG infection. Contaminated airborne, dust, droplets, feathers, poor farming biosecurity and poor personnel practices can also lead to MG infection.

The MG infection usually affects nearly all chickens in a flock but it is variable in the severity and duration of infection. It tends to infect younger birds more severely than mature birds. Beside that, this infection causes reduced feed conversion efficiency, drop in egg production in layers and increase in the medical costs in infected flocks. In broilers, the mortality may range from low in uncomplicated disease to 30% in complicated outbreaks. Downgrading of the carcass, condemnations and retarded growth contribute further to economic losses. Since MG can be egg-transmitted, maintaining chicken and turkey flocks free of MG infection is only possible by obtaining replacement flocks that are known to be free of MG infection. Good farming biosecurity such as all-in and all-out farming management, keeping the birds according to the age groups and control of population density should be implemented to prevent MG infection.

There is still a lack in information and reports regarding MG infection in chicken embryos, which can support the theory of vertical transmission of MG infection. This preliminary study was conducted to establish the pathogenicity of MG infection in chicken embryos and the appropriate viscera organs to be sampled in the detection and isolation of MG in hatched chicks.

Materials and Methods

Embryonated chicken eggs
Specific pathogen free (SPF) embryonated eggs were purchased from Veterinary Research Institute (VRI), Ipoh, Perak, Malaysia. Seventy-eight (78) eggs were used in this study. Out of 78 SPF eggs, 30 eggs were used for MG reference strain inoculation, 30 eggs for MG field isolate inoculation, and 18 eggs as uninoculated controls. To achieve the second objective of the study, six SPF eggs from MG reference group, six from MG field group, and five from uninoculated control group were allowed to hatch at 21 days of incubation.

Mycoplasma strains
Reference strain and field isolate of MG were used in this study. The reference strain (MG-S6) was obtained from Veterinary Research Institute (VRI), Ipoh, Perak, Malaysia, and the field isolate (I-29) was isolated by Tan (2004). Strain MG-S6 was reported to be one of the highly pathogenic MG strains (Jordan, 1979; Power, 1976). Pathogenicity of I-29 (field isolate) is still under investigation (Tan, 2005). The current study was to determine the pathogenicity of reference strain and field isolates of MG.

Determination of Inoculum
The number of viable mycoplasma organism inoculated was determined by microbroth dilution using “pleuropneumonia like organism (PPLO)” broth. Growth was indicated by a change in the phenol red indicator in the medium, from red to yellow. The number of viable organisms in the original cultures was determined by using the tables published by Meynell and Meynell (1970) and expressed as the most probable number of color-changing unit (CCU)/ml. The amount of the inoculum in this study was 6.2×10^5 CCU/ml, based on the study by Kleven (1999).

Embryo Inoculation

All 78 SPF eggs were labeled according to groups, reference, field, or control. The eggshell was swabbed with 70% alcohol before inoculation of the organism into the yolk sac. The blood vessels of the eggs were viewed with the help of egg candler. These vessels may appear as nothing more than an array of faint lines, orange in color, extending from a clear halo. The embryos were within the area of the halo close to the margin of the air cell. A hole was made with an egg punch at the top of the shell. Two groups of 30 SPF embryonated eggs were inoculated via yolk sac by using 26G, 1½-inch needle, at day 6 of incubation with approximately 6.2 x 10^5 CCU/ml, one with reference and the other with field strain isolates in 0.2 ml PPLO broth. The 18 control eggs were not inoculated. Embryonated eggs were maintained in an incubator at 37.5 ºC with 60% humidity. The eggs were candled daily.

Postmortem Examination of Embryos

MG-inoculated and control noninoculated embryonated eggs were examined at necropsy on days 7, 10, and 13, postinoculation. The embryos that died prior to the necropsy (PI 7, PI 10 and PI 13) were kept in the refrigerator at 4 °C for 24 h and examined the day after. Amnioallantoic fluid was cultured on the PPLO broth for mycoplasma isolation and on blood agar to verify bacterial contamination. During necropsy, the embryos were weighed and the body cavities incised. The viscera organs such as liver, heart, lungs, spleen, bursae, trachea, kidneys, gizzard, yolk sac and chorioallantoic membrane were examined before fixing the whole embryo in 40% neutral buffered formalin. Midsagittal sections of embryo heads and bodies were embedded in paraffin. The wings and legs were embedded separately. Tissue sections were cut at 3 to 5 µm and stained with haematoxylin and eosin for observation under the light microscopy. For the eggs that hatched completely after 21 days of incubation, postmortem was carried out on the chicks from reference, field, and control groups. Gross lesions were examined and viscera organs fixed using 40% formalin before embedding in paraffin and stained with haematoxylin and eosin for observation under light microscopy.

Reisolation of Mycoplasma Strain

Amnioallantoic fluid and yolk from each postmortem sample were collected and reinoculated into PPLO broth to determine the presence of mycoplasma. The broth was kept in the incubator at 37 °C for 3 to 5 days. The presence of mycoplasma was indicated by change in colour of PPLO broth from red to yellow.

Results and Discussion

A study was carried out to determine the pathogenicity of *Mycoplasma gallisepticum* in Specific Pathogen Free (SPF) embryonated eggs. For this purpose, gross and histopathological lesions of embryos from embryonated eggs inoculated with reference strain of MG and field isolates were compared with embryos from noninoculated control group. The mean embryo weights of the reference and field groups are lower than the mean embryo weight of the control group during the first, second, and third sampling. At first sampling (day 7 postinoculation), mean weight of control eggs was 7.61 g, for the reference group was 7.14 g and for the field group was 7.32 g. At second sampling (day 10 postinoculation), mean egg weight of the control group was 18.28 g, of the reference group was 16.35 g, and of field group was 13.02 g. At third sampling (day 13 postinoculation), mean egg weight of the control group was 28.48 g, for the reference group was 24.92 g, and and or the field group was 26.21 g. Mean egg weight of the reference group at second sampling was higher than the mean egg weight of field group by 20.37%. Mean egg weight of field group at first sampling

is higher than mean regg weight of reference group by 2.46%. Mean egg weight of the reference group at third sampling was lower than mean egg weight of field group by 4.92%.

The gross lesions observed in embryos of the reference strain of MG and field groups were dwarfing, head oedema, slightly enlarged and pale coloured liver, slightly enlarged and pale coloured spleen, and curled toes. These gross findings were also reported in other studies (Bradbury, 1983; Wakenell, 1995). In comparison, based on severity of gross lesions, the reference strain seems to be more pathogenic than the field isolate.

Histopathological lesions of embryos from reference and field groups revealed that there were mild to moderate inflammatory cells infiltrations such as neutrophils and lymphocytes. These histopathological lesions were also reported by other researchers (Michael e*t al.*, 1996; Kleven *et al.*, 1999; Mahmood *et al*., 1991). In this study, histopathological lesions in the viscera organs of embryos of the reference group showed more severe lesions than the field group, based on the lesion scoring of various viscera organs such as liver, chorioallantoic membrane, gizzard, lungs, heart, spleen, trachea, yolk, kidneys, and bursa. This indicates that the reference strain of MG is more pathogenic than the field MG isolate.

Mycoplasma gallisepticum were successfully reisolated from the air-sac, trachea, yolk, and chorioallantoic membrane of the incomplete hatched chicks from pipped embryos and dead-in-shell embryos. This suggests that MG could possibly cause systemic infections in embryos. The reisolation of MG from some internal organs of experimentally infected embryos were also reported by other researchers, however, the liver and brain were not examined (Bradbury et al., 1983; Reis et al., 1971)

Ten viscera organs that were taken for histopathological lesions such as liver, chorioallantoic membrane, gizzard, lungs, heart, spleen, trachea, yolk, kidneys, and bursa showed that MG from the reference and field groups were pathogenic to embryos. Lungs, trachea, spleen, liver, chorioallantoic membrane, and yolk showed mild to moderate lesions histopathologically, thus, are suitable to be taken as samples for MG isolation. This study showed that the lesion scores in these organs were more significant than in other viscera organs.

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BIRD SPECIES COMPOSITION AND FEEDING GUILD DETERMINATION AT AYER HITAM FOREST RESERVE, PUCHONG, SELANGOR

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A study on bird species composition and feeding guild determination was conducted at Ayer Hitam Forest Reserve (AHFR), Puchong, Selangor. The forest is located at Puchong area, about 25 km from Universiti Putra Malaysia (UPM) and 45 km southwest of the Kuala Lumpur. The study was conducted at four compartments (C12, C13, C14, and C15) of the AHFR area. Mist-netting method was used to capture the birds in the study area. In this study, 158 birds were captured. The captured birds belonged to 26 families consisting of 56 species. The bird composition comprised of insectivorous (6 families), carnivores (5 families), frugivores (2 families), nectarivores (2 families), and combination of above feeding guilds (11 families).

Keywords: Ayer Hitam Forest Reserve, bird species

Introduction

A previous study conducted study on bird species composition in Puchong, Selangor by Zakaria and Rahim (1999) recorded 160 species of bird that belonged to 38 families. The objectives of the present study were to identify the bird species in this area by using morphology of their head, bill, feather, color, tail, feet and special appearance and the feeding guild of the species identified by their bills and feet topography.

Materials and Methods

The study was conducted at four compartments (C12, C13, C14, and C15) of AHFR area. Mist-netting method was used to capture the birds in the study area. Nets were opened just before sunrise about 6.00 to 6.30 am and closed one hour before sunset, at about 6.00 to 6.30 pm. The nets were checked every two or three hours.

Results

In this study a total of 158 birds were captured. The captured birds belonged to 26 families consisting of 56 species (Table 1).

All birds were evaluated for feeding guild characteristics. The bird composition comprised of insectivorous (six families), carnivores (five families), frugivores (two families), nectarivores (two families) and combination of above feeding guilds (11 families) (Table 2). The feeding guilds were identified based on their bill and foot morphology (Table 2).

Discussion

During the four weeks of study period, 56 species of birds were recorded from the 158-individual captured. In a previous study, Zakaria and Rahim (1999), bulbul was the commonly found species. The most abundance species identified in this study was Collared Scoop Owls. This might be due to the abundance of prey in the forest such as rats, squirrels and small size birds (Sures, 2005). One of the factors determining species diversity is the pattern of resource availability (Zulhadzelan, 1998). Lowland tropical forest environment with relatively stable temperature and high humidity support extremely diverse terrestrial organisms, as many of the bird species exist at low density and are expected to be susceptible to any form of disturbance that alters the pattern of resource availability (Karr, 1976). According to Daud (1999), AHFR is still full with edible and potential edible fruits trees for the frugivores, nectarivores and the omnivores groups. The situation is also the same for the insectivore group because of the presence of migratory species such as Flycatcher which typical insect dependent species. Tropical forest such as AHFR is suitable for insect growth (Zulhadzelan, 1998). The availability of fruits in the forest is not only a food source for birds, but also for small mammals such as rats and squirrels that provide food source for carnivore birds (Sures, 2005). Based on this short-term study, the results will provide veterinarian some familiarity with wild bird species and their diet for future medical management program.

Table 1: Number of Species According to Families

FAMILY	COMMON NAME	SCIENTIFIC NAME	LOCAL NAME
ACCIPITRIDAE	Japanese Sparrow hawk	*Accipiter gularis*	Lang Sewah
ALCEDINIDAE	Blue Eared Kingfisher	*Alcedo meninting*	Pekaka Bintik
	Blacked Backed Kingfisher	*Ceyx erithacus*	Pekaka rimba
	White Throated Kingfisher	*Halycon smyrensis*	Pekaka belukar
	Ruddy Kingfisher	*Halcyon coromoda*	Pekaka Belacan
CAMPHEPAGIDAE	Pied Triller	*Lalaga nigra*	-
CAPRIMULGIDAE	Large Tail Nightjar	*Caprimulgus macrurus*	Tukang Kubur
COLUMBIDAE	Spotted dove	*Streptopedia chinensis*	Terkukur
	Zebra dove	*Geopelia striata*	Merbuk
	Green winged Pigeon	*Chalcophas indica*	Punai Tanah
CUCULIDAE	Rusty Breasted Cuckoo	*Cacumantis sepulcralis*	Sewah
	Chestnut Breasted Cuckoo	*Clamator coromandus*	Sewah
CHLOROPSEIDAE	Green Iora	*Aegithina viridissina*	Kunyit Bakau
	Common Iora	*Aegithina tiphia*	Kunyit Kacat
DICAEDAE	Crimson Breasted Flowerpecker	*Prionochilus percussus*	Sepah Puteri Pelangi
HIRUNDIDAE	Pacific Swallow	*Hirundo tabitta*	Sualo batu
	Barn Swallow	*Hirundo rustica*	Sualo Api
PICIDAE	Rufous Piculet	*Sasia abnormis*	Belatuk Kecil
	Rufous Woodpecker	*Micropternus brachyurus*	Belatuk Biji Nangka
	Rufous Woodpecker	*Picus mintalis*	Belatuk Ranting
	Banded yellownape	*Picus miniacens*	Belatuk Merah
	Buff-necked Woodpecker	*Meiglyptes tukki*	Belatuk Tuki-tuki
DICRURIDAE	Bronzed Drongo	*Dicrurus aeneus*	Cecawi keladi
	Crow-Billed Drongo	*Dicrurus annectans*	Cecawi sawai
	Greater Racquet- tailed Drongo	*Dicrurus rennifer*	Cecawi Anting-anting
MOTACILLIDAE	Richard's Pipit	*Anthus novaeseelandiae*	Pipit Tanah
MUSCICAPIDAE	Asian Brown Flycatcher	*Muscicapa latirostris*	Sambar Asia
	Ferruginous Flycatcher	*Muscicapa ferru*ginea	Sambar Rimba
	Narcissuss Flycatcher	*Ficedula narcissini*	Sambar Bunga
NECTARINIIDAE	Purple napped Sunbird	*Hypogramma*	Kelicap Rimba
	Little Spiderhunter	*hypogrammicum*	Kelicap Jantung
		Arachnothera longirostra	
FALCONIDAE	Black-Thighed Falconet	*Microbierax fringillarius*	FalkoRajawali
TYTONIDAE	Oriental Bay Owl	*Phodilus Badius*	Hantu Jampok Pantai

STRIGIDAE	Collared Scops-Owl	*Otus bakkamonea*	Hantu Reban
	Reddish Scops-Owl	*Otus rufescens*	Hantu Merah
	Brown Boobook	*Ninox scatulata*	Hantu Bertemak
	Mountain Scop Owl	*Otus spilocepalus*	Hantu Gunung
	Oriental Scop Owl	*Otus sunia*	HantuKuang Kuik
TIMALIIDAE	Chestnut- winged Babbler	*Stachyris erythroptera*	Rimba Merah
	Black- throated Babbler	*Stachyris nigricollis*	Rimba Bertam
	Short -tailed Babbler	*Trichastoma malaccense*	Rimba Ekor Pendek
	Black Capped Babbler	*Pellorneum capistratum*	Rimba Ekor Hitam
MEROPIDAE	Blue- tailed Bee-eater	*Merops philippinus*	Berek-berek Carik Dada
PLOCEIDAE	White headed Munia	*Monticola gularis*	Pipit Uban
ORIOLIDAE	Black- napped oriole	*Oriolus chinensis*	Dendang
STURNIDAE	Asian Glossy Starling	*Aplonis panayensis*	Perling Mata Merah
	Purple -backed Starling	*Sturnus sturninus*	Perling Belakang Unggu
PYCNONOTIDAE	Yellow-Vented Bulbul	*Pycnonotus goaivier*	Merbah Kapor
	Cream Vented Bulbul	*Pycnonotus simplex*	Merbah Mata Putih
LANIIDAE	Brown Shrike	*Lanius cristatus*	Tirjup Tanah
	Tiger Shrike	*Lanius tigrinus*	TirjupRimau
PITTIDAE	Hooded Pitta	*Pitta sordida*	Pacat Gembala Pelanduk

Table 2: Numbers of family identified according to their feeding guild

Feeding Guild	**Number of families**
Carnivores	5
Insectivores	6
Frugivores	2
Nectarivores	2
Insectivores/Frugivores	3
Insectivores/ Carnivores	1
Insectivores/Omnivores	1
Frugivores/Omnivores	2
Insectivores/Nectarivores/Frugivores	1
Insectivores/Frugivores/Omnivores	3

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COLD ANAESTHESIA IN TROPICAL INDIGENOUS ORNAMENTAL FISH

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The efficacy of cold anaesthesia in conducting physical examination, sampling and minor surgical procedures in tropical indigenous ornamental fish was evaluated. Two species, *Trichogaster tricopterus* and *Puntius schwanenfeldii*, were used. They were acclimatized in water tank at 27 to 28 °C for 1 week prior to the experiment. Cold anaesthesia was achieved by lowering the water temperature using ice packs and maintained at 14 °C. Both species became anaesthetized in less than 3 minutes, and they remained unconscious for 5 minutes out of water. The recovery time was relatively rapid with survivability rate of 100%. No physical side-effect was observed postanaesthesia. Cold anaesthesia was found to be effective in the immobilization and anaesthesia both species of fish, allowing for physical examination, external sampling and minor surgery to be conducted with ease.

Keywords: cold anaesthesia, ornamental fish

Introduction

Trichogaster trichopterus (*sepat ronggeng*) is a freshwater tropical fish, found in muddy and clear rivers, lakes, ponds, drainage canals and paddy fields in Indonesia, Malaysia, Thailand and Vietnam (Rainbroth, 1996). They live in water with temperatures ranging between 22 to 28 °C and can grow to a maximum size of 15cm. *T. trichopterus* is omnivorous, feeding mostly on plants and algae, and occasionally zooplankton, crustaceans, worms and insect larvae (Rainbroth, 1996). This fish is distinguished by a labyrinth organ that permits the fish to breathe air directly from the atmosphere. They are categorized as an obligate air-breather that is they need air to survive (Bond, 1996).

Puntius schwanenfeldii (Lampam sungai) is also a tropical indigenous fish and can be found in rivers, streams, canals, and ditches of Malaysia, Sumatera and Thailand (Kottelat *et al*., 1993). They can grow to a maximum size of 41 cm, live on aquatic macrophytes, submerged land plants, algae and insects. *P. schwanenfeldii* does not have labyrinth organ and is categorized as a normal water-breather fish (Bond, 1996).

The handling of fish both in and out of their natural environment is usually difficult. They characteristically struggle during capture and handling, and this usually strongly affects their physiology. Therefore, it is often necessary to immobilize or anaesthetized the fish before performing any procedures on them. Various anaesthetic techniques such physical, chemical, and psychological method can be applied (Ross and Ross, 1999). Cold anaesthesia or hypothermia is a simple technique where the body temperature of fish is reduced by lowering the temperature of water using a chiller or by the addition of ice. Lowering the temperature of fish will reduce oxygen consumption and body metabolism; blocks nerve conduction and reflexes, and eventually anaesthetize the fish (Roots and Prosser, 1962; Prosser and Farhi, 1965).

Materials and Methods

Ten of each group of *Trichogaster tricopterus* (Sepat Ronggeng) and *Puntius schwanenfeldii* (Lampam Sungai) was used in this study. They were placed in 4 aquarium tanks and acclimatized at 27 to 28°C for 1 week prior to the experiment. The water was extensively aerated and filtrated. No food was given two days before the commencement of the experiment.

The experiment began by putting a pack of ice into the water to lower the temperature until it reached 14 °C. Then, the ice pack was removed from the water to prevent further decrease in water temperature. However, if the water temperature rises during the cold treatment, the ice pack was again placed into the tank. Five fishes from each species were directly transferred one at a time into the tank for induction. The other 5 fishes from each species were used as controls and anaesthetized with MS 222 (Tricaine methane sulphonate). The time taken for each anaesthetic stage was recorded (Table 1). The time for full recovery was also recorded.

Table 1: Anaesthethic stages for cold anaesthesia

Anaesthesia		**Category**	**Activity**
Stage	**Plane**		
0		normal	swimming actively
I	1	Light sedation	became sluggish
	2	Deep sedation	total loss of reactivity to visual and tactile stimuli
II	1	Light narcosis	increase in respiration rate
	2	Deep narcosis	belly up
III	1	Light anaesthesia	stop movement, stay at bottom of tank
	2	Surgical anaesthesia	Immobile and stiff
IV		Medullary collapse	stop breathing, cardiac arrest

Adapted from Stoskopf (1985)

Results

When the water temperature was lowered to 14 °C, both species of fish went through a sequence of anaesthetic stages; Stage 0 (normal) to Stage III Plane 2 (surgical anaesthesia). The fish was sluggish (light sedation) then went into deep sedation (unresponsive to external visual and tactile stimuli). The respiration rate and muscle tone gradually decreased as they went through the stage of light narcosis. In Stages II and III, the fish went belly-up, showed sign of locked-jaw (Plate 1), ceased to respond to stimuli, and remained at the bottom of the tank. They soon ceased all motions and became stiff and immobile. The respiration and opercular movement completely stopped. The time to anaesthetize the fish was significantly different among species (Tables 2 and 3). The fishes did not exhibit any side-effects after revival 24 h later. The rate of survival of the fishes after 1 week was 100%.

Plate 1: *T. tricopterus* showing sign of locked-jaw

Table 2. Time taken to reach the stages of cold anaesthesia in fishes

Fish	Plane	Stage of Anaesthesia (sec)			Duration of Action (sec)	Recovery Time (sec)
		I	II	III		
T. tricopterus	1	27 ± 4	109 ± 33	155 ± 31	287 ± 53	426 ± 48
	2	58 ± 15	130 ± 27	183 ± 31		
P. schwanenfeldii	1	27 ± 9	67 ± 5	95 ± 10	348 ± 24	403±49
	2	46 ± 5	80 ± 3	139 ± 10		

All values are expressed as mean ± std. dev.

Cold anaesthesia is a simple anaesthetic method that allows induction to take place during immersion of fish in cold water (Roots and Prosser, 1962; Prosser and Farhi, 1965). In this study, a few preliminary tests were carried out to determine the ideal temperature for cold anaesthesia. Both species required a longer time to be anaesthetized when the water temperature was high (18 °C). They took approximately 14 minutes to be fully anaesthetized. For practical purposes, this time was too long and may not be suitable for experimental work. When the water temperature was too low (10 °C), lethal shock can occur due to disruption of osmoregulatory capabilities, which may result in major and lethal ion imbalances over short periods (Ross and Ross, 1999). Lower temperatures also cause haemorrhages, for instance, 2 of the *P. schwanenfeldii* showed signs of haemorrhage in the eyes when they were anaesthetized at 10 °C. In this study however, no fish were found dead from thermal shock.

Both species of fish in the control groups took longer and a high dose of MS 222 (150 ppm for *P. schwanenfeldii* and 200ppm for *T. trichopterus;* normal: 50 to 100ppm) to be anaesthetized compared to cold anaesthesia. This could be due to the air-breathing characteristic of *T. trichopterus*, where they have an accessory organ (labyrinth) that can extract oxygen direct from the atmospheric air. Thus, this would interfere with the uptake of anaesthetic agent where a less quantity passed through the gills, which eventually delayed the induction time. Unlike *T. trichopterus, P. schwanenfeldii* does not have labyrinth organ to extract oxygen, but they can prolong the induction time by breathing from the surface of the water, which contain a high concentration of oxygen.

In cold anaesthesia, air-breathing does not contribute to the prolongation of induction time. The fish is in contact with water all the time during cold anaesthesia. When the temperature is low enough, the fish would become immobile and unconscious due to blockage of nerve conduction and lack of oxygen and this leads to anaesthesia. The time of induction of *T.*

trichopterus and *P. schwanenfeldii* was significantly different ($p \leq 0.05$). *T. trichopterus* took longer to be anaesthetized compared to *P. schwanenfeldii*. The exact mechanism of how *T. trichopterus* can remain conscious longer in cold water was not clear. However, the significant difference ($p \leq 0.05$) in duration of action and recovery time among species strongly suggest a correlation with with their air-breathing and normal water-breathing characteristics.

This study showed that the stages of cold anaesthesia cannot be measured by the standard anaesthetic stages described previously by various authors (McFarland 1959; Stoskopf 1985; Brown 1993). This is because, the fishes exhibit body spasm and completely stop breathing while in Stage III Plane 2, whereas this behavioral response does not occur under MS 222 or other chemical agents. Therefore, the anaesthetic stages have to be redefined and modified accordingly to suit the criteria and characteristics of cold anaesthesia in fish.

In conclusion, cold anaesthesia can be an effective form of anaesthetic method for *Trichogaster trichopterus* and *Puntius schwanenfeldii*. At 4 °C, there is a high degree of efficacy of anaesthesia in the fishes. The fish remained unconscious out of water for approximately 5 minutes, enough time for physical examination, external sampling, and minor surgical procedures. Recovery time is relatively rapid at 5 to 6 minutes. Both species survive postanaesthesia without showing any side effect.

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INFECTIVITY OF AVIAN ADENOVIRUS IN SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS

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Group I avian adenoviruses are widely distributed throughout the world and the incidence of disease outbreaks associated with adenovirus infection has been reported at a high rate recently. The virus is easier to grow in primary chicken embryo kidney or liver cell culture than chicken embryos. However, under certain circumstances that limit the use of cell culture, the chicken embryos can alternatively be used for isolation of the virus. It was the objectives of the study to determine the lesions of specific pathogen free (SPF) embryonated chicken eggs following three different routes of avian adenovirus (UPM 04317) inoculation, and to determine the most sensitive route for isolation and identification of the virus. Forty, 9-day-old SPF embryonated chicken eggs were divided into four groups; A, B, C and D. The eggs in group A, B and C were inoculated with adenovirus of the Malaysian isolate (UPM 04317) via the allantoic sac, yolk sac and chorioallantoic membrane (CAM), respectively. Group D acted as the control group. The study showed that that embryonic death was characteristic of the adenovirus infection. It occurred within 5 to 7 days, 5 to 6 days and 4 to 5 days pi in the allantoic sac, yolk sac and CAM routes, respectively with total mortality of 100% within the period. The dead embryos showed haemorrhage and congestion of body with pale and multiple to diffuse white foci observed on the liver and presence of intranuclear inclusion bodies in the CAM, liver, spleen and yolk sac in all groups. No significant difference was observed among groups. It was concluded that SPF embryonated chicken eggs are sensitive and reliable in adenovirus isolation while the CAM appears to be the better route of inoculation followed by the yolk and allantoic sac in the isolation of virus.

Keywords: Specific pathogen free, embryonated chicken eggs, avian adenovirus, allantoic sac, yolk sac and chorioallantoic membrane routes, intranuclear inclusion bodies, virus isolation

Introduction

Adenovirus is a nonenveloped double-stranded DNA virus with icosahedral structure of 70 to 90 nm in diameter (Zsak and Kisary, 1984; McFerran, 2003). The adenovirus family is divided into the genus Mastadenovirus and Aviadenovirus, virus strains that infect mammals and birds, respectively (Ritchie and Carter, 1995). The first avian adenovirus isolated from a distinct clinical condition in birds was from a fatal outbreak of respiratory disease in quail in 1950 (Olson, 1950). Since then, the adenoviruses have been widespread throughout avian species and been isolated from chickens, turkeys, ducks, geese, guinea fowl, pigeon and psittacine birds (Ritchie and Carter, 1995). The avian adenoviruses have been associated with quail bronchitis, inclusion body hepatitis in chickens, turkey viral hepatitis, duck hepatitis, chicken egg drop syndrome, egg drop syndrome in ducks, chicken splenomegaly, marble spleen disease of pheasants and haemorrhagic enteritis in turkeys (Ritchie and Carter, 1995).

The avian adenovirus infections are believed to cause economic losses and may be involved in immunosuppression leading to increased incidence of secondary infection (Rabbani and

Naeem, 1996; Silk *et al*., 1996; Van Eck *et al*., 1976; Xie *et al*., 1998). Hydropericardium-hepatitis syndrome in chickens characterised by high morbidity and mortality was reported previously (Rabbani and Naeem, 1996; Xie *et al*., 1998). Many diagnostic methods have been developed for the diagnosis of avian adenovirus infection including virus isolation in cell culture (Cowen *et al*, 1978; Domermuth *et al*., 1980), the virus neutralisation test and polymerase chain reaction (Xie *et al*., 1998). Generally primary chicken embryo kidney or liver cell culture is a more sensitive growth medium than chicken embryos for the isolation of avian adenoviruses. However, under certain circumstances that limit the use of cell culture, the chicken embryos can alternatively be used for isolation of the avian adenoviruses (Cowen, 1987).

It was the objectives of this study to determine the gross and microscopic lesions of specific pathogen free (SPF) embryonated chicken eggs following three different routes of avian adenovirus of the Malaysian isolate (UPM 04317) inoculation and to determine the most sensitive route for isolation and identification of the virus.

Materials and Methods

Viruses

The avian adenovirus of Malaysian isolate identified as UPM 04317 was isolated from the liver of commercial broiler chickens in a farm in Perak in 2004 (Hair-Bejo, 2005). The liver was homogenised by using mortar and pestle in sterile sand and diluted with phosphate-buffered saline in a ratio of 1:4 (w:v). The liver homogenate was then centrifuged at 3000 rpm for 15 minutes at 4 °C (Sigma 4 kD, B. Braun). The supernatant was collected, syringe-filtered through 0.45 μm, and mixed with antibiotic-antimycotic solution (GIBCO Lab., USA) in a ratio of 1:10 (v:v). The supernatant was kept and stored in the –20 °C freezer until used.

Embryonated Chicken Eggs

Forty, 9-day-old SPF White Leghorn embryonated chickens' eggs (SPAFAS, USA) were divided into four groups namely the groups A, B, C and D. The embryonated eggs in the groups A, B and C were inoculated with 0.1mL avian adenovirus of Malaysian isolate (UPM 04317) via the allantoic sac, yolk sac and chorioallantoic membrane (CAM), respectively following an established technique reported previously (Senne, 1989). The group D acted as the control group. The eggs were monitored twice daily for viability and necropsy was conducted on the dead embryo. The control group was sacrificed at day 7 postinoculation (pi) for gross and histological examination.

Necropsy

The gross pathological changes of the embryos were recorded. Samples of CAM, yolk sac, liver and spleen of the embryo were collected and fixed in 10% buffered formalin for histological examination.

Histopathology

The tissue samples were trimmed and subsequently dehydrated in series of alcohol, cleared with xylene and embedded in paraffin wax using an automatic tissue processor. Tissues were sectioned and mounted on glass slides, dewaxed and stained with haematoxylin and eosin (HE). Tissues were carefully examined under microscope. Five views from each tissue were obtained under 100x objectives, and the number of intranuclear inclusion bodies from each view was recorded.

Results

Mortality and Gross Lesions

Total mortality of 20%, 70% and 100% were recorded in group A at day 5, 6 and 7 pi, respectively. The dead embryos showed haemorrhage and congestion of body with pale and multiple to diffuse white foci observed on the liver. The embryos from the group B showed the highest mortality at day 5 pi (89%) and 100% total mortality by day 6 pi, whilst the embryos from group C started to die at day 4 pi (10%) and 100% total mortality at day 5 pi (Figure, 1). The lesions observed in the embryo from groups B and C were similar to those in the group A. The embryo in the group D (control) remained normal through the trial.

Histopathology

The CAM, liver, spleen and yolk sac of the dead embryo in group A showed haemorrhages, congestion, degeneration and necrosis. Intranuclear inclusion bodies were found in these tissues. Similar lesions were recorded in the groups B and C. There were no significant lesions observed in the group D.

Intranuclear Inclusion Bodies

The number of intranuclear inclusion bodies recorded in the CAM, liver, spleen and yolk sac in the group A were 1.3 ± 2.2, 4.70 ± 3.1, 5.0 ± 3.2 and 4.6 ± 4.0, respectively. In the group B, the number of intranuclear inclusion bodies in the CAM, liver, spleen and yolk sac were 1.0 ± 1.1, 6.6 ± 3.2, 5.0 ± 0.0 and 3.4 ± 1.9, respectively. Meanwhile, in the group C, the number of intranuclear inclusion bodies in the CAM, liver, spleen and yolk sac were 2.1 ± 3.0, 5.8 ± 2.3, 11.0 ± 0.0 and 4.4 ± 2.9, respectively (Figure, 1). In the group D, no intranuclear inclusion bodies were found in the organs. The total number of intranuclear inclusion bodies in the groups A, B and C in all organs examined was not statistically significance ($p < 0.05$).

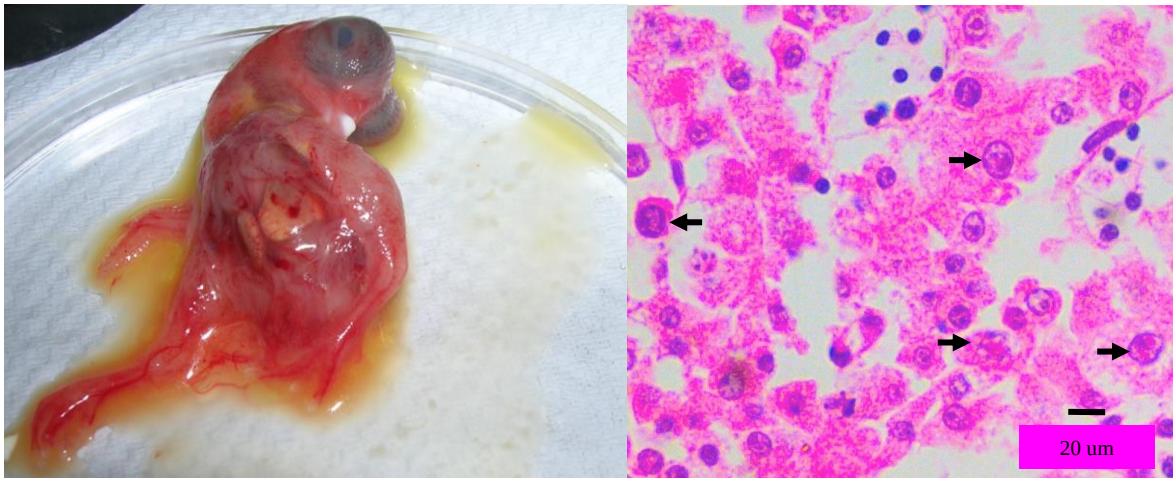

Figure 1: SPF embryonated chicken egg inoculatated with adenovirus (UPM 04317) via CAM showed embryo with pale liver, haemorrhage and congestion of the body and intranuclear inclusion bodies in the hepatocytes at day 5pi.

Discussion

The study showed that embryonic death is characteristic of adenovirus infection. It occurred within 5 to7 days, 5 to6 days and 4 to 5 days pi in the allantoic sac, yolk sac and CAM routes,

respectively. It appears that the CAM route is the better route for isolation of field avian adenovirus followed by the yolk sac and allantoic sac routes. This is in agreement with the previous report that found the CAM route was more sensitive than the allantoic cavity in the virus isolation (McFerran, 2003). However, it has also been shown that the yolk sac was a sensitive route for isolating laboratory strains of adenoviruses representative of 11 serotypes (Cowen, 1987).

The gross and histological lesions of the dead embryo in all groups were compatible with published findings for avian adenovirus (Ritchie and Carter, 1995). Although the total intranuclear inclusion bodies presence in the organ examined was slightly higher in group C then groups A and B, statistically there was no significance difference among the groups.

It was concluded that SPF embryonated chicken eggs are sensitive and reliable in adenovirus isolation with the CAM appearing to be the better route of inoculation followed by the yolk and allantoic sac routes in isolation of virus.

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EFFECTS OF SHORT-TERM SUPPLEMENTATION OF DIETARY N-3 FATTY ACIDS ON PLASMA FATTY ACID PROFILE AND LEARNING ABILITY OF LABORATORY RATS

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The objective of this study was to investigate the effects of a three-week dietary n-3 PUFA supplementation on the plasma fatty acid profile and learning ability in rats. Twenty-four six-week-old Sprague-Dawley rats were assigned randomly to four treatment groups of six animals each. Rats were fed either rat chow only (Control), rat chow containing 10.0% w/w butter (BT), rat chow added with 2.0% w/w menhaden oil and 8.0% w/w soybean oil (S6) and rat chow added 8.0% menhaden oil and 2.0% w/w soybean oil (M3). All diets were isonitrogenous and isocaloric to each other except the control diet. Feed intake per rat was fixed at 15 g/day and water was provided *ad libitum*. Spatial memory was tested using the Morris Water Maze. It was found that the M3 rats had significantly higher ($P<0.05$) percentage of plasma docosahexaenoic acid (DHA) and total n-3 fatty acids when compared to unsupplemented BT and Control rats. The BT rats, which were fed with 10 % butterfat, demonstrated an increase in plasma saturated fatty acid levels. However, there was no significant difference in maze learning ability between groups. In summary, short term dietary fatty acid supplementation were able to induce detectable plasma fatty acid profile changes in rats, but imparted no effect on their Morris Water Maze performance.

Keywords: n-3 fatty acids, learning ability, rats

Introduction

The dietary polyunsaturated fatty acids (PUFA) have clinically significant effects on the fatty acid (FA) composition of blood plasma (Jin *et al*., 2004), and the learning abilities in animals (Ikemoto *et al*., 2000). PUFA are essentially components of the structural membrane lipids, and they are able to modulate the composition and propensity of signal transduction in mammalian cells (Innis *et al*., 2001). The docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6) are the predominant n-3 and n-6 PUFA, respectively, in the mammalian central nervous system (CNS). DHA is selectively enriched in synaptic plasma and retinal membranes, whereas ARA is distributed in second messenger, cell signaling and eicosanoid pathways. Numerous studies have shown that dietary deficiency in DHA and ARA resulted in decreased visual function and alterations on learning ability in rats, apart from alterations of the plasma fatty acid profile (Chalon et al., 1998). However, the focus of this project is mainly on the n-3 fatty acids as these fatty acids have been shown to be critical to the mammalian nervous system (de Wilde *et al*., 2002). The objective of this study was to investigate the effects of a three-week dietary n-3 PUFA supplementation on the plasma fatty acid profile and learning ability in rats.

Materials and methods

Twenty-four six-week-old Sprague-Dawley rats were assigned randomly to four treatment groups of six animals each in this four-week trial, inclusive of a one-week adaptation period. Rats were fed either rat chow only (Control), rat chow containing 10.0% w/w butter (BT), rat chow added with 2.0% w/w menhaden oil and 8.0% w/w soybean oil (S6) and rat chow added 8.0% menhaden oil and 2.0% w/w soybean oil (M3). All diets were isonitrogenous and isocaloric to each other except the control diet. Feed intake per rat was fixed at 15 g/day and water was provided *ad libitum*.

Spatial memory was tested using the Morris Water Maze, which was a circular tank with a diameter of 70 cm and a height of 27 cm. A circular escape platform, measuring 12.5 cm in diameter and 8 cm in height, was submerged 2 cm below the surface of the water hidden from the rat's view. Four points, equally spaced along the circumference of the pool, were arbitrarily assigned as: N, E, S and W, on this basis, the pool area was divided into 4 quadrants (NE, SE, SW and NW). Visual cues were placed above the water level of each quadrant except the NE quadrant. A video camera was mounted next to the NW quadrant. The swim path and time taken to discover the hidden platform was recorded and displayed on a television. The Morris Water Maze Test (MWMT) was performed on all rats upon entry into the trial and at the end of the three-week supplementation period. About 1.5 ml of blood was also obtained via cardiac puncture for plasma fatty acid profile determination.

Results

It was found that the M3 rats had significantly higher ($P<0.05$) percentage of plasma docosahexaenoic acid (DHA) and total n-3 fatty acids when compared to unsupplemented BT and Control rats. The BT rats, which were fed with 10 % butterfat, demonstrated an increase in plasma saturated fatty acid levels. However, there was no significant difference in maze learning ability between groups. There was generally an improvement in time spent locating the platform three weeks after treatment for all groups, which was a clear testament that the rats were adapting well to the test.

Discussion

The plasma fatty acid profiles demonstrated a diet-dependent relationship. It was clear that the plasma fatty acid profiles mirrored those of the diet after only three weeks of feeding. In fact, it is not impossible to induce plasma fatty acid profile changes within two to six weeks of dietary fatty acid supplementation (Wainright *et al.*, 1999).

Generally, the MWMT results showed that all rats had similar mental ability across treatment group base on their maze-solving performance. Although the M3 rats, which were fed a diet enriched with n-3 polyunsaturated fatty acids for a 3-week period had higher plasma levels of DHA, they did not demonstrate significantly ($P>0.05$) better performance in the Morris Water Maze Test when compared to rats from other treatment groups. This finding was inline with that described by Hafandi *et al*., (2005). The long-chained n-3 fatty acids are known to modulate and improve nervous functions through a series of mechanisms related to interneuron signal transduction by cellular membranes (Horrobin and Bennet, 1999). However, the earliest signs of superior Morris Water Maze Test performance can only be seen only after six weeks of n-3 polyunsaturated fatty acid feeding (Hafandi *et al*., 2005).

This is because the central nervous system and more specifically the brain is very resistant to changes in their fatty acid composition due to the blood brain barrier (Bourre *et al*., 1993).

In summary, short term dietary fatty acid supplementation were able to induce detectable plasma fatty acid profile changes in rats, but imparted no effect on the their Morris Water Maze performance.

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CHICKEN ANAEMIA VIRUS INFECTION IN COMMERCIAL BROILER CHICKENS: VIRUS DETECTION BY REAL-TIME POLYMERASE CHAIN REACTION, CLINICAL SIGNS AND HISTOPATHOLOGY CHANGES IN THYMUS

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Previous studies indicated a high seroprevalence of chicken anaemia virus (CAV) in commercial chickens in Malaysia. However, the clinical implication of CAV infection in commercial chickens is not known. This study was undertaken to detect the presence of CAV DNA and its correlation with the clinical signs and histopathological changes in the thymus. A total of 50 commercial broiler chickens were grouped into three different statuses; normal, sick and found dead. Thymus samples were collected for real-time PCR detection of CAV and histopathological examination. Out of 50 chickens, 25 were detected positive for CAV by real-time PCR. Samples that were detected positive for CAV had various levels of viral DNA concentration. However, there were no significant differences in the amount of CAV DNA in thymus obtained from chickens regardless of their health conditions. In addition to that, all the chickens do not exhibit specific lesions that were characteristic of CAV suggesting that the chickens were showing subclinical or latent infection of CAV. Histopathological examination of the thymus alone is not a good indicator to detect CAV in commercial chickens. Real-time PCR offers an alternative approach for rapid and sensitive method to detect the presence of CAV in commercial chicken flocks.

Keywords: CAV, Real-time PCR, Broilers, Thymus.

Introduction

Chicken anaemia virus (CAV) is an economically important avian pathogen with a worldwide distribution (Yuasa *et al*., 1979). The disease is characterized by aplastic anaemia, generalized lymphoid atrophy and concomitant immunosuppresion. Hence, secondary bacterial and/or viral infections are commonly observed in chickens infected with CAV (Rosenberger and Cloud, 1989). In addition, the virus is vertically transmitted and present latently in specific-pathogen-free (SPF) chickens. Clinical implication of CAV infection in chicks with maternal antibody is not clear as the disease exhibits a complex pathogenesis. Studies have also shown that CAV can remain latent in reproductive tract of SPF chickens with high level of neutralizing antibody (Cardona *et al.,* 2000).

A tentative diagnosis can be made with the support of clinical signs and gross pathological lesions. However, to confirm the presence of CAV infection, laboratory diagnosis based on virus isolation, serological and molecular detection is required (Chowdhury *et al*., 2002). The first serological study on CAV was carried out in several broiler and layer farms in Malaysia and the result indicates a high prevalence of the virus in Malaysia (Rozanah *et al*., 1995). Subsequently, researchers have isolated and characterized several local CAV isolates (Chowdhury *et al*., 2002, 2003; Hasmah *et al*., 2004). However, there is lack of study in detection of CAV in Malaysia using real-time PCR. Conventional PCR is highly specific for detection of CAV at a certain amount of viral load. However, real-time PCR offers a much

higher sensitivity compared to conventional PCR. Hence, this study was undertaken to detect the presence of CAV DNA and its correlation with the clinical signs and histopathological changes in the thymus.

Materials and Methods

Commercial broiler chickens
Fifty broiler chickens aged between 26 to 43 days from four farms in the northern area of Johore were used in this study. The boilers were categorized into three groups as normal, sick and found dead (if available). All the farms practiced an open-housed system and with raised floor except for one farm that practiced closed-house system with deep litter floor. However, the farms have different histories of infectious diseases.

Necropsy and histopathological examination
Necropsy was performed on all 50 broilers. The carcasses were examined and any gross lesions found were recorded. Particular attention was paid to lesions with atrophied and pale thymus. Thymus from each bird was collected and fixed in 10% buffered and processed for histopathological examination.

Thymus DNA extraction
Extraction was carried out using method previously described by Chowdhury *et al.* (2002).

SYBR Green I real time PCR
The primers, real-time PCR mixtures and profiles used in this study have been previously described by Wan Keng Fei (unpublished data). The primers are expected to amplify amplicon of 180 bp product. Threshold cycle (Ct) was set at 0.05 to exclude the background fluorescence level that was generated from known negative samples. The real-time PCR results were also verified by agarose gel electrophoresis.

Standard curve
In order to quantify the CAV DNA in the thymus, a standard curve was established using 10-fold serially diluted of a DNA sample that was extracted from thymus of a previously confirmed CAV positive case, MB008/05 (Biologics Lab, FPV).

Results and Discussion
Out of 50 chickens, 25 were detected positive for CAV by real-time PCR. Samples that were detected positive for CAV had various levels of viral DNA concentration. The lowest CAV DNA detected was at 0.3916 ηg and the highest CAV DNA detected was 70600 ηg. However, majority of the broilers that were positive for CAV had viral DNA concentration between 1 to 10 ηg whilst broilers under the normal group had CAV DNA ranging from 2.77 to 8.84 ηg. On the other hand, broilers grouped under sick status had CAV DNA ranging from 1.97 to 6.73 ηg and broilers found dead had CAV DNA ranging from 1.69 to 4.17 ηg. Hence, there were no significant differences in the quantity of CAV DNA in thymus obtained from chickens regardless of their health conditions (Table 1).

In addition, none of the chickens exhibited specific gross or histopathological lesions characteristic of clinical CAV infections (McNulty, 1991). Out of 25 samples that were tested positive for CAV, 12 samples had no histopathological lesions whilst 9 samples has mild histopathological lesions and four samples had severe histopathological lesions. Thus, there is a poor correlation between real-time PCR result and histopathology results.

Histopathological examination of the thymus alone is not a good indicator for CAV infection in commercial chickens. Laboratory tests such as determination of haematocrit values and serum antibodies against CAV are essential to support a tentative diagnosis against CAV. Nevertheless, real-time PCR offers an alternative approach to diagnosis CAV. The real-time PCR proved to be rapid, sensitive, and specific. The detection limit of the real-time PCR was at least 1 ηg but less than 0.1 ηg. In addition, all the 50 extracted thymic DNA samples were analyzed simultaneously and results were available for analysis and interpretation after 5 hours. However, further study on the importance of real-time PCR in detecting clinical and sub-clinical CAV infections in commercial chickens remains to be determined.

Table 1: Relationship between the health status of chickens, real-time PCR detection of CAV and histopathological changes of thymus samples in commercial broiler chickens.

Sample	Status	Thymus lesion	Ct	Tm (°C)	Quantity (ηg)	Remarks
E1	Normal	NSF	16.03	87.6	811.8	NIL
E2	Normal	NSF	17.88	87.6	270.14	NIL
E3	Normal	NSF	24.71	87.6	4.650	NIL
E4	Normal	NSF	19.91	87.6	80.77	NIL
E5	Normal	NSF	23.63	87.6	8.84	NIL
F1	Sick	NSF	24.09	88.4	6.733	vvIBDV
F2	Sick	Atrophied	25.58	88.4	2.771	vvIBDV
F3	Sick	NSF	11.87	87.6	9690	vvIBDV
F4	Sick	Atrophied	25.84	88.0	2.373	vvIBDV
F5	Sick	Atrophied	21.03	87.6	41.66	vvIBDV
G1	Dead	Atrophied	23.58	87.6	9.123	vvIBDV
G2	Dead	Atrophied	22.38	87.6	18.61	vvIBDV
G3	Dead	Atrophied	23.20	87.6	11.42	vvIBDV
G4	Dead	NSF	23.07	87.6	12.34	vvIBDV
G5	Dead	Atrophied	21.55	87.6	30.55	vvIBDV
H6	Normal	Atrophied	25.58	87.6	2.771	NIL
H8	Normal	Atrophied	28.87	87.6	0.3916	NIL
H9	Normal	NSF	24.69	87.6	4.7051	CCRD
I6	Sick	Atrophied	25.88	87.6	2.318	CCRD
I9	Sick	NSF	26.14	87.6	1.986	NIL
J6	Dead	Atrophied	4.66	88.0	70600	NIL
J7	Dead	Atrophied	24.89	87.6	4.172	CCRD
J8	Dead	Atrophied	7.83	87.6	10700	CCRD
J9	Dead	Atrophied	22.46	87.6	17.77	CCRD
J10	Dead	Atrophied	26.41	87.6	1.694	CCRD

NSF = Nonsignificant Finding; vvIBDV = very virulent infectious bursal disease virus; CCRD = complicated chronic respiratory disease.

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A STUDY OF GROWTH KINETICS OF HERPESVIRUSES ISOLATED FROM CAPTIVE WILDLIFE

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The main aim of this study was to determine the growth kinetics of three strains of herpesviruses isolated from captive wildlife animals (V4/05, V19/05 and V5/05). Growth kinetics is one of the important biological determinants of herpesvirus. The influence of various temperatures on the growth kinetics of the isolated viral strains was also studied. The isolated virus was propagated and prepared in stock. Growth kinetic of each strain of virus was incubated at 30 °C and 37 °C and was harvested at different periods. Observation of cytopathic effect (CPE) of each strain revealed differences in the shape and plaque-forming unit. The growth kinetic also differed between each strain. This study also showed that 37 °C is an optimal temperature for viral growth.

Keywords**:** herpesvirus, growth kinetics, cytopathic effect

Introduction

The family herpesviridae consists of three major subfamilies namely, α-herpesvirus which can localized lesion on skin, mucosa and respiration disease, β-herpesvirus can cause respiration and generalized disease and γ-herpesvirus which can cause systemic and tumour disease.

In most natural hosts, herpesvirus produces a fatal infection, but the occurrence of clinical symptoms is inversely related to the age of the affected animals. Herpesvirus infection can affect all types of animal either pet-animal, livestock or wildlife and humans.

The range of replication cycle and cytopathic effect produced on cell culture vary between groups of herpesviruses (Gary *et al*., 1999). A study on the kinetics of infection of cells by animal viruses provides a means for exploring the kinetics and mechanism of virus penetration. It is also important in timing the following events of virus multiplication (Thorne, 1962).

Recently, the virology laboratory in Faculty Veterinary Medicine (FPV) has received many clinical samples from captive wildlife such as bear, monkey, and gaur for herpesviruses screening. Thus, the main aim of this study is to determine the virus growth kinetics of these herpesvirus isolates from these wildlife samples.

Materials and Methods

Viruses
Three cell-culture-purified herpesvirus isolates were obtained from virology laboratory, Faculty of Veterinary Medicine, University Putra Malaysia. They were isolated from cases of

wildlife animals. Isolate V19/05 was obtained from an oral swab of a growth on the mandibular region of a bear, whilst isolates V4/05 and V5/05 were obtained from blood samples of two gaurs.

Cell culture preparation

Vero cell lines were used for tissue culture repassage. Cell cultures were prepared according to the standard method. For growth kinetics study of each virus isolate, 26 Vero cell culture were prepared in 25 cm^3 tissue culture flasks (TPP® Switzerland).

Virus stock preparation

Each viral isolate was propagated in a cell culture and prepared in stock. The stock titer was determined by plaque-forming assay.

*Negative Contrast Electron Microscopy (NCEM***)**

Electron microscopic examination of the purified virus was carried out according to the method described by Ibrahim and Lai (1981).

Cell count

Haemocytometer cell counter was used to determine the number of cells harvested from the tissue culture flask. The cells are counted to allow estimation of required virus inoculation dose for growth kinetics study. A standardized amount of one plaque-forming unit (pfu) of virus was required to infect every 100 cells.

Growth curve study

One hundred microlitre of virus (2 $\log_{10}$ pfu/ml) was inoculated in 25 cm^3 tissue culture flask. Seventy-eight flasks were inoculated in this experiment. At each time-interval of 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 96 and 120 h, two replicates of tissue culture flask were harvested. The supernatant containing extracellular virus ware collected in 10 ml bejo bottle and stored at -70°C (Heto Ultra Freeze, UF375, Denmark). The pellet containing the cell-associated virus was resuspended in 1 ml serum free MEM medium and stored in sterile vial at -70 °C. Both extracellular virus and cell-associated virus were used as a stock for subsequent studies.

Titration of virus by plaque forming assay

Samples from growth kinetics study were serially diluted and the standard plaque-forming assay was performed in 24 wells flat-bottomed culture plates (TPP®, Switzerland). After incubation for 3 days, the plates were fixed and stained with crystal violet (BDH stain, England) to visualize the plaques.

Results and Discussion

Cytopathic effect (CPE)

There are differences in the types of CPE observed between the strains of viruses studied. Strain V4/05 produced syncytial cell both at early and later stages of infection. Strain V19/05 produced numerous ballooning cells at early stage and produced large ballooning cells with giant cells and cytoplasmic extension during the later stages of infection. For V5/05, there was prominent ballooning during early stage of infection and after 72 h there was syncytium formation with peripheral cytoplasmic extension in cell culture.

Growth kinetics

To gain an understanding of the replication of isolates V4/05, V19/05 and V5/05 in Vero, the growth kinetics experiment was conducted. The growth curve exhibited a variation in the growth kinetics of isolate V4/05, V19/05 and V5/05. The findings are summarized in Table 1.

Influence of temperature in growth kinetics of isolates V4/05, V19/05 and V5/05

Studies in cell culture have demonstrated a marked effect by the temperature on the growth and release of herpesvirus (Farnham and Newton 1959). The results showed that at a lowered incubation temperature at 30 °C, the latent period was greatly lengthened. The observed difference in peak titer in the growth curve also indicated the release of infected cells into the medium more efficient at 37 °C compared to 30 °C. The markedly greater stability of the virus at 37 °C could also account partly for greater propensity of the virus to spread through the cell monolayer. Harkness *et al*. (1981) observed that the effective incubation temperature for alcephalines herpesvirus was 32 °C to 34 °C. However, in this study, conducted at 30 °C and 37 °C seems to suggest that later was the optimum incubation temperature.

Table 1 : Summary table of growth kinetics of isolates V4/05, V19/05 and V5/05

Isolate	**Type of CPE**	**Latent period**	**Early detection**	**Peak titer, hours**	**Drop titer, hours**
V4/05, 37 °C	Rounded, ballooning & syncytial cell formation	CA, 2-10 h EC, 2-10 h	CA, 8 h EC, 8 h	CA, 72 h EC, 72 h	CA, 96 h EC, ~↑
V4/05, 30 °C	Rounded, ballooning & syncytial cell formation	CA, 2-12 h EC, 2-24 h	CA,12 h EC, 12 h	CA, 120 h EC, 120 h	CA, ~↑ EC, ~↑
V19/05, 37 °C	Rounded, ballooning	CA, 2-8 h EC, 2-8 h	CA, 8 h EC, 8 h	CA, 72 h EC, 72 h	CA, 96 h EC, ~↑
V19/05, 30 °C	Rounded, ballooning	CA, 2-18 h EC, 2-18 h	CA, 10 h EC, 18 h	CA, 96 h EC, 48 h	CA, 96 h EC, 96 h
V5/05, 37 °C	Numerous, clear rounded cell	CA, 2-24 h EC, 2-18 h	CA, 24 h EC, 8 h	CA, 96 h EC, 48 h	CA, 120 h EC, 120 h

CA=cell-associated virus**;** EC=extracellular virus**;** ~↑=still increased

In conclusion, there were differences in the type of CPE and growth kinetics pattern of isolates V4/05, V19/05 and V5/05. This study also revealed that temperature influences the growth kinetics of the virus isolate. Nevertheless, additional data on the pathogenicity and genomic characteristics of isolates should be obtained for better understanding of their biological properties and classification of viruses.

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IN VITRO EFFICACY OF ANTIMICROBIAL PROPERTY IN GINGER FAMILY AGAINST *BRUCELLA MELITENSIS*

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The present study was carried out to evaluate the efficacy of antimicrobial property of five different species of ginger (*Zingiberaceae*) against five field isolates and strains and one reference strain of *Brucella melitensis*. The minimum inhibitory concentration (MIC) of ginger extracts on Brucella was studied by the broth microdilution method. The ginger species exhibited minimal or no antimicrobial effect on all strains of *Brucella melintensis* tested.

Keywords: *Brucella melitensis*, *Zingiberaceae*, minimum inhibitory concentration (MIC),

Introduction

Brucellosis is a zoonosis caused by several species of the genus *Brucella.* This infection affecting mainly domestic animals can be transmitted from infected animal to humans. In most developed countries, brucellosis remains the most common zoonotic infection although eradication programmes have managed to minimize its prevalence. *Brucella melitensis* biovar 1, 2 or 3 is the main causative agent of caprine and ovine brucellosis. *Brucella melintensis* is one of the most virulent *Brucella* species and has proven to be very difficult organism to eliminate. *Brucellae* are localized intracellularly and infection with these bacteria should be treated with high concentrations of antibiotic to ensure penetration into the cell (Ohan *et al*., 2004). Researchers have been forced to discover new drugs and treatment regimens due to deficiencies in the present therapies, relapses, and difficulties related to the antibiotics used, such as adverse effects, absorbance disturbances and the limited ability of certain age groups to use certain antibiotics. In recent years, multiple drug-resistant microorganisms had increased due to indiscriminate use of commercial antimicrobial drugs in the treatment of infectious diseases. This situation had created the need for new antimicrobial substances from various sources, like medicinal plants, which can be good sources of novel antimicrobial chemotherapeutic agents. Ginger is one of the medicinal plants that are readily available in Malaysia since the plants are widely distributed in the tropical region. A few studies on antimicrobial activities of ginger species on several microorganisms have been conducted previously (Habsah *et al.,* 2000). Efficient utilization of nature's resources in order to produce valuable products such as antibacterials may beneficial to humans and animals alike. The present study was conducted to investigate antimicrobial properties of ginger against *B. melitensis*.

Materials and Methods

Antimicrobial Agents
Five antimicrobial agents from the ginger family namely, *Camptarida ovata*, *Etligera elatior*, *Globba patens*, *Scaphoclymys sp*. and *Zingiber sp*., were use in this study.

Microorganisms
One reference strain and five local isolates strains of *Brucella melitensis* namely, isolates 2, 24, 25, 56, 67 and 16 M (reference strain) were used in this study.

Broth Minimal Inhibitory Concentration (MIC) Test
Solid ginger extracts were dissolved in the DMSO at 100 mg in 1 ml 10% DMSO to obtain a concentration of 100,000 µg per ml. The solution was diluted to a final concentration of 25,000 µg per ml. A serial two-fold dilution was made in sterile 96-wells microplates containing *Brucella* broth in order to obtain concentrations ranging from 12.21 to 12500 µg per ml. One hundred microliters of broth containing 10^8 CFU per ml of *Brucella* was added to all wells. The plate was covered with sterile sealer. The content of each well was mixed on the plate shaker and the plates incubated at 37 °C for 72 h. Microbial growth was determined by plating 5 µl samples from each well on *Brucella* agar. The agar plate was incubated at 37 °C for 48 h. The minimum inhibitory concentration was recorded as the lowest concentration of compound to inhibit the growth of microorganisms.

Results

In the present study, the results showed that all ginger extracts tested has no inhibition effect on the growth of *B. melitensis* isolates and *B. melitensis* (16M) reference strain.

Discussion

Brucella is susceptible to many antibiotics but, as the organism is localized intracellularly, treatment against the infection requires combined regimens of antibiotics and drugs that are able to penetrate the cells (Ohan *et al*., 2004). There are three principal components of the treatment of brucellosis. Adequate intracellular concentration of antibiotic should be achieved, the antibiotic combination should be chosen because of its synergistic effect, and thirdly, *in vitro* susceptibilities of the antibiotics should be evaluated. The most effective antibiotics against *Brucella sp* are the tetracyclines. World Health Organization (WHO) still considered the gold standard for treatment of brucellosis is the combination doxycycline and rifampin or doxycycline with streptomycin. Each of these regimens has disadvantages, which was high incidence of relapses, toxicity and side effects especially in children and pregnant women (Trujillano *et al*., 1999). Antimicrobial resistant strains of *Brucella sp* are reported; however, their clinical implications are not fully understood. The present worldwide occurrence of resistant strains of pathogenic *Brucella sp* had created the urgent need for an alternative treatment for brucellosis, using antimicrobial agents not employed for brucellosis.

This present study focused on *in vitro* susceptibilities of ginger extracts against *B. melitensis.* The antimicrobial property in ginger family against *B. melitensis* was evaluated. Ginger or *Zingiberaceae* family is among the plants family that are widely distributed throughout the tropical region, particularly Southeast Asia. It has been reported that the major active components of ginger was gingerols (Tyler, *et. al,* 1981). Ginger has been used for thousands of years for relief from arthritis, rheumatism, sprains, muscular aches and pain, catarrh, congestion, coughs, sinusitis, sore throats, diarrhea, colic, cramps, indigestion, in appetence, motion sickness, flu, chills and infectious diseases. The antimicrobial activities of ginger family against *B. melitensis* examined in the present study were assessed by MIC values. In this study, extracts from ginger *Camptarida ovata, Etligera elatior, Globba patens, Scaphoclymys sp*. and *Zingiber sp*. were unable to kill or inhibit *B. melitensis* even at a high concentration (12,500 µg/ml). It seems like the antimicrobial component was lacking in

ginger extract. Although no antimicrobial effect against *B. melitensis* was detected in this present study, Chrubasik *et al*., 2005 suggested that some of the ginger species do have antimicrobial property. Ginger extracts exhibit potent antibacterial against Gram-positive and Gram-negative bacteria including *Staphylococcus aureus, Streptococcus pyogenes, Salmonella sp*. and *E. coli* (Chrubasik *et al*., 2005). The finding of the present study suggested that the antimicrobial activity varies widely, depending on the species of ginger and microorganism. Comparisons between the antimicrobial activities of ginger (*Z. officinale*), fingerroot (*B. pandurata*) and turmeric (*C. longa*) showed ginger to have the lowest antimicrobial activity against *Salmonella sp* and *Listeria monocytogenes*. The antimicrobial effect of fingerroot and turmeric may be evidient if the concentration of ginger extract is increased. However, relatively high concentrations of antimicrobials often lead to adverse side-effects.

In conclusion, there is no component in the ginger extracts that specifically targets *B. melitensis.* Based on these findings, it is not recommended to used *Camptarida ovata, Etligera elatior, Globba patens, Scaphoclymys sp.* and *Zingiber sp.* to treat brucellosis due to *B. melitensis.*

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ISOLATION AND IDENTIFICATION OF SALMONELLA IN GREEN IGUANAS

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In Malaysia, keeping iguanas as reptiles are becoming very popular. However, most iguana owners are unaware of the risk of contracting Salmonellosis from these reptiles. *Salmonella* infections usually cause gastroenteritis and can result in invasive illnesses (e.g., septicaemia and meningitis), especially in infants and immunocompromised persons. In iguanas, *Salmonella* resides in the intestine, shedded with the faeces, can be picked-up by humans and cause disease, and therefore is zoonotic. Nine *Salmonella* isolates were obtained from 34 Green iguanas cloacal samples taken from zoos, petshops and individual pet-owners. The isolates were identified and confirmed as *Salmonella* by conventional tests that included cell and colony morphology, characteristics on MacConkey, Brilliant Green and xylose lactose deoxycholate agars, biochemical tests such as triple sugar iron, urease, oxidase, sulphide indole motility tests, and slide agglutination test with *Salmonella* poly-O antisera.

Keywords: Iguana, S*almonella*, Salmonellosis, zoonotic, antibiotic

Introduction

Salmonellosis is an important worldwide health problem, affecting both humans and animals. Illness can occur if live *Salmonella* bacteria enter the body, usually through consumption of foods containing the bacteria. There are about 2000 different species in the genus *Salmonella*, all of which are considered pathogenic, or disease causing. The genus includes *S. typhi* (which causes typhoid fever), *S. choleraesius*, and *S. enteritidis*, which are the most frequent causes of gastroenteritis. *Salmonella* infections can also result in invasive illnesses (e.g., septicemia and meningitis), especially in infants and immunocompromised persons.

Salmonella is a Gram-negative bacterium, often found in the faeces of some animals, particularly reptiles. Reptiles can be an asymptomatic carrier for *Salmonella* and they have been recognized as a source of human salmonellosis for decades. *Salmonella* species were first isolated from snakes, turtles, and lizards in the1940s and recent studies have shown that at least 50% to 90% of these animals are carriers of *Salmonella*. The bacteria are excreted intermittently in the faeces and can be isolated from the cloacae, skin, and throat of water-living reptiles.

Green iguanas have been known to carry *Salmonella marina* that resides in the intestine, that are shed with the faeces, and can be picked up by humans and cause disease. However, most owners are unaware of the risk of contracting Salmonellosis from these reptiles.

Handling of the reptiles can be one of the contributing factors that cause infection in man. People who have these animals as pets have a greater risk of contracting salmonellosis from touching faeces-contaminated skin of the reptile. As with all types of *Salmonella*, the risk of contracting salmonellosis can be reduced with careful attention to hand washing.

While numerous studies have been carried out on the occurrence of *Salmonella* in iguanas elsewhere, no similar study has been done in Malaysia. Therefore, this study was conducted to isolate and enumerate *Salmonella* from Green iguanas and to determine antimicrobial susceptibility pattern of the *Salmonella* isolates.

Materials and Methods

Sampling

Thirty-four Green iguana cloacal swab samples were taken from different locations. The samples were obtained mainly from zoos (27 samples), pet shops in Selangor (4 samples) and from individuals who keep Green iguanas as pets (3 samples). Cloacal swabs were taken using sterile custom-made cotton swabs. All swabs were premoistened with either sterile normal saline or buffered peptone water. The cotton swabs were inserted slowly into the rectum during sampling to prevent any occurrence of rectal prolapsed in the animal. Two cloacal swabs were taken from each Green iguana, one swab was placed into 2 ml of buffered peptone water while the other placed into 2 ml normal saline. Samples were kept in an icebox (4 °C) and immediately transported to the laboratory.

Isolation and identification of Salmonella

Preenrichment and enrichment stage

In the laboratory, the swab samples from the buffered peptone water were incubated at 37 °C for up to 24 h. After the preenrichment stage, 100 µl of the solution was transferred to the Rappaport Vassiliadis (RV) enrichment broth and further incubated up to 24 h at 37 °C.

Isolation of Salmonella species

One loopful of the culture from Rappaport Vassiliadis (RV) enrichment broth was inoculated onto xylose-lysine-deoxycholate (XLD) agar, MacConkey agar and Brilliant Green agar (BGA) and incubated at 37 °C for 24 h. Upon completion, plates were examined for presumptive *Salmonella* colonies by Gram-staining and colony morphology. Pure colonies suspected to be *Salmonella* colonies were subjected to a series of biochemical tests such as triple sugar iron (TSI), urease and sulphide indole motility (SIM) for identification. Colonies showing typical characteristics of the *Salmonella* spp. were then subjected to a slide-agglutination test using *Salmonella* polyvalent 'O' Antiserum A-S (Seiken Co) for confirmation (Quinn, 1994).

Results

Nine (26.5%) *Salmonella* isolates were obtained from 34 iguana cloacal samples. Typical *Salmonella* colonies on xylose-lysine-desoxycholate (XLD) agar are characterized by formation of pink colonies with or without black centers. Colonies on MacConkey agar appear transparent and colourless, sometimes with dark centers. On BGA, colonies of Salmonella appeared red.

All 9 *Salmonella* isolates gave similar results with biochemical tests. On TSI reaction, the test showed positive growth with alkaline slant and acid butt, variable gas production, H_2S-positive and H_2S-negative result with oxidase test. All 9 isolates were negative with urease test and showed similar reactions with the SIM reaction (sulphide-positive, indole-negative, and positive motility).

All presumptive *Salmonella* colonies were confirmed as *Salmonella* by agglutination when mixed with *Salmonella* polyvalent 'O' antisera. All 9 isolates were found to be positive by the rapid agglutination that occurred within seconds.

Discussion

Salmonellosis associated with reptiles is a continuing public health concern. Even though the major source of salmonellosis is food, up to 5% of the cases have been estimated to be associated with exotic pet exposure (Mack, 1998; Zapor 2005). Zapor (2005) reported that *Salmonella* infections typically produced 1 to 3 distinct syndromes: gastroenteritis, typhoid (enteric) fever, or focal disease. Among the exotic pets, green iguanas are among those that can transmit *Salmonella* infection to humans.

As much as 90% of all reptiles are carriers of *Salmonella*. Exposure to iguanas have been associated with infection by *S. marina, S. chameleon, and S. java* (Akhras and Murthy 2000). The main serotypes isolated from patients with reptile-associated salmonellosis include *Salmonella enterica* ssp. *diarizonae* (IIIb) serovars, the *S. enterica* ssp. *houtenae* (IV) serovars *chameleon* and *marina*, and the *S. enterica* ssp. *enterica* (I) serovars *java*, *stanley* and *Poona* (Ackman *et al*. 1995).

Nine (26.5%) *Salmonella* isolates were obtained from 34 faecal material samples. This finding is relevantly lower than what was reported by Chiodini and Sundberg (1981) where they found 84% to 94% of faecal materials contained *Salmonella*. The low prevalence of *Salmonella* in this study may due to the intermittent shedding of organism and also the limited number of samples studied. Thus, faecal and cloacal cultures of Green iguanas may not identify an existing infection.

Selective Medias, BGA and XLD were found to be effective in differentiating *Salmonella* from other bacteria. On BGA, *Salmonella* appears as red colonies while non*Salmonella* appears yellow. Phenol red in BGA will turn colonies into red color if it does not ferment the sugar in the medium (alkaline) (Hirsh, 1990). On XLD, *Salmonella* appears as red colonies with black center. Phenol red substance in this agar will be the indicator for differentiation between acid colonies (non*Salmonella*), and alkaline colonies (possible *Salmonella*) will appear red (Hirsh, 1990). Some researchers use the same agars in isolating and identifying *Salmonella* while others use different selective agars like xylose-lysine-tergitol 4 agar (XLT4), and Hekteon enteric (HE). The sensitivity of XLT4 was comparable to that of HE and with nearly 100% specificity and it can be regarded as an alternative for the isolation of nontyphoid *Salmonella*e from stool samples (Dusch and Altwegg, 1995)**.**

Zoonotic implication of *Salmonella* should be considered and taken seriously. Most study reported that infant and children below the age of 10 are most susceptible to being infected especially with reptile-associated *Salmonella*. Older people are no exceptions. *Salmonella* survives well in the environment and can be isolated for prolonged periods from surfaces contaminated by reptile faeces. For this reason, even minimal indirect contact with reptiles can result in illness.

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CASEOUS LYMPHADENITIS IN GOATS IN SELANGOR

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Two hundred twelve serum samples were obtained from goats in seven farms in Selangor. Two serological tests were done, which were the indirect enzyme-linked immunosorbent assay (ELISA) and the agar-gel precipitation test (AGPT) to detect the antibodies for caseous lymphadenitis (CLA). The overall prevalence was high by the ELISA method but low by the AGPT. Forty-eight (22.6%) samples were positive by ELISA while only seven (3.3%) samples were positive by AGPT. High prevalence of infection was shown in one farm (72.7%) whilst the prevalence is low in the other six farms (3.3% to 30%).

Keywords: caseous lymphadenitis (CLA), goats, Selangor, ELISA, AGPT

Introduction

Caseous lymphadenitis (CLA) is a chronic disease of small ruminants caused by *Corynebacterium pseudotuberculosis*. The disease causes economic losses due to loss of body condition and subsequent reproductive failure. The disease can also affect other mammals including horses, cattle and man (Brown and Oleander, 1987, Hommez *et al*., 1999). In goats, *C. pseudotuberculosis* infection leads to abscess formation in the superficial lymph nodes, although frequently disseminates into the visceral lymph nodes and organs. The organism was first isolated in Malaysia in 1970 in a goat and in 1971 in a sheep. Sheikh-Omar and Chulan (1980) and Tham and Sheikh-Omar (1981) have shown the economic importance of this disease in goats and sheep in Selangor. The prevalence of CLA in goats in Peninsular Malaysia was almost 7% in which of the 3484 serum samples tested by the gel diffusion test, 242 samples were positive (Bahaman *et al.* 1989). Diagnosis of CLA is based on the characteristic clinical signs and the detection of *C. pseudotuberculosis* in the abscess (Kaba *et al*., 2001). Serological diagnosis is very important since subclinically infected animals could be a potential source of infection for healthy goats. Several serological tests have been described for diagnosis of CLA such as a microaglutination assay, immunodiffusion test, gel precipitation test, complement fixation assay, indirect haemaglutination test and enzyme-linked immunosorbent assay (ELISA).

The purpose of this present study was to determine the presence of CLA in goats in Selangor by using ELISA and agar gel precipitation test (AGPT).

Materials and Methods

Blood sample
Seven goat farms from different areas in Selangor were selected. Males and female goats from various age groups and breeds were selected randomly from each farm and blood samples were taken from the jugular veins into plain blood tube. The blood samples were centrifuged at 3000 rpm for 15 min to obtain the serum. The serum samples were then kept in the sterile tubes and stored at -20°C until used.

Agar gel precipitation test (AGPT)
The method used in this test was as described by Zaki (1968). One-hundred microlitre (100 µl) of test serum samples were placed in the six wells cut into the ion agar plate. The central well was filled with 100 µl *C. pseudotuberculosis* exotoxin. One peripheral well of each plate was filled with 100 µl positive control serum. The plates were incubated at room temperature in moist environment for three days. The plates were examined for presence of precipitation lines.

Enzyme linked immunosorbent assay (ELISA)
Pure cultures of *C. pseudotuberculosis* were subcultured in the BHI and incubated at 37 °C for 24 h. Then the BHI culture contains cell density of approximately 1 x 10^8 cells/ml was centrifuged at 10000 rpm for 30 min to pellet. The pellet was washed three times with PBS. The pellet was then resuspended in 1.5 ml lysis buffer. The suspension was boiled 5e min, cooled and centrifuged at 10000 rpm for 5 min. The supernatant was then used as the ELISA antigen. The ELISA procedure used in this study was as described by Maki *et al.*, 1985. Optical Density (OD) was measured at 405 nm and serum was recorded as positive if the OD reading is two times higher than the OD reading of the negative control serum.

Results

Thirty-three percent (7/212) of the samples were positive for CLA by the presence of precipitation lines on AGPT. Goats from three farms were negative by this test, while the highest percentage is from Farm 7 (8.8%). Forty-eight (22.6%) sera were positive for CLA as detected by ELISA. The highest percentage is from Farm 4; almost 73% of the goats were positive for CLA, while the lowest percentage is from Farm 5 (3.3%). Seven sera were tested positive for CLA by both indirect ELISA and AGPT, while 164 samples were negative by both tests. Of all 212 samples, 41 sera were positive by indirect ELISA but negative by AGPT.

Table1: Prevalence of positive caseous lymphadenitis amongst goats in different farms in Selangor

Owner	**No. of Samples**	**ELISA Positive**	**% Positive**	**AGPT Positive**	**% Positive**
Farm 1	22	16	72.7	0	0
Farm 2	47	10	21.2	1	2.1
Farm 3	19	1	5.2	1	5.2
Farm 4	10	3	30.0	0	0
Farm 5	30	1	3.3	0	0
Farm 6	50	8	16.0	2	4.0
Farm 7	34	9	26.4	3	8.8
Total	**212**	**48**	**22.6**	**7**	**3.3**

Discussion

The overall prevalence of CLA in goats in Selangor is 22.6% and 3.3% as detected by ELISA and AGPT respectively. This study showed that ELISA is more sensitive compared to

AGPT. However, the specificity of the test needs to be ascertained. The prevalence of CLA in goat in the present study is considered low. One of the factors is that the antibody titer cannot be detected in animals with new infection that occur within two weeks. In naturally infected animals, it has been observed that some animals with lesions of CLA have no detectable antibody titer, even when the causative agent can be isolated from the lesions (Brown *et al*., 1986; Kuria, 1989). The prevalence of infection within farms depends on the vaccination status of the goats, management and source of animals in the herd. Many of the goats in the farms were imported from various countries. The goats were not previously screened for CLA before mixing with the goats that already existed in the farms. The goats in Farm 3 and Farm 5 were imported from Australia but they were not screened for CLA. Some of the goats were brought into the herd from another herd that may have history of CLA. These goats may act as asymptomatic carrier that can contribute to the introduction and spread of infection in the farm. The AGPT has several advantages as well as an provide an alternative and newer method for the diagnosis CLA. However, the result of this test is subjective compared to ELISA method. The ELISA may overcome the problem faced using the AGPT method. In our study, 19.3% of the goats were positive by the indirect ELISA but negative by AGPT. The sensitivity of ELISA were 85%, however the specificity is yet to be determined. The ELISA has several advantages over the other serologic tests for diagnosis or screening of CLA (Ter Laak *et al*., 1992). The test is easy to perform and many sera can be tested in a short time. It was suggested that ELISA technique can be used as a diagnostic tool to detect CLA in goats.

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ASSESSMENT OF ANTIMICROBIAL PROPERTIES OF CULINARY PLANTS EXTRACTS ON BACTERIA ISOLATED FROM FISH ULCER

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Aquaculture is an important business as protein supplier for human consumption. Unfortunately, the main hindrance in aquaculture activity is the disease outbreak especially bacterial diseases. To treat bacterial diseases, a variety of antibiotics was used, in many instances indiscriminate and uncontrolled. Inevitably, the problem of antibiotic-resistance bacteria development and antibiotic toxicity in fish and human as consumer are of major concern. Thus, alternative antibacterial materials must be found. Accordingly, the objective of the study was to evaluate the antibacterial properties of culinary herbs viz., garlic, ginger and shallot to replace the use of synthetic antibiotics. The herb extracts were tested against two bacteria namely *Aeromonas hydrophila* and *Shewanella putrefaciens* isolated from fish ulcerative disease. The assessments were made using bacterial sensitivity to the herb extracts, disk sensitivity test and assessment of treatment response in the fish injected with the bacteria. From the findings, garlic showed better antibacterial properties than either ginger or shallots. Garlic is bactericidal at low concentrations. In the disk sensitivity test, garlic showed better growth inhibition properties. In experimental infection through subcutaneous injection, *Shewanella putrefaciens* induced more prominent ulcer lesions compared to *Aeromonas hydrophila.* In conclusion, garlic can be used as an antibacterial agent against *Shewanella putrefaciens* and *Aeromonas hydrophila.*

Keywords: antibacterial properties, herbs, fish, ulcerative disease

Introduction

Ulcerative disease is one of the commonest and most problematic diseases in cultured fish. The disease is manifested by superficial and deep necrotizing ulcers on the fish body and on the head. These lesions initially appear as a 'bruise' in the skin and over the period of several days progress to the loss of scales and dermal tissue often extending to exposure of underlying body muscle or cartilage. Secondary invasion of the ulcer by bacteria and fungi is common. The severity of the disease varies but significant mortality may occur. This disease is a particular feature of cultured fish and is known by several names such as "Motile Aeromonad Septicaemia" (M.A.S), "Hemorrhagic Septicaemia," "Ulcer Disease," or "Red Sore Disease." The many synonyms of this disease related to the lesions caused by the invading bacteria include septicemia where the bacteria or bacterial toxins are present within organs of the fish, and ulcers of the fish's skin. Basically, the disease is caused by *Aeromonas hydrophila*. This Gram-negative bacterium is an opportunistic pathogen that invades tissues of the host, rendered susceptible by stress or other disease processes. In fact, most bacteria that infect fish are Gram-negative including *Aeromonas salmonicida, Vibrio sp., Shewanella putrefaciens, Edwardsiella tarda, Yersinia ruckeri Pasteurella piscicida* and *Pseudomonas sp.*

Antimicrobial agents have been widely used in aquaculture worldwide to treat infection caused by a variety of bacterial pathogens of fish. The evidence is clear that aquaculture use of antimicrobial agents has raised resistant bacteria in the exposed bacterial flora in the fish and in the environment (Inglish *et al*., 1991; Midtvedt & Lingas, 1992; Barnes *et al*., 1994). Furthermore, since antimicrobial agents are administered by mixing with feed that is then dispersed in the water or as in the immersion treatment, directly dosing the environment had resulted in additional toxicification to the ecosystem. Hence, alternative treatments or medicines using bioactive materials from plants as antibacterials are much sought after**.** Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions from hundreds, if not thousands, of indigenous plants, dating back to prehistory (Freiburghaus *et al*., 1996; Cowan, 1999). Thus, the main objective of this project was to assess the antibacterial properties of three culinary herb extracts, namely garlic (*Allium sativum*), ginger (*Zinziber officinale*) and shallot (*Allium ascalonium*) against two bacteria isolated from ulcerative disease in fish.

Materials and Method

Bacterial Preparation
Two aquatic bacteria viz., Aeromonas *hydrophila* and *Shewanella putrefaciens* were cultured onto Tryptic Soy Agar and incubated at 30 ^{o}C for 24 h prior to use. These bacteria were originally isolated from skin ulcer lesions in Koi carps. .

Experimental Fish
One hundred catfish (*Clarias batracus*) with an average length of 4 inches were used. Thirty fishes were divided into six 3 x 2 x 2 ft size aquarias and the rest were kept as a control. The fish was maintained with internal box filters and fed pellets twice a day at 2% body weight. The fish were injected subcutaneously with the bacteria singly and in combination (10^{6} cfu/ml) and observed for development of skin ulcers.

Herbs Preparation
The herb was skinned and washed thoroughly before blending in sterile double-distilled water. The resultant homogenates were then centrifuged twice at 250 x *g*, at 4 ^{o}C for 10 min. The clear supernatants obtained were then diluted (in ppm) and tested against the two bacteria. The antimicrobial evaluations were made by mixing different concentrations of herbs (1, 5, 10, 25, 50, 100 ppm) against known concentrations of bacteria (diluted at 10^{-3}, 10^{-6}, 10^{-9}) for 30 min to 1 h, and overlying on agar plates, by the disk diffusion method (disk impregnated with herbs extracts at different concentrations), and by healing responses in the fish injected with the bacteria.

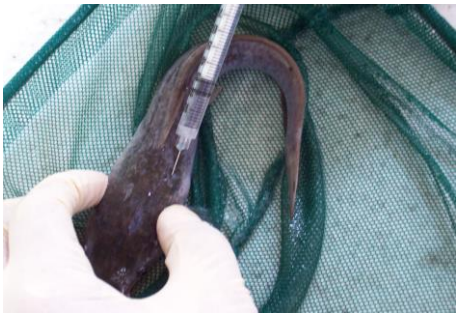

Figure 1. Subcutaneous injection of fish

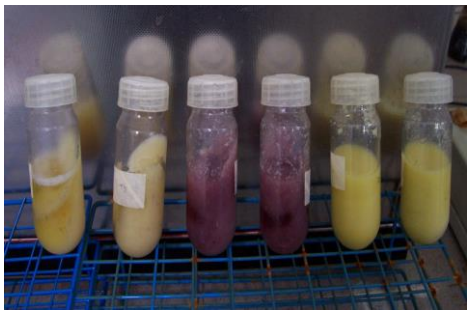

Figure 2. Herb extracts after centifugation

Results and Discussion

In the pathogenicity test, the results showed that *S. putrefaciens* induced more prominent ulcer lesions than *A. hydrophila* in catfish subcutaneously injected with the bacteria. In the healing evaluations, garlic showed better antibacterial properties than the other two culinary herbs (ginger and shallots). Garlic extract induced most rapid healing i.e. in 2 days at 50 ppm compared to ginger extract which took 3 days at 100 pm and shallots extracts taking 6 days at 100 ppm. In direct exposure method, it was shown that, generally garlic extracts killed or inhibited both bacteria at lower concentrations (25 to 50 ppm) and in a shorter time (30 min exposure). While, in the disk diffusion test, only garlic and ginger extracts at 100 ppm, showed mild inhibition (halo ring formation) on *A. hydrophila* agar lawn.

In conclusion, the study showed that garlic could be used as an antibacterial agent against *S. putrefaciens* and *A. hydrophila*. The garlic extract can be used as a topical application or as permanent bath for ulcerative diseases in freshwater fishes. Future in-depth studies need to be done to assess the natural active chemicals of these herbs as potential fish antibacterials. The current findings presented a promising future for culinary herbs as an alternative to synthetic antibiotics in the treatment of microbial diseases in food fish.

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SPECIES OF FLIES FEEDING AND BREEDING ON CHICKEN CARCASSES

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Carrion-breeding flies is of ecological, medical, veterinary and forensic importance. In this study, 12 two-month-old apparently healthy jungle fowls were slaughtered and flies were allowed to feed and breed naturally on the carcasses. Three of the carcasses were placed individually on a bed of sand in an uncovered pail. Three other carcasses serving as controls were similarly treated but the pail was covered with a net to prevent flies from reaching the carcasses. The experiment was replicated. The adult flies caught feeding on the exposed carcasses were identified as *Chrysomya megacephala*, *Musca domestica*, *Sarcophaga* and *Lucillia* species. Total disintegration of fly-infested carcasses occurred after 120 to 168 hours. In the control group, the carcasses totally disintegrate after 144 to 240 hours. There was no significant difference in total carcass disintegration between the fly-infested and control group. After the total disintegration of the carcasses, the larvae of the flies were collected and identified. Most of the larvae were identified as *Chrysomya megacephala*. This preliminary study suggests that larvae of the flies may play a minor role in the disintegration process of exposed carcasses.

Keywords: Chrysomya megacephala, carcass disintegration

Introduction

Forensic entomology is an important investigative tool providing an estimation of the post-mortem interval (PMI) particularly in homicide cases. The application of entomology in investigation of criminal cases demands accurate PMI estimations which may be based on the identification of insect specimens harbouring on the rotting flesh (Prins 1982; Wallman 2001). Many species of blowfly are attracted to the dead animal and human bodies (Greenberg and Singh 1995). The adult flies will feed on secretions, including blood and gravid females rapidly lay their eggs on the bodies. *Calliphora vicina* for example hatches their eggs at temperatures above 15°C within 24 hours of infestation. The larvae feed on the rotting tissue and once fully grown usually leave the body to pupariate elsewhere. Some fly species pupariate on or close to the carcasses (Greenberg, 1971).

The biology of flies breeding on carrions is important, especially in forensic science. The life-cycle of the flies provides the coroner with information on the time of death. The insect specimens may be removed from the corpses for identification. The size of the larva, usually represented by its length, is related to its age and is a function of time. In forensic investigation, this is used to determine the PMI.

Materials and Methods

Animals
Twelve, two-month-old apparently healthy cross-bred jungle fowl were used in this study. The birds were slaughtered and each carcass was weighed and placed in a pail on a 1 cm deep sand bed. All pails with carcasses were left exposed in an open field. Three of the carcasses-

containing pails were left uncovered and served as the experimental group while three others were covered with nets to prevent flies from assessing the carcasses. The experiment was replicated.

Collection and identification of adult flies

To collect and identify the flies, three additional two-month-old cross-bred jungle fowls were slaughtered and placed in similar type of pails. The carcasses were place about 5 feet away from the experimental site. The flies landing on the exposed carcasses were caught and identified based on a pictorial key (Zump 1965). The species and number of flies caught were counted every hour between 8 am to 5 pm daily.

Carcasses

The total disintegration of carcasses was determined by tissue disintegration leaving only the skin and bones. The disintegrated carcasses were then weighed. At this time the fully matured larva had began to migrate into the sand bed. The larvae and pupae were collected, counted and separately preserved in 70% alcohol.

Identification of larvae

The larvae were carefully sorted based on size and morphology by stereomicroscopy. The heads of larva containing mouth-parts (cephalopharyngeal apparatus) and posterior spiracles were cut with a sharp scalpel blade and placed in Hoyer's medium on a glass slide and covered with a coverslip. The glass slides were dried in an oven at 37 °C for three days. The larvae were then identified by compound microscopy.

Determination of species of flies emerging from the pupae

A sample of pupae was collected and vertically planted on a 10 cm sand bed in a plastic jar. The mouth of the jar was covered with a net to prevent newly hatched flies form escaping. The time required for the pupa to hatch was recorded. The flies were identified using a pictorial key (Zump 1965)

Results

Carcass disintegration

The carcasses of the chicken disintegrated leaving skin and bones after 120 to 168 hours in the fly-infested and after 144 to 240 hours in the control group. The weights of fresh and disintegrated carcasses are presented in Table 1. Total disintegration of exposed carcasses occurred after 120 to 168 hours. In the control group in which the carcasses were not assessable to flies, total disintegration occurred after 144 to 240 hours.

Table1. Weights of fresh and totally disintegrated chicken carcasses

Treatment	Weight of carcass (g)			
	Replicate 1		Replicate 2	
	Fresh	Disintegrated	Fresh	Disintegrated
Control	200 ± 87	93 ± 6	300 ± 0	73 ± 6
Fly-infested	217 ± 29	47 ± 6	300 ± 50	47 ± 6

All values expressed as mean ± std. dev.

The result suggests that the final disintegrated weight of the fly-infested carcasses was lower than the controls.

Fly species, larva and pupa on disintegrated carcasses
The adult flies caught feeding on the exposed carcasses were identified as *Chrypsomya megacephala*, *Musca domestica*, *Sarcophaga* and *Lucillia* species. The most common fly species was the Chrysomyia spp. followed by the *Musca domestica* and Sarcophaga spp (Table 2).

Table2. Fly species on exposed chicken carcasses

Days	Fly species (number)					
	Sarcophaga spp.		Chrysomya spp.		*Musca domestica*	
	R1	R2	R1	R2	R1	R2
1	1	-	2	-	1	1
2	2	-	50	-	10	-
3	-	-	80	120	10	20
4	-	2	30	140	-	20
5	-	-	-	60	25	10
6	-	-	-	30	-	5
7	-	-	-	10	-	5
Total	**3**	**2**	**162**	**360**	**46**	**61**

R1=Replicate 1; R2=Replicate 2

Table 3. Total number of flies collected from carcasses during morning and evening periods

Replicate	Morning				Evening		
	8-9	9-10	10-11	11-12	12-1	1-2	2-3
1	-	70	105	77	70	-	-
2	-	140	190	306	475	477	475
Total	**-**	**210**	**295**	**383**	**545**	**477**	**475**

The result suggests the largest number of flies were found on the carcasses during midday when the temperature was the hottest (Table 3).

The carcasses were dominated by the larvae of Chrysomya spp which was identified as the *Chrysomya megacephala*. A total of 7740 larva and 1613 pupa were collected during the period of the study.

Discussion

The time taken for total carcass disintegration was slightly faster in the fly-infested than the control group. In this study, the majority of flies found on the carcasses were the Chrysomya spp, later identified through the larvae to be the *Chrysomya megacephala*. A few larvae were identified to belong to the Calliphora spp. It is suggested that the dominancy of the Chrysomya megacephala on the carcasses had suppressed Calliphora and other species. The larvae of the Chrysomya spp are active feeders, and are expected to cause faster carcass disintegration than the Calliphora spp (Wall and Shearer, 2001).

Although there was greater disintegration of the fly-infested group carcasses, the difference in final weight of this group compared to the control group was not significant. This study was conducted on a small sample size, and this may have contributed to the lack of difference. It should be noted that the rate of carcass disintegration is dependent on several factors which include temperature, moisture and bacteria flora (Vass, 2001). Disintegration a result of larva feeding on the carcasses is just one of the contributing factors which may hasten the process (Davies, 1999).

The study also showed the feeding habit of flies seems to be most prominent at midday. This was evident by the number of flies collected which was highest at 12 to 1 in the afternoon.

In conclusion, the major fly species found on the carcasses in this study was *Chrysomya megacephala*. Other species such as Sarcophaga, *Musca domestica*, Calliphora and Lucillia species play a less significant role. The total disintegration of chicken carcasses did not seem to solely depend on fly larvae. However, by the marginally faster disintegration of the fly-infested carcasses compared to control, it may be concluded that fly larva contributes to carcass disintegration.

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EVALUATION OF PREDATORY ACTIVITY OF INDIGENOUS (UPM) MESOCYCLOPS AND MICROCYCLOPS (CRUSTACEA: CYCLOPOIDA), AS A BIOLOGICAL CONTROL FOR *AEDES* LARVAE

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Predatory activity of indigenous (UPM) *Mesocyclops* and *Microcyclops* (Crustacea: *Cyclopoida*) as biological control agent for *Aedes* larvae was evaluated. Both cyclopoids thrived in in-vitro culture using FAAPW and maintained with fish fillet and pellets. Their populations sustained more than three months. For predatory experiments, each adult cyclopoid was inoculated into 3.5cm vials containing 10mLFAAPW with 10 *Aedes* larvae, either L1 (1-3mm), L2 (3-5mm) or L3 (5-7mm) for 12 hours. The highest larval mortality (34%) was observed in L1, caused by *Mesocyclops.* Both cyclopoids failed to kill L2 and L3 significantly ($P<0.05$). These were affected by duration of experiment, cyclopoid:larvae ratio, volume of water and size of vial. There was no difference in larval mortality between day and night predation. *Mesocyclops* was more aggressive and efficient in attacking *Aedes* larva than *Microcyclops*. The temperature, pH, oxygen concentration, ammonia and nitrate levels of waters where cyclopoids and *Aedes* larvae thrived were measured. Both cyclopoids and *Aedes* larvae thrived in a wide range of these parameters. Based on its performance and survival in the experiments, *Mesocyclops* merit consideration as biocontrol agents of *Aedes*.

Keywords: *Mesocyclops*, *Microcyclops*, *Aedes*, larva, predation

Introduction

Every year, there are between 50 and 100 million new dengue infections, with 250,000 to 500,000 cases of dengue haemorrhagic fever, which can be fatal. In October 2005, Malaysia reached the epidemic status with 1201 and 1023 dengue cases reported in two consecutive weeks (Anonymous, 2005).

Ultra-low-volume malation fogging was widely favoured, but it has limited effect on the immature forms of mosquitoes (Chua *et al*., 2005) while the adult mosquitoes returned to pre-treatment level within two weeks (Pant, 1971: cited by Chua *et al*., 2005). Some mosquito larvae even became resistant to chlorphyrifos and temephos (Teng *et al*. 2005).

Current biological control using freshwater fish produced high predation of native aquatic organisms (Russell, 2001) while entomopathogenic bacteria (*Bacillus thuringiensis var israelensis*) only temporarily reduce mosquito larvae population (Chansang *et al*., 2004).

An emerging natural predator of mosquito larvae are the freshwater cyclopoids which could survive, thrive and maintain long-term populations in various types of water (Marten, 1994), even at 0^{o}C or 40 ^{o}C (Rey *et al.,* 2004) and under low substrate moisture, as low as 13.8% (Kay *et al*., 2002). Cyclopoids could survive in field releases without providing food sources (*et al.*, 2004) allowing them to be cultured relatively easily and at low cost (Suarez *et al.*, 1992: cited by Manrique-Saide *et al*., 1998) while controlling the larvae population for a period of 4 to 6 months (Chansang *et al*. 2004; Schaper, 1999).

In Laos People's Democratic Republic, *Mesocyclops guangxiensis* was used for the *Ae. aegypti* control programme (Jennings *et al.* 1995: cited by Chansang *et al.*, 2004). In Vietnam, *Mesocyclops* succeeded in eliminating *Ae. aegypti* from a number of villages. As a result, no dengue cases have been detected in these communities since 2002 (Kay *et al.*, 2002; Nam *et al.*, 2005).

Materials and Methods

Preparing Cyclopoids
Using a soft net, the pond water in UPM was scooped and then screened for cyclopoids in 5 cm petri dishes under stereomicroscope (x10), followed by identification of genus under compound microscope (x400 and x1000) using the Key to Genera and Species of Cyclopoid. *Mesocyclops* and *Microcyclops* were identified. A gravid female of each genus was cultured in a 80 ml beaker of filtered, autoclaved and then aerated (at least 10 minutes) pond water (FAAPW) to produce a pure culture, and fed 0.01g fish powder and 0.01 g of fish fillet every four days. At least 10 adults emerged in a week.

Aedes colony and larval rearing conditions
Five dark containers filled with 600 ml of dechlorinated water with organic debris and oviposition substrate were placed in dark, sheltered spots around the UPM Aquatic Laboratory. The containers were inspected daily and *Aedes* larvae were identified under stereomicroscope and reared *in vitro* (26 to 29^{o}C). The larvae were inoculated for experiment once they had grown to the required lengths. The L1 (1 to 3mm) consisted of first and second instars (1 to 2 days after hatch), L2 (3 to 5 mm) mostly third instars (3 to 4 days after hatch) and L3 (5 to 7 mm) fourth instars (5 days after hatch) (Dieng *et al.*, 2002).

Cyclopoid predation experiment
An adult cyclopoid of each genus (previously starved for 12 hours) was inoculated into vials (3.5 cm diameter, 10 ml), which contained 10 L1, L2 or L3. The rate of predation was compared between the day (8 am to 8 pm) and night (8pm to 8am) periods. Twelve hours postinoculation, the number of surviving (still able to wiggle) *Aedes* larvae were counted. One L1 *Aedes* was inoculated into a drop of water with one adult cyclopoid, and the interaction was continuously observed under x40 for 6 hours.

Environmental parameter
The pH, temperature, oxygen, ammonia and nitrate levels of waters where cyclopoids or *Aedes* larvae were found were recorded. Cyclopoids were then exposed to extreme pH's (5 and 10, using HCl and NaOH repectively) and temperatures (20 ^{o}C and 35 ^{o}C, maintained with ice flakes or hot water bath) by inoculating one cyclopoid into FAAPW (3.5 cm vial, 15 ml) for 12 hours. The results were recorded as "alive and active", "alive but passive" or "dead".

Results and Discussion

Cyclopoid predation on Aedes larvae

Mesocyclops killed L1 significantly ($F=7.087$, $P<0.05$) while *Microcyclops* did not ($F=0.225$, $P<0.05$). Overall, the mortality for L2 and L3 was approximately 1%, with or without

cyclopoids, showing no significant interaction with the cyclopoids. There was no significant difference in larvae mortality between day and night.

Marten *et al.* (1994) defined a good cyclopoid predator as one that kills more than 20 L1 in 24 hours, which was not meet by either cyclopoid in this study. The criterion was however, not appropriate for this study due to various factors. In this study, the duration of experiment was only 12 hours. A longer experimental time would allow more time for cyclopoids to acclimatise and weak larva to die. Although the killing rate for *Mesocyclops* against L1 was 34%, most surviving L1 were weakened and died 24 hours later. The cyclopoid:larvae ratio in this study was 1:10, while Mittal *et al.* (1997) indicated that the ratio required to produce over 90% predation in 24 hours was 1:2. Smaller containers and water volume produce higher predation rates due to higher frequency of encounter between predator and prey (Dieng *et al.*,2002).

Table 1. *Aedes* larvae mortality 12 hours post inoculation with cyclopoids

Cyclopoid	*Larva Sizes*		
	L1 (1-3mm)	**L2 (3-5mm)**	**L3 (5-7mm)**
Microcyclops	$1.8^{ab} \pm 2.25$	0.1 ± 0.32	0.2 ± 0.42
Mesocyclops	$3.4^{b} \pm 1.90$	0.2 ± 0.42	0.0 ± 0.32
Control	$1.4^{a} \pm 1.43$	0.0 ± 0.0	0.1 ± 0.32

10 larvae: 1 cyclopoid. n=50 (5 replicates)
All values are expressed as mean ± SD
Mean value with different superscripts differ significantly at $P<0.05$.

Table 2. L1 *Aedes* morality 12 hours postinoculation with cyclopoids

Cyclopoid	**L1 larvae**	
	Day	**Night**
Microcyclops	2.60 ± 2.79	1.00 ± 1.41
Mesocyclops	3.40 ± 2.30	3.40 ± 1.67
Control	1.60 ± 1.67	1.20 ± 1.30

10 larvae:1 cyclopoid. (n=50; 5 replicates)
All values are expressed as mean ± SD

The study shows that the predation of *Mesocyclops* was significant, suggesting that *Mesocyclops* is an effective predator of *Aedes* L1 supporting the results obtained by other researches (Chansang *et al.*, 2004; Nam *et al.*, 2005). The present study showed that Microcyclops is a poor *Aedes* larvae predator. Currently there is no data available on Microcyclops' predation to substantiate this finding.

Predation rates also depend on other factors, namely species and size of cyclopoid and mosquito (Rey *et al.*, 2004), weather (Marten *et al.* 1994), physical and biological milieu of the experimental container (Marten *et al.* 1994, Tietze *et al.* 1994), and habitat structure, such as presence of hiding spaces for prey (Rey *et al.*, 2004). Most cyclopoids' predations drop considerably when the mosquito larvae were 4-day old or older (Tietze, 1994; Rey *et al.*, 2004).

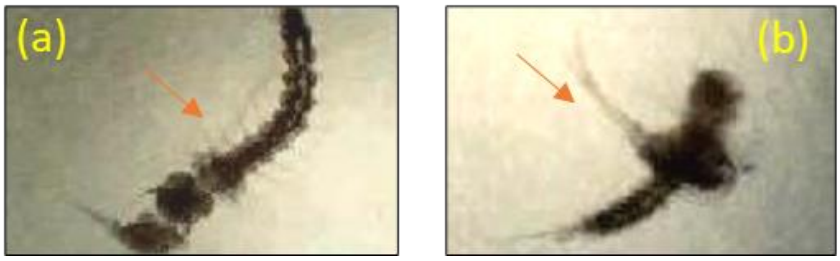
Observations of cyclopoid predation
Prior to attack, cyclopoids swam near L1 and remained stationary for a few seconds, presumably analyzing the prey. Then they pounced unto L1, grasping and biting and then quickly dashed away. The larva immediately coiled its body after each attack, seen as a form

of self-defense by escaping and avoidance. *Mesocyclop*s' attacks were speedy and forceful as compared to that of *Microcyclops*. Generally, Mesocyclops was a much more aggressive predator than *Microcyclops*, supported by an average of 15 strikes in 10 minutes, while *Microcyclop*s two times or less.

Environmental parameter

Both cyclopoids and *Aedes* were found to thrive between pH 6.42-9.54, oxygen concentration of 1.2 to 6.3 mg/l, ammonia level up to 0.18 mg/l and nitrate up to 400 mg/l. *Mesocyclops* remained "active" at water temperature between 19 to 36 ^{o}C and pH 4.9 to 10.1. *Microcyclops* showed similar behaviour except that at 19 to 21 ^{o}C, most *Microcylops* became "passive". This proved that both cyclopoids could thrive and multiply under a variety of conditions.

Figure 1. *Mesocyclops* (a) and *Microcyclops* (b)seizing *Aedes* larva.



Mass-culturing

Mesocyclops spp. have been known for their predation upon mosquito larvae for decades. However, no such investigation has been carried out in Malaysia. In this study, two indigenous Malaysian strain of cyclopoid was chosen to obtain baseline data to determine the potential of this predator in dengue vector control programmme in Malaysia. Both *Mesocyclops* and *Microcyclops* were successfully cultured using FAAPW or dechlorinated water. The population thrived for at least 3 months. Cyclopoids can be mass-cultured using the methods in this study, and the gravid females can be inoculated into *Aedes* breeding sites to reduce *Aedes* population. Further studies are needed to maximize *Mesocyclops*' predation, as well as to test the feasibility of incorporating *Mesocyclops* culture in the efficiently control of *Aedes* population.

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A PRELIMINARY STUDY ON HAEMATOLOGY AND CLINICAL BIOCHEMISTRY OF CAPTIVE ROCKHOPPER (*Eudyptes chrysocome/crestatus*) AND AFRICAN PENGUINS (*Spheniscus demersus*) IN MALAYSIA

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Haematology and clinical biochemistry are aids in disease diagnosis in avian species. In this study, selected haematology and clinical biochemical parameters of 18 captive Rockhopper penguins and 17 African penguins in Malaysia were determined. The haematological parameters included packed cell volume, haemoglobin concentration, total erythrocyte count, total leucocyte and differential counts. The clinical biochemistry parameters analysed were plasma protein, albumin, inorganic phosphate, glucose, cholesterol, creatinine, urea, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), uric acid sodium, potassium, chloride and calcium. Most of the blood parameters were within the ranges reported by other researchers except for plasma ALT and CK concentrations from Rockhopper which were significantly higher than those reported earlier. Comparisons between Rockhopper and African penguins showed significant differences in all the parameters, except for mean cell haemoglobin concentration (MCHC) cholesterol and albumin concentrations. The blood cell morphologies of the Rockhopper and African penguins in this study were similar to other avian species. However, it is difficult to distinguish between heterophils and eosinophils.

Keywords: Penguins, Haematology, Clinical biochemistry, blood cell morphology

Introduction

All penguins belong to the class Aves, order Sphenisciformes and family Spheniscidae. They are medium to large flightless seabirds with thick-set body, short robust legs and wings compressed to form flippers. Most adult birds have plumage with black or bluish grey upper and white under parts. There are 6 genera and 17 species of penguins. The penguins live mainly in the cold waters of Antarctic and sub-Antarctic (del Hoyo *et al*., 1992). Their diets in the wild vary, but fish is the main staple food in captivity (Cranfield, 2003).

Rockhopper penguins (*Eudyptes chrysocome/crestatus*) are characterised by its drooping yellow crest with a black, wedge-shaped crest in between. Their feet are pinkish on top and black at the bottom. The African or Black-footed penguin (*Spheniscus demersus*) has a black face extending to behind the eyes and into the white supercilium. Some birds have a second black band on upper breast. Adult penguin weighed between 2 to 4 kg (del Hoyo *et al*., 1992).

Haematology and clinical biochemistry parameters are useful in disease diagnosis in birds. Although studies have been conducted on the blood parameters of captive penguins (Karesh *et al.*, 1999), haematological and clinical biochemistry data of penguins kept in tropical countries under controlled conditions are inadequate.

This preliminary study was conducted to establish reference value for blood parameters of healthy captive penguins in Malaysia.

Materials and Methods

Animals
This study was conducted at the Underwater World, an aquarium on Langkawi Island, Kedah. The subjects were 18 (9 males, 9 females) Rockhopper penguins (*Eudyptes chrysocome/crestatus*) and 17 (8 males, 9 females) African penguins (*Spheniscus demersus*). The Rockhoppers were about 4 years old while the African penguins ranged between 5 and 22 years old. The penguins were kept in large separate tanks (79 m^2 per tank) for display. Rockhopper penguins were kept under 15-16ºC while the African penguins under 18-20ºC ambient temperature. They were fed twice a day with fish, occasionally squids and supplemented with multivitamin in tablet form once per day.

Blood sampling was done in the morning before they were fed their first meal of the day. Blood samples were collected from the medial metatarsal vein or from the feet web using 3 ml sterile syringes and 23G needles into EDTA tubes.

Haematology and Plasma Biochemical Parameters
All laboratory analyses were done at the Haematology & Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Smears were made from the EDTA-anticoagulated blood immediately after sampling, air-dried and stained with Wright's stain to perform the leucocyte differential counting and examination of erythrocyte, leucocyte and thrombocyte morphologies.

Total erythrocyte and leucocyte counts were done by the haemocytometer method. The haemoglobin concentration was determined by the standard cyanmethaemoglobin method. The Packed cell volume was obtained by the microhaematocrit method. Plasma protein was estimated by refractometry.

The plasma biochemical parameters analysed were urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), total bilirubin, glucose, cholesterol, inorganic phosphate, plasma protein, albumin and uric acid. The plasma sodium, potassium, chloride and calcium concentrations were also determined. These parameters were analysed using the Hitachi 902® chemistry analyzer.

Results and Discussion

The haematology and clinical biochemistry findings for the Rockhopper and African penguins are presented in Tables 1 and 2 respectively. The penguin blood cells are shown in Figure 1.

Blood Collection in Sphenisciformes
Blood sampling in penguins is difficult because of the evolutionary anatomical modifications associated with lost of its flight ability. Based on the structure of the circulatory system of Sphenisciformes, the optimal site for blood sampling is the brachial vein on the ventral surface of the flipper (Samour *et al.* 1983).

In our study, blood was collected from the medial metatarsal vein or from the feet web vein. However, only small amounts of blood could be obtained. Previous studies reported that, with proper restraint, up to 20ml blood can be withdrawn form a 3kg bird through the right jugular vein. Other suitable sites were the branchial and tibeotarsal veins (Cranfield, 2003).

In general, the haematological and clinical biochemistry parameters of the penguins in this study are mostly in agreement with those obtained by previous researchers (Hawkey 1989; Gheberemeskel *et al.* 1989; Karesh *et al.* 1999).

Table 1: Haematological parameters of Rockhopper and African penguins

Parameters	**Rockhopper**	**African**
Erythrocytes	1.66 ± 0.22	2.05^{a} ± 0.23
Haemoglobin (g/L)	198.4 ± 18.8	172.8^{a} ± 14.4
PCV (L/L)	0.51 ± 0.03	0.43^{a} ± 0.04
MCV (FL)	310 ± 49	213^{a} ± 24
MCHC (g/L)	392 ± 32	399 ± 16
Leucocytes	17.67 ± 5.17	10.35^{a} ± 5.67
Heterophils	10.60 ± 3.72	-
Lymphocytes	5.14 ± 1.70	-
Monocytes	0.95 ± 0.50	-
Eosinophils	0.79 ± 0.56	-
Basophils ($x10^{9}$/L)	0.23 ± 0.26	-

Values expressed as mean ± std. dev.
For each row, means with different superscripts are significantly different ($p<0.05$).

Table 1: Clinical Biochemistry parameters of Rockhopper and African penguins

Parameters	**Rockhopper**	**African**
Urea (mmol/L)	2.33 ± 0.30	3.90^{a} ± 0.90
Creatinine	47.8 ± 8.3	35.2^{a} ± 10.8
Glucose (mmol/L)	14.1 ± 1.5	10.4^{a} ± 1.3
Cholesterol	9.47 ± 1.17	8.52 ± 1.35
Plasma protein	58.8 ± 7.3	69.6^{a} ± 9.3
Albumin (g/L)	20.9 ± 1.3	24.4 ± 15.6
ALT (U/L)	236 ± 77	738^{a} ± 201
AST (U/L)	265± 72	-
CK (U/L)	478.8 ± 183.3	-
Uric Acid (µmol/L)	518.8 ± 152.3	-
Inorganic Phosphate	1.43 ± 0.34	-
Sodium (mmol/L)	143 ± 18	-
Potassium	25.5 ± 4.5	-
Chloride (mmol/L)	122 ± 23	-
Calcium (mmol/L)	2.46 ± 0.40	-

Values expressed as mean ± std. dev.
For each row, means with different superscripts are significantly different ($p<0.05$)

The results also suggested that different species of penguins may have different blood profiles. There is however, no great variability in values of the parameters between genders for both the Rockhopper and African penguins.

The morphology of penguin heterophils is typical of the avian heterophils accept that the granules are neither distinctly rod nor oval shaped. Some granules appeared more rounded compared to those of other avian species. This has created difficulties in differentiating heterophils from eosinophils. The eosinophil granules are also round and stained lighter and the nucleus darker than those of the heterophils.

Reference plasma biochemistry values are currently inadequate. In the African penguins the plasma biochemical parameters higher than those reported for Magellanic penguins were ALT, plasma protein and albumin concentrations. The plasma ALT and CK concentrations in Rockhoppers were generally higher than those reported earlier (Hawkey 1989; Ghebremeskel et al., 1989; Karesh et al., 1999). These differences are most likely caused by variations in age, season, environment, physiology, nutrition and methodologies used in the studies.

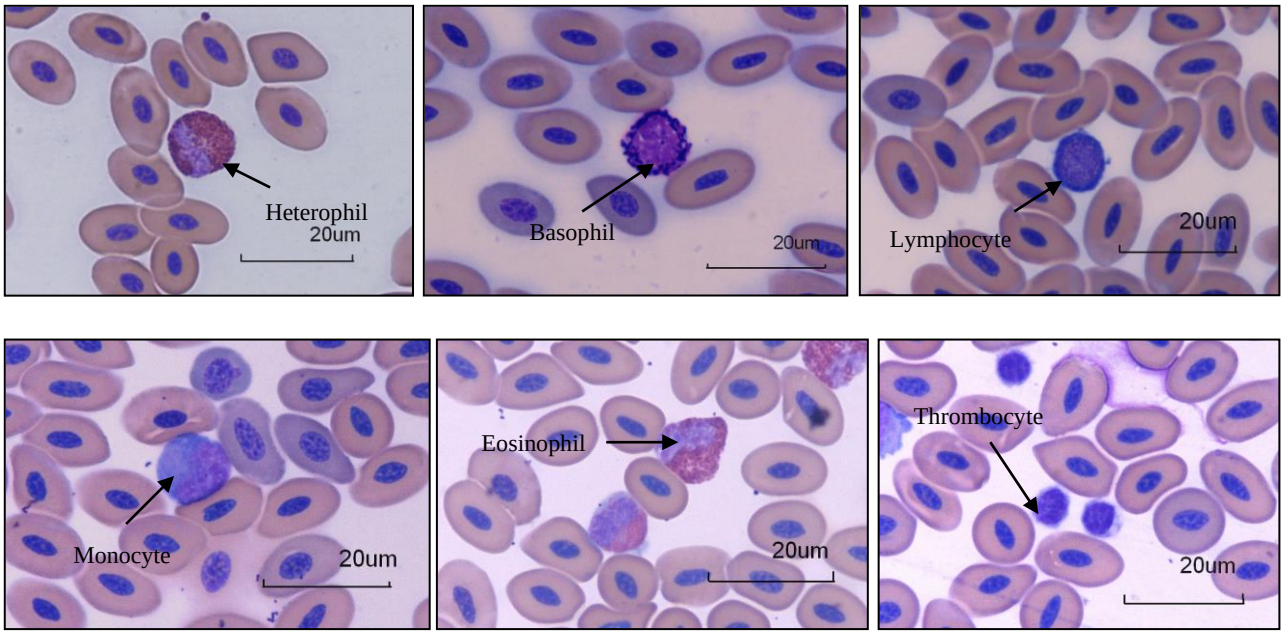

Figure 1. Blood cells of penguins

Although only selected haematology and clinical biochemistry parameters were analysed, they can still form the basis for development of reference values for penguins in tropical countries. Nevertheless, large sample sizes and better-quality controls are necessary to ensure more reliable sets of data.

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DETECTION AND DIFFERENTIATION OF INFECTIOUS BURSAL DISEASE VIRUS STRAINS ISOLATED FROM COMMERCIAL BROILER CHICKENS USING REAL TIME POLYMERASE CHAIN REACTION

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Infectious bursal disease (IBD) is one of the most important immunosuppressive diseases in chickens causing high mortality and immunosuppression. IBD outbreaks continue to occur despite of vaccination against the disease. The IBD virus (IBDV) strains; classical (ca), variants (va) and very virulent (vv) strains vary in their virulence and pathogenicity. Furthermore, chicken vaccination against one strain may not be protected or fully-protected against IBDV challenge of the other strains. It was the objective of the study to detect and differentiate IBDV strains of field isolates using real-time polymerase chain reaction (real-time PCR). The lesions in the bursa of Fabricius were also determined. The study showed that 67%, 33% and 22% of cases submitted to our laboratory from March to May 2005 were positive for vvIBDV, IBDV vaccine (caIBDV) and both, respectively. Field study in another 4 broiler chicken farms showed that vvIBDV and IBDV vaccine were detected in Farms 2 and 3, whilst no virus was detected in Farms 1 and 4. Severe acute and chronic bursal lesions were recorded in farms 3 and 4, respectively. Complicated chronic respiratory disease (CCRD) was commonly observed in the chickens in Farm 4. It was concluded that the real-time PCR and histopathology are important rapid techniques in detection and differentiation of IBDV strains and diagnosis of the disease.

Keywords: infectious bursa disease (IBD), IBD virus (IBDV), real-time PCR.

Introduction

Infectious bursal disease (IBD) or Gumboro disease is a highly infectious viral disease of chickens and causes high economic losses worldwide due to high rate of mortality in acute clinical form of the disease or as a consequence of severe immunosuppression (Muller *et al.,* 2003). Immunosuppressed chickens perform poorly, increased their susceptibility to other pathogens and decreased vaccination efficacy. There are three strains of IBD virus (IBDV) namely; very virulent (vv), variant (va) and classical (ca) IBDV. Outbreak of IBD due to caIBDV was first reported in USA in 1957 (Cosgroove, 1962). The disease was then relatively under control by proper the vaccination programmes practiced in the hens and their chicks.

However, in late 1980's outbreaks of IBD due to vvIBDV and vaIBDV were reported in Europe (Chettle *et al.,* 1989) and USA (Snyder *et al.,* 1988)), respectively. The disease has spread worldwide and vvIBDV infection first described in Asia in early 1990's (Nunoya *et. al.,* 1992) including Malaysia in 1991 (Hair- Bejo, 1992). To date, despite the wide use of vaccines, IBD outbreaks continue to occur in the chicken farms. It is important to note that the virulence and pathogenicity of the vvIBDV, caIBDV and vaIBDV strains vary. Furthermore, chicken vaccination against one strain may not be protected or fully-protected against IBDV challenged of the other strains.

It was the objective of the study to detect and differentiate IBDV strains namely, the vvIBDV and vaccine strain (caIBDV) using real time-PCR.

Materials and Methods

Chickens

Samples of bursa of Fabricus from 9 chicken farms submitted to Biologic Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia from 28 Mac 2005 to 12 Mei 2005 were processed and analysed for detection and differentiation of IBDV strains namely, the vvIBDV and vaccine strain (caIBDV). Further field study was conducted in another 4 broiler chicken farms without (Farms 1 and 2) and with (Farms 3 and 4) history of IBD outbreaks. On necropsy the bursa of Fabricius were collected for histopathology examination, and detection and differentiation of IBDV strains by real-time PCR.

Real-Time PCR

Bursa of Fabricius was processed and the RNA of the virus was extracted. The real-time PCR technique was according to an established technique previously described (Omar *et al.*, 2005). Briefly the PCR conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 45 cycles of a repetitive program of 30 seconds denaturation at 95°C, 30 seconds annealing at 60°C, and 30 seconds extensions at 72°C. The correlation coefficient and PCR efficiency were calculated by using computer software (iCycler Software; Bio-Rad). The results were expressed in the threshold cycle value (C_T). Each sample was checked both for vvIBDV and vaccine IBDV (caIBDV) strains.

Histopathology

Bursa of Fabricius were fixed in 10% buffered formalin, trimmed, dehydrated in a series of alcohol concentration, and embedded in paraffin wax using an automatic tissue-processor. The tissues were then sectioned, mounted on glass slides, dewaxed and stained with Hematoxylin–Eosin and examined under the light microscope (Hair-Bejo *et al.*, 2004).

Results

Real-tim PCR

Six or 67% of the bursal samples submitted to the laboratory were positive for vvIBDV, despite vaccination programme against IBD practiced in the farms. Three or 33% of the samples were positive for IBD vaccine, while two or 22% of the samples were positive for both vvIBDV and IBD vaccine. Only three or 33% of the samples were free from IBDV infections.

Neither the vaccine nor vvIBDV strains were detected in the bursal samples from Farms 1 and 4. However, the vaccine strains were detected in Farm 2 and both the vvIBDV and vaccine strains were detected in Farm 3. Complicated chronic respiratory disease (CCRD) was commonly recorded in the chickens in Farm 4.

Histological Changes in the Bursa of Fabricius

There were mild lesions observed in Farm 1 and moderate to severe lesions in Farm 2. Severe acute to sub-acute necrotizing bursitis was recorded in Farm 3, whilst subacute to chronic bursitis recorded in Farm 4.

Discussion

The study demonstrated that IBD outbreaks continue to occur in the chicken farms despite of the vaccination programme against the disease. Reasons for these are not clearly established, although it could be closely associated with vaccination failure and/or poor biosecurity. The virus is highly stable and has a tendency to persist in the environment even with thorough cleaning and disinfection. The use of vaccines antigenically nonsynonymous to the strain present in the flock's environment will decrease vaccination efficacy. In this case, the field IBDV can break through the passive or active immunity and cause disease outbreaks (Nurulfiza *et al.,* 2006; Tan *et al.,* 2004). The development of new vaccine antigenically similar to the field strains such as MyVAC UPM93 could offer better protection and promising approach in the control and prevention of IBD (Hair-Bejo *et al.,* 2006).

The real-time PCR technique is relatively simple to perform, economically feasible and provides very fast results and can detect and differentiate IBDV strains; vvIBDV and vaccine or caIBDV especially in acute or subacute clinical cases of IBD. Consequently, this approach may assist in the development of more effective vaccination strategies, which may improve the control of IBD. However, it has some limitation in diagnosis of subclinical cases of IBD as demonstrated in Farm 4. This could be due to the low concentration or absent of the virus in the bursa of Fabricius of chickens recovered from acute episodes of IBDV infection. It was demonstrated that the virus could be detected in the bursa of Fabricius of specific pathogen free chickens up to 12 days vvIBDV postinoculation (Hair-Bejo and Thu-Zar, 1996).

The chickens in the Farm 4 had a history of IBD outbreak in the previous cycle. In the present cycle, clinical signs of IBD and high mortality were observed in the 24-day-old. The affected chickens (35-day-old) were immunosuppressed and died of complicated chronic respiratory disease (CCRD) (Hair-Bejo, 1994). The histological lesion in the bursa of Fabricius is consistent with vvIBDV infections (Hair-Bejo *et al.,* 2004). The lesions observed were clearly different from vaIBDV infections, although it may not be easily differentiated from the caIBDV infections.

It was concluded that real-time PCR and histopathology are important rapid techniques in detection and differentiation of IBDV strains and diagnosis of the disease, and thus should be used in the control and prevention of IBD.

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A COMPARISON OF FOUR SKIN CLOSURE TECHNIQUES IN THE DOG

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Four female, adult local dogs were used in this study. Eight craniocaudal linear incisions, 3 cm. long were made at the dorsum, four on the left and another four on the right, 4cm. apart and 4cm away from spinous processes. Incisions were closed with stapler, nylon, chromic catgut and tapes respectively for primary intention wound healing. Skin biopsies were taken on days 0, 3, 6 and 9 postsurgery for histological evaluation of fibroblast population and epidermis thickness. Biopsies for tensiometry were harvested on day 9. The results showed that stapler provided good skin apposition, minimal scar formation and moderate tensile strength. Nylon caused prominent scar formation but had good tensile strength. Chromic catgut evoked inflammatory reaction but its tensile strength was equal to that nylon. Tapes failed to appose skin especially on the hairy surface and highly mobile regions.

Keywords: skin closure, stapler, nylon, chromic catgut, tensile strength, scar formation

Introduction

Wound closure techniques have evolved from the earliest development of suturing materials to comprise resources such as synthetic sutures, absorbables, staples, tapes, and adhesive compounds. The engineering of synthetic material along with standardisation of traditional materials viz. catgut and silk were directed towards aesthetic results. Similarly, the creation of natural glues, surgical staples, and tapes as substitutes for sutures has supplemented the armamentarium of wound closure techniques. Aesthetic closure is based on the knowledge of healing mechanisms and skin anatomy as well as suture materials and closure techniques. Choosing the proper material and wound closure technique ensure optimal healing (Galli *et al*., 2004).

The objective of this study was to compare the healing progress in primary intention wound healing using various skin closure techniques, *viz*: staples, tapes, chromic catgut with intradermal suturing technique and nylon with horizontal mattress suture pattern.

Materials and Methods

Animal and Anaesthetic Protocol
Four female stray dogs identified as Dog A, B, C and D were used in this study. The dogs were housed in individual cages, fed on commercial maintenance dry food twice daily and water *ad libitum.* Before surgery the animals were induced with ketamine (20mg/kg) and xylazine (1.1mg/kg) intramuscularly and maintained with pentobarbitone (30mg/kg) intravenously.

Surgical Protocol
On sternal recumbency, the dorsolateral area from the caudal aspect of the scapula to the lumbosacral junction was prepared for aseptic surgery. The locations of eight experimental

craniocaudal linear wounds were marked, measuring 3cm in length, 4 cm apart and 4 cm away from spinous processes of the vertebrae. Four linear incisions on the right dorsum were made and identified as R1, R2, R3 and R4 accordingly. Another four linear incisions were made on the left dorsum and identified as L1, L2, L3 and L4.

Skin Closure Techniques
Skin closure adopted in this study were single use skin staplers with stainless steel staples, 4.8 mm in width and 3.4 mm in height (ROYAL™), tapes (URGOSTRIPS®), chromic catgut 3-0 with intradermal skin closure technique and monofilament nylon 2-0 (Ethilon) with horizontal mattress suture pattern.

Gross Evaluation
Each wound was examined daily and photographed on days 3, 6 and 9. The progression of wound healing for each incision was recorded.

Histological Evaluation
Skin biopsies of the wounds were taken on days 0, 3, 6 and 9 for histological evaluation using 4mm diameter of skin biopsy punch. Skin biopsies fixed in the Bouin's solution were processed and stained with hematoxylin and eosin.

Tensiometer
On day 9, samples from the stapled, taped and sutured surgical incisions were harvested each measuring 2 cm in width and 4 cm in length. The samples were placed between gauze sponges moistened with normal saline (0.9% NaCl) solution. To test for maximum load, the samples were then positioned in the jaws of the tensiometer (Instron Model 5567) with the surgical incision line parallel to the jaws.

Results

Gross Evaluation
On day 3, skin closure using staples showed well-apposed incised skin surfaces with minimal swelling and devoid of skin tension. By day 9, the incision had healed completely and scar formation was hardly visible.

With chromic catgut and nylon, swelling around the suture sites was obvious on day 3 following wound closures. However, on day 9 wound closure using chromic catgut showed complete apposition and healing while closure using nylon showed elevation of the incised edges. On day 9, wound closure using tape failed to appose the incised edges.

Histological Evaluation
Fibroblast population and epidermis thickness between groups and within group over the duration studied in the four skin closure techniques are as shown in Tables 1 and 2.

Histological Evaluation
Histological sections to depict epidermis thickness and incidence of fibroblast and collagen fibers on day 9 for the four techniques studies are as shown in Figures 1, 2, 3 and 4.

Table 1: Postsurgical skin fibroblasts number in dogs with different skin closures

Suture Material	Fibroblasts (cells/mm^3)			
	Day 0	Day 3	Day 6	Day 9
Staples	49 ^{a,x} ± 6	100 ^{a,x} ± 40	166 ^{a,x} ± 69	201 ^{a,x} ± 224
Nylon	96^{b,x} ± 17	169 ^{a,x,y} ± 115	220 ^{a,x,y} ± 77	285 ^{a,y} ± 130
Chromic Catgut	43^{a,x} ± 10	87 $^{a\ x,y}$ ± 23	215 ^{a,y,z} ± 137	299 ^{a,z} ± 104
Tapes	65^{a,x} ± 18	85 $^{a\ x,y}$ ± 17	255 ^{a,z} ± 195	228 ^{a,y,z} ± 85

All values are expressed as mean ± std. dev.
$^{a, b}$ For each row, mean values with different superscripts differed significantly ($p<0.05$) within time.
$^{x, y, z}$ For each column, mean values with different superscripts differed significantly at $p<0.05$ within group.

Table 2: Postsurgical epidermis thickness in dogs with different skin closures

Suture Material	Epidermis thickness (mm)			
	Day 0	Day 3	Day 6	Day 9
Staples	0.036 ^{a,x} ± 0.069	0.064 ^{a,y} ± 0.017	0.093^{a,y} ± 0.25	0.074 ^{a,y} ± 0.038
Nylon	0.029 ^{a,x} ± 0.006	0.083 ^{a,y} ± 0.034	0.090^{a,y} ± 0.182	0.116 ^{a,y} ± 0.037
Chromic Catgut	0.029 ^{a,x} ± 0.002	0.104 ^{a,y} ± 0.026	0.138^{b,z} ± 0.01	0.108 ^{a,y,z} ± 0.033
Tapes	0.039 ^{a,x} ± 0.02	0.108 ^{a,y} ± 0.046	0.091^{a,y} ± 0.127	0.091 ^{a,y} ± 0.027

All values are expressed as mean ± std. dev.
$^{a, b}$ For each row, mean values with different superscripts differed significantly ($p<0.05$) within time.
$^{x, y, z}$ For each column, mean values with different superscripts differed significantly at $p<0.05$ within group.

Table 3: Mean maximum load on day 9 postsurgical harvested skins

Suture Material	Mean Maximum Load (N)
Staples	34.66 ± 16.95 ^{b}
Nylon	40.27 ± 5.54 ^{b}
Chromic Catgut	40.51 ± 10.63 ^{b}
Tapes	19.51 ± 13.83^{a}

All values are expressed as mean ± std. dev.
$^{a, b}$ Mean values with different superscripts differed significantly (<0.05).

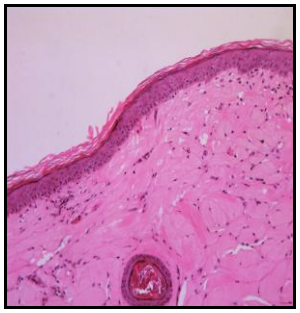

Figure 1: Staples

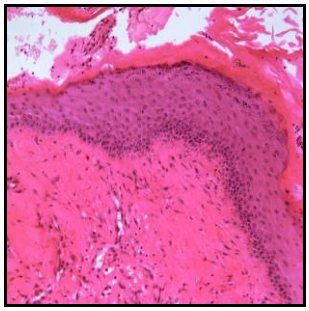

Figure 2: Chromic Catgut

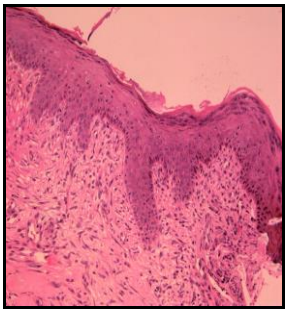

Figure 3: Nylon

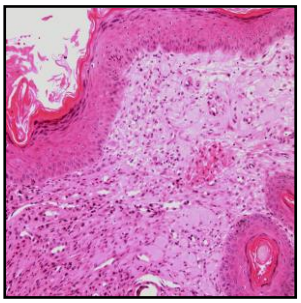

Figure 4: Tapes

Discussion

Based on the gross and histological evaluations and tensiometry it was evident that skin closure using staples provided the most encouraging results. From the gross evaluation study, staples did not press on the apposed surfaces but instead the staples merely held the incised edges together leaving a space between them. Absence of tissue strangulation promoted angiogenesis from the deeper dermis with subsequent revascularization of the incised surfaces. This observation concurred with a previous study by Nagamachi (1988) who reported that a study using the hydrogen gas clearance method showed that wound closed with staples elicited better blood flow compared to that of nylon.

On day 9, a scar was hardly visible at the site of incision closed by the staples. Scar formation is attributed to the thickened epidermis of the incised edges, which in this case is the least compared to the other three wound closure techniques. Although there was an increased epidermis thickness from day 0 to day 6, the thickness was markedly reduced on day 9. Hence,it can be deduced that the reduced epidermis thickness and the low fibroblast count in the dermis contribute to the absence of a scar in wound closure using staples.

Wounds gained only about 20 % of their final strength in the first three weeks of healing (Singer, 1999). At maximum strength, a scar is only 70 % as strong as that of normal skin. In the present study there is a strong correlation between a high maximum load and a high fibroblast count in wound closures using nylon and chromic catgut.

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PREVALENCE OF *SALMONELLA SPP.* AND *MYCOPLASMA GALLISEPTICUM* IN POULTRY MULTI-SPECIES FARMS

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This study was undertaken to investigate the prevalence of *Salmonella spp.* and *Mycoplasma gallisepticum* (MG) in typical Malaysian poultry multispecies farms. In this study, 156 samples were taken from two farms in Selangor and Perak. A total of 122 samples were taken from 113 samples of village chickens, ducks, and turkeys including 9 environmental poultry droppings in Farm A. In Farm B, 28 samples were taken from village chickens, French geese and 6 environmental poultry droppings. Cloacal and choanal cleft swabs from 141 birds and 15 environmental poultry droppings were taken separately. *Salmonella spp.* was isolated from 10 (6.4%) out of 156 samples, where three isolates (30.0%) were identified as *Salmonella enteritidis* and the remaining 7 isolates (70.0%) were unidentified *Salmonella spp.* Mycoplasma was isolated from 110 (78.0%) out of 141 birds. Ninety-seven (88.2%) samples were positive for glucose fermentation tests. Haemadsorption test revealed all samples were nonpathogenic Mycoplasma, which could be *M. gallinarum*. However, Indirect Fluorescent Antibody (IFA) test revealed that only one (1) sample was positive for MG. Based on Salmonella and Mycoplasma isolation results, there were significant differences in the prevalence of Salmonella among the species of poultry. However, the prevalence for MG was not significant among the species of poultry since the isolation of MG was very low. Salmonella and MG infection were more predominant in chickens, followed by ducks, geese and turkeys.

Keywords: *Salmonella spp., Mycoplasma gallisepticum, multi-speciess farm, poultry.*

Introduction

In recent years, poultry farming has been hampered by the outbreak of fatal infectious diseases caused by bacteria, viral, Mycoplasma and other agents. Among bacterial diseases, Salmonellosis is one of the most important diseases in poultry that causes serious economic loss due to mortality and reduced egg production (Khan *et al*., 1998). Mycoplasmosis is also an important disease in poultry since it can cause respiratory problems. *Salmonella spp*. and *Mycoplasma gallisepticum* (MG) have been recognized among the most important pathogens in avian. They cause infection in many species of animal including pets and livestock leading to great economic loss.

Avian salmonellosis is divisible into three diseases, which are; pullorum disease (*Salmonella pullorum* infection), fowl typhoid (*Salmonella gallinarum* infection), and paratyphoid (non-host adapted salmonellae including *Salmonella arizonae* infection). Although many different Salmonellae can infect poultry from time to time, one or two particular species occur more frequently than others and account for the majority of outbreaks. The importance of Salmonellosis lies not only in its economic effects on the poultry industry but also in its disease-causing potential in the human population, in which the Salmonellae are one of the prime causes of food poisoning.

Mycoplasmas are also well-known pathogens that cause diseases in chickens, turkeys, and ducks; and mycoplasmosis is an economically important disease in avian production. It is caused by four commonly recognized pathogenic Mycoplasma, namely *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis* and *Mycoplasma iowae* (Bradbury, 2001). *Mycoplasma gallisepticum* is one of the avian pathogens that well-known to cause chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys, especially in intensively housed poultry where it can cause considerable economic loss, which is exacerbated if the birds are under stress. When present in a flock, *M. gallisepticum* often causes poor production efficiency of growth and egg-laying, and also increases medication costs. Mycoplasma isolated from geese and ducks in France were shown to be closely related to *M. gallisepticum* (Bradbury *et al*., 1993).

Since Salmonella and Mycoplasma are highly pathogenic and contagious, there is a potential for poultry raised in multi-species farming system to have a cross-infection through direct contact, oral-faecal route or inhalation.

This purpose of this study was to determine the presence of *Salmonella spp.* and *Mycoplasma gallisepticum* in poultry multi-species farm and to compare the occurrence of Salmonella spp. and *Mycoplasma gallisepticum* in different species of poultry in multi-species farming.

Materials and Methods

Sampling
Two semi-intensive, multi-age flocks, multi-species poultry farms were chosen in the area of Selangor and Perak. Farm A consists of village chickens, turkeys and ducks, while Farm B consists of village chickens and French goose. All the birds were sampled at random by using stratified random sampling technique, where the poultry population was divided into groups of chickens, ducks, turkeys or geese, and a sample of unit is drawn from each group. Within these two farms, 104 samples were taken from the village chickens, 15 from ducks, 14 from French goose, 8 from turkeys and 15 samples of environmental poultry droppings were collected and analysed. Altogether, a total of 141 samples were collected from poultry along with 15 environmental poultry droppings.

In this study, cloacal swabs were taken for *Salmonella* isolation whereas for *Mycoplasma gallisepticum*, the choanal cleft region was swabbed as suggested by Branton *et al*., (1984) and Shah-Majid and Nihayah, (1987).

Isolation and Identification Methods for Salmonella spp.
At the sampling site, cloacal swabs from individual birds were placed in 10 ml Rappaport-Vassiliadis (RV) Enrichment broth (Oxoid). Each sample was then incubated for 18 to 24 hours at 37ºC. The samples were then inoculated onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid) and MacConkey Agar No. 3 (Oxoid) for 24 to 48 hours. Salmonella colonies on the plates (red colonies, translucent with a black centre on XLD agar and colourless colonies on MacConkey agar were confirmed by oxidase test. Suspected Salmonella colonies which were oxidase-negative were subjected to purity check by serological test. Serological characteristics of the isolates were investigated by the slide agglutination test using a commercial polyvalent O, H and specific antisera.

Isolation and Identification Methods for Mycoplasma gallisepticum (MG)
The choanal swabs were inoculated directly onto mycoplasma agar (Modified K agar) plates containing antibiotic (Penicillin G) and thallium acetate. The agar plates were incubated in 5% CO_2 at 37ºC. All plates were examined for growth of Mycoplasma colonies at days 3, 7, 10 and 14 post-incubation, under 40x magnification by using a stereomicroscope according to the methods described by Jordan (1985). Colonies which were approximately 0.1 - 1 mm in diameter with a central, elevated, denser portion or with the shape similar to fried-egg appearance were identified as Mycoplasma. The samples with Mycoplasma growth were subcultured to broth and only the glucose fermenters were subcultured for another 2 passages before proceeding to the Haemadsorption test followed by Indirect Fluorescence Antibody (IFA) test.

Results

Isolation of Salmonella spp.
Salmonella spp. was present in 10 out of 156 (6.4%) samples cultured and out of 10 isolates, 3 isolates (30.0%) were identified as *Salmonella enteritidis* and the remaining 7 isolates (70.0%) were unidentified *Salmonella spp.*

Tables 1 and 2 show the present of *Salmonella spp*. in chickens, turkeys, ducks, geese and environmental faecal dropping isolated from Farms A and B. The highest isolation (80.0%) of Salmonella was from Farm A as compared to Farm B (20%). Out of these two farms, chickens and ducks from Farm A were harbouring *Salmonella spp*. which included *Salmonella enteritidis* (37.5%), and *Salmonella spp*. was also isolated from faecal dropping (Table 1). While in Farm B, only unidentified *Salmonella spp.* was present in chickens and geese. However, Farm B has higher percentage (6.0%) of isolation of *Salmonella spp.* as compared to Farm A (4.0%).

Table 1: Recovery of *Salmonella spp.* from faecal droppings (Farm A)

Source	**No. of samples submitted**	**Total Salmonella positive sample (%)**	**Salmonella-positive (%)**		**Whole population positive samples (%)**
			S. enteritidis	***Salmonella spp.***	
Chicken	90	5 (5.6)	1 (20.0)	4 (80.0)	**4.1**
Turkey	8	0 (0)	0 (0)	0 (0)	**0**
Duck	15	2 (13.3)	2(100.0)	0 (0)	**1.6**
Faecal	9	1 (11.1)	0 (0)	1 (100.0)	**0.8**
Total	122	8 (6.5)	3 (37.5)	5 (62.5)	6.5
Prevalence		0.07 (7)	0.03 (3)	0.04 (4)	-

Table 3: Recovery of *Salmonella spp.* from faecal droppings (Farm B)

Source	**No. of samples submitted**	**Total Salmonella positive sample (%)**	**Salmonella-positive (%)**		**Whole population positive samples (%)**
			S. enteritidis	***Salmonella spp.***	
Chicken	14	1 (7.1)	0 (0)	1 (100.0)	2.9
Goose	14	1 (7.1)	0 (0)	1(100.0)	2.9
Faecal dropping	6	0 (0)	0 (0)	0 (0)	0
Total	34	2 (5.8)	0 (0)	2 (100.0)	5.8
Prevalence		0.06 (6)	0 (0)	0.06 (6)	-

Isolation of Mycoplasma galllisepticum (MG)
Tables 3 and 4 show the presence of *Mycoplasma gallisepticum* (MG) and other *Mycoplasma spp*. in chickens, turkeys, ducks and geese sampled from Farms A and B. Mycoplasmas were isolated from 110 out of 141 (78.0%) birds. Ninety-seven (97) samples were positive for glucose fermentation tests and the other 13 samples were non-glucose fermenters. Only 100 samples were viable and were subjected to haemadsorption test and IFA test.

MG was isolated from 1 chicken (0.9%) in Farm A using the IFA test. Thirty-three (33) isolates were identified as MGN and the remaining 76 isolates were unidentified Mycoplasma spp. The highest isolation of Mycoplasma was from Farm A with a prevalence of 0.9 (86.7%) followed by Farm B with a prevalence of 0.4 (42.9%). MGN was isolated higher (26.5%) than *Mycoplasma spp.* in Farm A (Table 3), while *Mycoplasma spp.* was isolated higher (32.1%) than MGN in Farm B (Table 4). Overall, the highest isolation was the *Mycoplasma spp.*, in both farms, with a prevalence of 0.5 (53.9%) followed by isolation of MGN and MG.

Table 3: Recovery of *Mycoplasma spp.* from Farm A.

Source	No. of samples submitted	Total Mycoplasma - positive samples (%)	Mycoplasma-positive (%)			Whole population positive samples (%)
			M. gallisepticum	*M. gallinarum*	*Mycoplasma spp.*	
Chicken	90	80 (88.9)	1 (1.2)	28 (35.0)	51 (63.8)	70.8
Turkey	8	7 (87.5)	0 (0)	0 (0)	7 (100.0)	6.2
Duck	15	11 (73.3)	0 (0)	2 (18.2)	9 (81.8)	9.7
Total	113	98 (86.7)	1 (1.0)	30 (30.6)	67 (68.4)	86.7
Prevalence		0.9 (86.7)	0.01 (0.9)	0.27 (26.5)	0.59 (59.3)	-

Table 4: Recovery of *Mycoplasma spp.* from Farm B.

Source	No. of samples submitted	Total Mycoplasma - positive samples (%)	Mycoplasma-positive (%)			Whole population positive samples (%)
			M. gallisepticum	*M. gallinarum*	*Mycoplasma spp.*	
Chicken	14	12 (85.7)	0 (0)	3 (25.0)	9 (75.0)	42.9
Goose	14	0 (0)	0 (0)	0 (0)	0 (0)	0
Total	28	12 (42.9)	0 (0)	3 (25.0)	9 (75.0)	42.9
Prevalence		0.4 (42.9)	0 (0)	0.1 (10.7)	0.3 (32.1)	-

Discussion

This study shows that *Salmonella enteritidis* and other *Salmonella spp.* were present in multi-species poultry farming. It shows that Salmonella is present in the alimentary tracts and faeces of poultry of different species, such as chickens, ducks and geese. One faecal dropping sample from the environment (floor) from one of the farms showed the presence of *Salmonella spp*. The predominant species isolated were unidentified *Salmonella spp.* (70.0%) and *S. enteritidis* (30.0%). *S. enteritidis* is one of the common several paratyphoid Salmonella serotypes that are consistently isolated at high incidence in chickens and turkeys either in broiler and layer flocks (Poppe, 1994). The presence of paratyphoid salmonellae species in the cloacal swab samples are not unusual since these organisms can infect a wide variety of

hosts, result in relatively asymptomatic intestinal carriage and the organisms are usually excreted in the faeces (Calnek *et al*., 1997). Cloacal swabs have been used to provide evidence of persistence intestinal colonization by salmonellae in individual birds. Besides that, culturing environmental samples of faecal droppings is a useful diagnostic tool to monitor for the introduction of salmonellae into poultry houses or its environment by vectors, personnel, equipments and other sources.

Salmonella that was isolated from faecal droppings indicated that Salmonella can spread horizontally within and between flocks through contaminated litter or environment. According to Gast and Beard (1990), they reported that *S. enteritidis* could be found in the faeces and internal organs of uninoculated laying hens in cage adjacent to those of orally inoculated birds. Contaminated poultry house environments are often implicated as among the principal sources of Salmonellae (Kumar *et al.*, 1971).

In this study, *Mycoplasma gallisepticum* (MG) and other *Mycoplasma spp.* were present in multi-species poultry farming. MG was detected from the choanal cleft of one single chicken in Farm A.

It is possible that the Salmonella and MG had been transmitted through infected birds, eggs, wild birds, vehicles and fomites. The most likely cause of infection as indicated in this study was by direct transmission through fertile eggs from infected parent stocks (Jordan, 1985). There were other possible factors that lead to the infection of Salmonella and MG in the flock, such as the type of management and stressors such as cold during the rainy season and flock health programmes including vaccination activity that cause stressful conditions to the chickens, which lead to reduced immunity against Salmonella and MG infection.

The significant difference in prevalence for both *Salmonella spp.* and MG among different species of poultry (village chickens, turkeys, ducks and geese) in both farms were observed. Village chickens were harbouring high amount of both *Salmonella spp.* and *Mycoplasma spp.* as compared to other species of poultry. This indicates that the village chickens are more susceptible in contracting the infection, probably due to the difference in genetic susceptibility to infection and immunity status. The prevalence of Salmonella in village chickens was 3 times higher than the ducks and 6.5 times higher than the geese. The prevalence of MG was 0.7 times higher in the village chicken than the ducks, turkeys and geese.

In summary, *Salmonella spp*. and *Mycoplasma gallisepticum* were common in village chickens under study. *Salmonella spp*. was more common compared to MG. The infection rates for both Salmonella and MG were different among the farms. The infection rates for both Salmonella and MG were also different among the category of poultry. The highest prevalence of both *Salmonella spp*. and Mycoplasmas was detected in the village chickens.

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PREVALENCE OF LEPTOSPIRAL INFECTION IN RATS AROUND SERDANG AND KUALA LUMPUR

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Sixty rats (27 from Serdang and 33 from Kuala Lumpur) were trapped and blood sample was collected from each rat for serology. All serum samples were examined for presence of leptospiral infection by the microscopic agglutination test (MAT) against sixteen live antigens. Fifty-four (27 from Serdang and 27 from Kuala Lumpur) of the sixty rats were euthanized and kidney samples collected for leptospiral isolation and polymerase chain reaction (PCR) assay. Serological study showed that one rat from Serdang had an antibody against *javanica* whilst six rats from Kuala Lumpur had antibodies against *javanica, icterohaemorrhagiae, autumnalis,* and *bataviae.* Ten leptospiral isolates were obtained from the kidneys and PCR assay showed 4 of the 54 kidney samples were positive for pathogenic leptospires.

Keywords: Rats, leptospirosis, diagnosis, isolates, MAT, PCR

Introduction

Leptospirosis is an important zoonosis throughout the world, particularly in tropical countries where the prevalence of infection is high. Leptospiral infection in humans (Fletcher, 1928; Tan, 1970), wildlife (Smith *et al.,* 1961) and the environment (Baker & Baker, 1970) in West Malaysia have been well-documented (Bahaman & Ibrahim, 1987). Many leptospiral serovars are being maintained by wildlife and only a few by domestic animals. Each serovar apparently causes characteristic clinical features and has its own natural course of infection (Faine, 1994). Rats are considered the most significant reservoir of leptospirosis worldwide (Bahaman and Ibrahim, 1988; Weekes *et al.,* 1997). The objectives of the present study were to determine the prevalence of leptospiral infection in rats in Serdang and Kuala Lumpur and to determine which leptospiral serovars are prevailing in the rat population in these areas.

Material and Methods

Samples
Twenty-seven rats from Serdang residential area and thirty-three rats from Kuala Lumpur area were trapped alive and used in this study. The rats were anaesthetized with chloroform. Sixty blood samples were obtained by cardiac puncture and the serum samples obtained were stored at -20°C until tested by the Microscopic Agglutination Test (MAT). All rats were sacrificed and kidney samples were collected for leptospiral isolation and polymerase chain reaction (PCR) assay.

Bacteriological examination
Each kidney sample collected was macerated in a 5 ml syringe and forced into universal bottle containing 10 ml liquid Johnson and Seiter (JS) medium. The suspension was then mixed and allowed to stand at room temperature for 30 to 60 min. Two drops of the lysate were inoculated into four bottles of semisolid JS medium. All cultures were incubated at 30

°C and examined after 2 weeks for presence of leptospires by placing a drop of the culture on a glass slide and examine under dark-field microscopy. Positive cultures were consecutively subcultured into new semisolid JS medium until a pure culture was obtained.

Serological examination
Serum samples were examined for the presence of leptospiral antibodies by the microscopic agglutination test (MAT). The MAT was performed using 16 live antigens: *icterohaemorrhagiae, canicola, pomona, shermani, hadjo bovis, javanica, pyogenes, autumnalis, bataviae, australis, grippotyphosa, celledoni, tarassovi, ballum, cynopteri,* and *djasiman.*

Detection of Leptospiral DNA
Homogenized kidney lysate was incubated at 65 °C for 30 min. Subsequently 3 µl of RNase Solution was added into 1.5 ml microcentrifuge tube containing 600 µl lysate and incubated the mixture at 37 °C for 15 min. It was then cooled to room temperature for 5 min before 200 µl Protein Precipitation Solution was added, followed by thoroughly vortexing and chilled on ice for 5 min. The precipitate protein was rapidly sedimented by centrifugation. The supernatant containing the DNA was carefully transferred to a new 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol, then mixed gently and the solution centrifuged for 2 min at 13,000 rpm to sediment the DNA. The DNA was washed with 70% ethanol after the removal of the supernatant. The solution was centrifuged again for 2 min at 13,000 rpm. Lastly the ethanol was removed and the white pellet of DNA was air-dry for 15 min. The DNA was rehydrated with 100 µl DNA Rehydration Solution and incubated for 1 h at 65 °C, and kept at 4 °C until used.

Polymerase Chain Reaction (PCR)
Forty-five microliters of PCR reaction mixture containing 2 µl $MgCl^2$, 5 µl $MgCl^2$ buffer, 1 µl dNTP, 10 µl primer G1 (5' CTG AAT CGC TGT ATA AAA GT 3') and primer G2 (5' GGA AAA CAA ATG GTC GGA AG 3') each, 16 µl distilled water and 1 µl Taq polymerase were added into 5 µl rehydrated DNA in PCR tube. PCR was performed in a Thermocycler (Bio-Rad) with the conditions consisted of 35 cycles of consecutive denaturation, annealing of primers and DNA chain extension (3 min at 94 °C, 1 min at 55 °C, and 90 sec at 72 °C) preceded by an initial 3 min denaturation at 95 °C and followed by final elongation step at 72 °C for 10 min (Khairani-Bejo, 2002). PCR amplification products were detected by gel electrophoresis. Five microlitres of the amplicons were mixed with 2 µl loading buffer, and transferred into the wells of 2% agarose gel. Positive and negative controls were also loaded into each gel.The gels were stained with ethidium bromide for 15 min and leptospiral DNA bands were analyzed using Gel Doc system.

Results and Discussion

Microscopic Agglutination Test (MAT) is the most widely used laboratory test for diagnosis of leptospiral infection. Sixteen serovars were used as antigens and tested against the rat sera in this study. One sample (3.7%) from Serdang and six samples (18.2%) from Kuala Lumpur were positive for leptospiral infection. Kuala Lumpur has a higher prevalence of leptospiral infection compared to Serdang. Rats from both areas were positive and this indicated that rats are commonly infected with leptospirosis. The results were interesting as there were some samples which were MAT-negative but cultures of the kidneys were positive. Bacteriological prevalence of leptospiral infection in Kuala Lumpur was higher (29.6%) than Serdang (7.4%). It appears that culture is more sensitive than MAT as shown in this study.

Based on PCR, only 4 of the 54 kidney samples tested from Serdang and Kuala Lumpur were positive. The two primers used in this study will only bind with pathogenic leptospiral DNA. In this study, PCR results showed that the rats from both areas were infected with pathogenic leptospires. Three of the PCR positives were also culture positive.

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EFFECT OF TRICAINE METHANESULPHONATE (MS222) AND CLOVE OIL ON IKAN PUYU (*ANABAS TESTUDINEUS*) AND CARP (*CYPRINUS CARPIO*)

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This study was performed to observe the effects of Tricaine Methanesulphonate (MS222) and clove oil on, ikan puyu (*Anabas testudineus*) and carp (*Cyprinus carpio*) under different temperatures. Sixty ikan puyu and 60 carps were divided into 20 groups of three fishes each. Eight groups of fish were anaesthetized using 50, 100, 150, or 200 ppm of MS222 at 27 °C and at 20 °C. Another eight groups of fish were anaesthetized using 40, 60, 80, or 100 ppm of clove oil at 27 °C and 20°C. Four groups of fish were used as controls where two groups were placed in water containing no anaesthetic at 27 °C and 20 °C and another two groups were placed in water with dissolved ethanol only at 27 °C and at 20 °C. The fish was transferred into the anaesthetic bath, observed for response until stage 4 anaesthesia was reached or the time taken to induce the fish to stage 4 (induction time) exceeded 30 minutes. It was found that the induction times for ikan puyu were significantly longer than carp at all test doses. Reducing the temperature did not show significant difference in induction time for carps (clove oil and MS222) but it did for Ikan puyu using clove oil at a dose of 40 ppm (1800 to 1304.7sec.) and 60 ppm (1199 to 648.7 sec).

Keywords: tricaine methanesulphonate, clove oil, ikan puru, carp

Introduction

Anaesthesia is a reversible state of unconsciousness. It can be achieved in fish with chemicals, hypothermia, or exposure to an electric current (Ross, 2002). A wide variety of compounds has been utilized to anesthetize fish in health, research, management, and in aquaculture. However, in practice, the vast majority of anesthetic procedures have been performed with a limited number of compounds. Marking and Meyer (1985) indicated the most commonly used anesthetic compounds were tricaine methanesulphonate (MS222), quinaldine, and carbon dioxide. In recent years there has been a number of reports describing the use of clove oil as an anaesthetic in fish and its usefulness as an alternative to MS222 (Cho and Heath, 2000). These same authors also mentioned the many advantages of using clove oil compared to MS222, which include easy to obtain, inexpensive and being an organic product, it would not require a withdrawal period for fish. The time interval between placing a fish in the anaesthetic bath to reach a particular stage of anaesthesia is referred to as the induction time. The time interval between removing the fish from the anaesthetic bath to complete recovery is referred to as the recovery time. The climbing perch, known locally as ikan puyu (*Anabas testudineus)* and *Cyprinus carpio*, or carp are two species of fish that have two different modes of oxygen uptake from water. The carp are fish that breathe through their gills, while ikan puyu uses the labyrinth organs along with the gills. These labyrinth organs are opercular air chambers resulting from the extension of the opercular diverticulum into the skull to take the form of a labyrinth-like organ or coral-like dendritic

formations. This labyrinth organ is seen also in species of *Clarias* (Johansen, 1970). Gills are the primary organ involved in the uptake of an anaesthetic drug into the fish's circulation and therefore it would be interesting to observe the effect of the anaesthetic drug on air-breathers. Locally ikan puyu are farmed and consumed as a source of protein and sold as ornamental fish. There is a very large ornamental fish trade and Malaysia is the one of the largest exporters of ornamental aquarium fish. During transportation, the fish undergo a certain amount of stress and to reduce the stress, the fish would be sedated with an anaesthetic agent, which would incur a considerable amount of expenses. In the light of this situation, there is a need for the use of cheaper anaesthetic drugs that are more readily available. Clove oil is essentially cheaper than MS222 as an anesthetic (Soto and Burhanuddin, 1995). Reducing the reducing the temperature of the water would also reduce the induction time of anaesthesia (Hikasa *et al.*, 1986). This mode of anaesthesia could be applied to different species of fish (Hoskonen and Pirhonen, 2004) including the carp and ikan puyu. Thus, the objective of this study was to observe the effects of Tricaine Methanesulphonate (MS222) and clove oil on, ikan puyu (*Anabas testudineus*) and carp (*Cyprinus carpio*) under different temperatures.

Materials and Methods

Sixty Juvenile carp and 60 ikan puyu were each divided into 20 groups of 3 fishes each and placed in separate holding tanks with water maintained at 27 °C. The water pH was 6. The fish were housed in the tanks for a period of 2 weeks where the water was treated with sodium chloride at 3 ppt. The fish were fasted for two days before treatment with the anaesthetic. Ten grams tricaine methanesulphonate (MS222) was dissolved in 200 ml of distilled water and clove oil [88% (w/v) eugenol]. Eight groups of fish were anaesthetized using 40, 60, 80, or 100 ppm of MS222 at 20 °C (cold water) and at 27 °C (room temperature). Another 8 groups were anaesthetized using 40, 60, 80, or 100 ppm clove oil at 20 °C and at 27 °C. The remaining 4 groups of fish were the controls. The behavioural changes (Table 1) shown by anaesthetised fish was used to identify the stages of anaesthesia, induction and recovery times.

Table 1: Stages of anaesthesia

Stages	Characteristic behaviour
1	Opercular movement visibly slows or becomes erratic
2	Sporadic loss of equilibrium, difficulty maintaining position while at rest
3	Complete loss of equilibrium; inability to regain upright position
4	No reaction to handling or a sharp prod in the peduncle
5	No reaction with complete loss of opercular movement
Recovery	Ability to remain upright, normal swimming behaviour

Results

Fish anaesthetised with MS222

Increasing the anaesthetic dose of MS222 significantly reduced the induction time for ikan puyu and carp. Stage 4 was seen at 100, 150, and 200 ppm. The time required for carp (271.2, 121.0, 102.3sec.) to reach stage 4 anaesthesia was shorter than for Ikan puyu (>1800.0, 582.7,

405.7sec.). The mean recovery time of *Ikan puyu* at room temperature was longest at 100 ppm, which was 977.3sec. Groups of fish anaesthetised at 200 ppm in cold water showed large variations in the recovery times. The induction time of Ikan puyu anaesthetised in cold water was shorter than fish anaesthetised at room temperature. At 100 ppm (cold water), the induction time was 1197.7sec. The groups anaesthetised at room temperature did not reach stage 4 anaesthesia after 1800 sec.

No difference between groups of carps anaesthetised at room temperature water and those anaesthetised in cold water. None of fish from the control group reached stage 4 anaesthesia after 30 minutes. Postshock test did not show significant difference between 150 and 200 ppm of MS222 in ikan puyu and carps at either species of fish at 20 and 27 °C.

Fish anaesthetised with clove oil

Ikan puyu at 40 ppm clove oil did not reach stage 4 anaesthesia even after 1800sec. However, in cold water it took the fish 1304.7 sec to reach stage 4 anaaesthesia. The recovery times of ikan puyu anaesthesised with 40, 60, 80, and 100 ppm clove oil were shorter at room temperature (1283.3, 980.0, 1260.0, 1446.0 sec respectively) compare to in cold water (866.3, 900.7, 928.3, 1170.7 sec respectively). None of the fish from the control group reached stage 4 anaesthesia after 30 minutes.

The induction time of aneasthesia at room temperature in the carps was lower at higher doses clove oil. All carp reached stage 4 anaesthesia. At 40, 60 and 80 ppm clove oil the induction times in carps anaesthetised at room temperature were higher (378.7, 183.0, 122.3 sec respectively) than in cold water (303.3, 148.3, 99.3 sec respectively). However, these differences were not significant ($p<0.05$). At 100ppm of MS222 and clove oil, there was an increase in the induction time (173.0 sec) of anaesthesia in the carps.

Discussion

There is a very clear difference between the two species in both induction time and recovery times from anaesthesia with MS222 and clove oil. For Carp anaesthetised with MS222 the most effective dose was 100 ppm and for ikan puyu it was 150ppm. The reason for this difference is probably their physiological differences where ikan puyu are air-breathers and with gill surface area to body weight ratio lesser than the carp (Ross and Ross, 1999). This means that the ikan puyu has a smaller surface area exposed for the uptake of the anaesthetic agent and that a higher anaesthetic dose is required for the fish to reach stage 4 anaesthesia. Another reason for ikan puyu requiring a higher dose could be that when the fish is placed in a noxious solution, they have a tendency and ability to hold their breath thus prolonging the induction time of anaesthesia. In fish, the movement of the operculum is usually taken as the respiratory rate. In this study for both carp and ikan puyu, as the dose of clove oil increased the respiratory rate decreased leading to increase in carbon dioxide in blood. Carbon dioxide has been also known to elicit anaesthesia in fishes. Therefore, it would be hard to say if the anaesthetic effect is purely due to the clove oil or from the effect of increased circulating CO2. In fish anaesthetised with MS222, there was an increase in the respiratory rate. This observation was similarly reported by Hikasa *et al*., (1986). From our study, it was observed that a dose of 100 ppm produced an average induction time of about 300 sec at 27°C and 20°C in carps. This can be a good dose for stage 4 anaesthesia in carp using MS222. With clove oil, however, the anaesthetic dose required for the same induction time in carps was only 60 ppm. This indicates that clove oil has a good deep anaesthetic level at a lower dose compared to MS222. In groups anaesthetised with clove oil, it was found that at higher doses

the induction time was shorter but the recovery time was prolonged. These findings were similar to previous studies (Hikasa *et al*., 1986; Munday and Wilson, 1997; Keene *et al*., 1998; Woody *et al*., 2002). This would make clove oil useful for restraint and handling procedures that require a long handling time such as physical examination and even short surgical procedures. Clove oil is also more readily available since it can be purchased from health and herbal shops locally compared to MS222, which required a prescription. Because of this, clove oil can be a good alternative to MS222. The price of clove oil is cheaper compared to MS222. Clove oil is also cheaper, because for every fish anaesthetised using MS222, about 14 fishes could be anaesthetised using clove oil.

Isoeugenol, a derivative of clove oil, has shown to have good anaesthetic effect and a rapid tissue clearance rate within 48 hours (Kildea *et al*., 2004). This means that there is no withdrawal period for clove oil compared to the 21-day withdraw period for MS222. Temperature influences oxygen consumption and oxygen-carrying capacity of the water (Ross and Ross, 1984). Consequently, lowering the water temperature will tranquillise or even immobilize fish, (Ross and Ross, 1984). This suggests that in some cases, lowering the water temperature may enhance the anaesthetic effect of a product by reducing the required dose. Reducing the water temperature in our study had reduced the induction time anaesthesia in ikan puyu. The reason for this effect may be because ikan puyu is a tropical fish and reducing the temperature to 20°C could have reduced the metabolism of the fish significantly enough to shorten induction time of anaethesia. In carps, reducing the water temperature did not significantly reduce the induction time.

In conclusion, the anaesthetic doses required by ikan puyu to reach stage 4 anaesthesia for MS222 and clove oil were higher than for the carp. The effective dose of MS222 was 100 ppm for carps and 200 ppm for ikan puyu. The effective dose of clove oil was 40 to 60 ppm for carps and 100 ppm for ikan puyu. Reducing the water temperature did not influence the induction time in the carp. In ikan puyu, reducing the water temperature reduced the induction time of anaesthesia with clove oil.

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SUSCEPTIBILITY OF *LEPTOSPIRA INTERROGANS* TO GINGER

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A study to determine the susceptibility of *Leptospira interrogans* to ginger was carried out. Five ginger species used were *Etlingera, Globba, Camptandra, Scaphochlamys* and *Zingiber*. Five *Leptospira* strains which are *L. autralis, L. canicola, L. icterohaemorrhagiae, L. javanica* and *L. pomona* were used to determine the minimal inhibitory concentration (MIC). All ginger extracts tested had antigenic properties against serovar strains tested. *Etlingera sp* had shown the lowest MIC value that was 1,562 µg/ml. *Etlingera* sp had lower MIC than other gingers for serovar strain *L. australis* (12,500 to 1,562 µg/ml). It is suggested that ginger may be used as an alternative treatment of leptospirosis.

Keywords: *Leptospira interrogans*, ginger, minimal inhibitory concentration (MIC)

Introduction

Leptospirosis is a worldwide zoonotic infection caused by *Leptospira sp*. The disease is one of major public health concern due to the high infection rate in fresh water contaminated with infected animal urine. In Malaysia, leptospirosis had occurred among some participants of the recent EcoChallenge Sabah 2000 competition (Levett, 2001). Hence, leptospirosis is economically important in our country, proper control and prevention should be undertaken to avoid great economic losses to the livestock industry. Currently, penicillin and doxycycline are the most common antibiotics used in the treatment of leptospirosis. However, resistance of the *Leptospires* against these antibiotics must be considered. The efficiency of these antibiotics will decrease under long-term use.

Natural herbs have been also been used as alternative treatment of leptospirosis. An *in-vitro* study has demonstrated that ginger root extracts containing the gingerols are able to inhibit the growth of *Helicobacter pylori* strains (McLoughlin *et al.*, 2004). Another study demonstrated *Zingiber officinale* had successfully exhibited antibacterial activity against four respiratory tract pathogens, which are *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and *Haemophilus influenzae* (Chrubasik *et al.*, 2005). However, none of the studies has been carried out to test the antibacterial activity of natural herbs to *Leptospira* strains. Thus, the objectives of the present study were to evaluate the antibacterial properties of ginger species and to determine the effectiveness of several ginger species to *Leptospira interrogans*.

Materials and Methods

Bacterial strains
Five *Leptospira* strains were used in this study, which were *L. autralis, L. canicola, L. icterohaemorrhagiae, L. javanica* and *L. pomona*. The *Leptospira* strains were inoculated into 20 ml Johson Seiter medium and incubated at 30 °C for 7 days.

Antibacterial agents

Five ginger extracts which are *Etlingera, Globba, Camptandra, Scaphochlamys,* and *Zingiber* species were used in this study. The ginger extracts were obtained from the Laboratory of Natural Product, Institute of Bioscience, Universiti Putra Malaysia.

Minimal Inhibitory Concentration (MIC) Determination

Two hundred milligram (200 mg) of ginger extract was dissolved in 1 ml 10% Dimethyl Sulfoxide (DMSO) to a concentration 200,000 µg/ml. Microtiter plates were used to make the serial dilutions. The MIC procedure used in this study was as described by Clinton and Duane (2004).

Results

The MIC of each ginger extracts was determined by using five serovars strains of the genus *Leptospira*. All five ginger extracts tested had antigenic properties against the five serovar strains tested. *Etlingera sp* had shown the lowest MIC value that was 1,562 µg/ml. *Etlingera* sp had lower MIC than did other gingers for serovar strain *L. australis* (12,500 to 1,562 µg/ml). *Etlingera sp* was found to be less active in the inhibition to *L. canicola. Zingiber sp, Globba patens, Scaphochlamys sp* and *Camptandra sp* has low effectiveness compared to *Etlingera sp*. The MIC value ranged from 6,250 to 50,000 µg/ml with the lowest values for serovar strain *L. australis*. Overall, *L. australis* was more sensitive to all ginger extracts compared to the other serovar strains of genus *Leptospira.*

Discussion

All ginger species are effective against serovar strain of genus *Leptospira. The g*inger species showed the ability to inhibit the activity of *Leptospires* strains in different concentration ranging from 1,562 to 50,000 µg/ml. The findings of this study showed that the *Etlingera sp* is the ginger of choice to treat leptospirosis because its MIC against five selected *Leptospira* strains was relatively lower than other gingers. *Leptospira autralis* was shown to be more sensitive to all ginger extracts compared to other strain of the genus *Leptospira.* Crude ginger contains up to 3% essential oil, up to 9% lipids or glycolipids and about 5 to 8% oleoresin. Twenty-five percent of oleoresin contributed to the pungent property of ginger (Chrubasik *et al.,* 2005). The active ingredients of ginger are though to reside in the essential oil. Essential oil comprises groups of bisabolene, cineol, phellandrene, citral, borneol, citronellol, geranial, linalool, limonene, zingiberol, zingiberene, camphene, phenol, proteolytic enzyme, vitamin B6, vitamin C, calcium, magnesium, phosphorus, pottasium, linoleic acid and oleoresin ((Yance, 2003; Balick, 2005). Oleoresin consists mainly of [6]-gingerol, which is more pungent than [8]-gingerol and [10]-gingerol. Recently, the diterpenoid galanolactone glycosides of geraniol-related compounds and two proteases were identified in the crude plant material. Shogaols contained in semi-dried ginger are more pungent than gingerols. They are a major degradation product of the thermally labile gingerols and rarely found in fresh ginger (Churbasik *et al.,* 2005). Hydroethanolic ginger extract exhibited potent antibacterial activity against Gram-positive and Gram-negative bacteria. The fraction of the crude extract containing the gingerol was the most effective. Thus, gingerol was believed to be the active ingredient of ginger that inhibits the activities of *Leptospira* strains in present study. Due to the increasing emergence of antibiotic resistant *Leptospires,* natural herb such as ginger can be used as alternative treatment of leptospirosis. However, the dosage of ginger extracts used to inhibit the *Leptospira* strains is much higher than the dosage of antibiotics had been used and the dosage ginger extracts against other antimicrobial that had been

mentioned in previous studies. Therefore, preclinical safety of ginger must be studied to determine the possible side-effect of ginger extract on animals or humans.

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PREVALENCE OF INTRAMURAL FAT IN THE FELINE STOMACH WALL

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Abstract

Histology examination of stomach wall of recently euthanized cats was performed to determine the prevalence of the feline gastric intramural fat. The stomach was divided into 6 equal regions (G1, G2, G3, L1, L2, and L3) for the histology study. All 41 carcasses have at least one region that contains gastric intramural fat. The percentage of gastric intramural fat observed for each regions were G1 (93%), G2 (95%), G3 (90%), L1 (98%), L2 (98%), and L3 (90%).

Keywords: feline, histology, gastric submucosal layer, fat

Introduction

Histologically, the stomach is subdivided into four layers namely tunica mucosa, tela submucosa, tunica muscularis and tunica serosa. The tela submucosa or submucosa is a connective tissue layer with blood vessels, lymph vessels, nerve plexi and may contain submucosal glands. Evidently only Frappier (1998) mentioned there is white adipose tissue within the gastric submucosa. The prevalence of this fat layer or in which species it can found is not clear.

This study was undertaken to determine the prevalence of white adipose tissue and its distribution in the regions of the stomach.

Materials and Methods

Forty-one stray cat carcasses obtained from two animal shelters after euthanasia were used in this study. The cats were of variable age and weighed above 1.0 kg.

To conduct histology examination, each stomach was divided into 6 approximately equal regions; G1, G2, G3 of the greater curvature and L1, L2 and L3 of the lesser curvature. Samples from each region were preserved in 10% formalin and kept frozen at -4ºC. The samples were trimmed, and later processed and were embedded. The samples were trimmed, and later processed (Leica®ASP300) and embedded (Leica®EG1160). They were then trimmed and sectioned (Leica®RM2115), positioned on the new slides in while in a water bath (Leica®HI1210) and on a heating plate (Leica®HI1220). The slides were then stained with Haematoxylin and Eosin and examined under the microscope for the presence of intramural fat and its distribution.

Results and Discussion

Gastric intramural fat was present in the submucosal layer in 38 out of 41 (93%) carcasses in the G1 region, 39 out of 41 (95%) carcasses in G2 region and 37 out of 41 (90%) in G3

region. Intramural fat is also present in 40 out of 41 (98%) carcasses in both L1 and L2 regions, and 36 out of 40 (90%) in L3 region. The L1 region had the thickest intramural fat layer followed by L2, G1, G2, L3 and G3 regions.

The distribution of fat within the submucosal layer was observed to be variable. This is because some of the fat globules were distributed discretely separated from each other. Some were present in streaks while others were in multifocal clusters. Some fat covered the entire width and length of a section forming a thick fat layer.

This study confirms the presence of gastric intramural fat within the submucosal layer of the cats' stomach. It was found that all the carcasses have at least one region containing fat globules. Although the presence of intramural fat in the submucosal layer is rarely mentioned in literature, this study showed that its prevalence is 100% and thus suggesting that the intramural radiolucent band seen on radiographs is due to this fat layer (Heng et al. 2005).

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EFFECT OF TRAMADOL ON PULSE RATE, BLOOD PRESSURE AND POSTANAESTHETIC RECOVERY IN SHEEP

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Effects of premedication with tramadol at 2 mg/kg on pulse rate, blood pressure and post-general anaesthetic recovery were evaluated in six 6-month-old male sheep. Sheep were anaesthetized three times using xylazine, ketamine and halothane. Arthrotomy was performed on two occasions of the general anaesthesia while the last occasion was without surgery. Premedication with tramadol or saline were assigned in a crossover manner. There was no difference in pulse rate, mean blood pressure, time from cessation of halothane to swallowing reflex, chewing reflex, head movement, extubation and quality of recovery in sheep that had tramadol compared to sheep without tramadol for each occasion. These findings suggest that tramadol at 2 mg/kg is safe to be used during premedication of sheep in the perioperative period.

Key words: general anaesthesia, tramadol, opioid, sheep

Introduction

Sheep are excellent model for the study of major physiological system, such as the cardiovascular, respiratory, renal, reproductive and endocrinological system (McMillen, 2001). At the Faculty of Veterinary Medicine in Universiti Putra Malaysia, there are increasing numbers of sheep being used as animal models in researches that involve painful surgical procedure. As such, appropriate analgesics should be incorporated in the anaesthetic protocol to reduce pain in research animals. One of the analgesics of choice for treatment of acute and severe pain is opioid (Gaynor and Muir, 2002). In Malaysia, tramadol is one of the opioid that is used in human to control severe pain, and it is easily available. However, tramadol is still not widely used in animals. The use of tramadol in animals had been studied and it is limited to rats (de Wolff *et al.* , 1999), dogs (Mastrocinque and Fantoni, 2003), cats (Teppema *et al*., 2003) and horses (Natalini and Robinson, 2000). Due to the lack of literature on the use of tramadol in sheep, it is necessary to evaluate the safety of the use of this drug in the peri-operative period in sheep. This study aimed to evaluate the effects of tramadol on pulse rate and blood pressure during anaesthesia, and the post-anaesthetic recovery following premedication of tramadol at 2 mg/kg, intramuscularly (IM) in sheep.

Materials and Methods

Animals
This study was carried out using six 6-month-old Malin cross rams, body weight 13 ± 2.85 kg. These sheep required general anaesthesia to perform stifle arthrotomy on two occasions, 3 weeks apart, for a separate study. Sheep were housed indoors, 2 per pen and fed on pellets and water *ad libitum*. Animals were fasted from food for 12 hours before the general anaesthesia.

Experimental protocol

Sheep were premedicated with xylazine, along with the assigned treatments. Treatments consisted of tramadol (Domadol, UNICHEM Laboratories Ltd, India), 2 mg/kg, IM or saline at equal volume, IM. Treatments were randomly assigned and were crossed-over on the second occasion. The observer (TKS) was blinded to the treatment. The first occasion involved stifle arthrotomy to harvest the stifle cartilage while the second to implant bioengineered tissue on the other stifle, and this required longer general anaesthesia. General anaesthesia without surgery was conducted on a third occasion to serve as negative control for the surgical factor.

Following onset of sedation, a cephalic vein was catheterized. Sheep were induced with ketamine (Ketamav 100, Mavlab, Australia) 5 mg/kg, IV, to effect, intubated, and maintained on halothane with 100% oxygen. They were placed on dorsal recumbency and ventilation was controlled. All sheep received Lactated Ringer's fluid at 10 ml/kg/hr, IV during general anaesthesia.

Data collection

Pulse rate and blood pressure were measured after premedication and after induction, for 20 minutes using the noninvasive, oscillometric technique (Surgivet v6004 NIBP Monitor). The rectal temperature was measured prior to the surgery and at the end of the surgery. Following the end of the surgery, the vaporizer was turned off. Time from cessation of halothane to swallowing reflex, chewing reflex, head movement, sternal recumbency and standing were determined. Quality of recovery was assessed based on the first attempt to stand, with scale ranging from 0 (worst ataxia/frequent falling) to 10 (well coordinated stand/normal walk).

Results

There was no significant difference in the pulse rate of sheep that received tramadol compared to sheep that did not received tramadol during anaesthesia for all three occasions of cartilage harvest, tissue implant or negative control (Table 1). All sheep had elevated pulse rate immediately after induction. Mean blood pressure in the sheep that received tramadol in all three occasions tended to be lower than those without tramadol, but was not statistically significant. The mean blood pressures in all sheep remained lower than pre-induction throughout the surgery. Temperature after surgeries were lower compared to the baseline temperature in all three occasions. Following the longer surgical procedure (second occasion), temperature dropped lower than 35°C.

There was no difference in the quality of recovery (standing score) between sheep that received tramadol and sheep that did not receive tramadol within each occasion of general anaesthesia. Standing scores differed among occasions. All sheep stood up well, with no sign of lameness following control general anaesthesia with no surgery. Following stifle arthrotomy to harvest cartilage, sheep also stood well but showed some degree of lameness and were unable to balance well. Sheep showed severe lameness and were unable to balance themselves following arthrotomy to implant tissue.

Table 1: Pulse rate, mean blood pressure, duration of halothane anaesthesia and recovery times in sheep premedicated with tramadol or saline following xylazine-ketamine-halothane anaesthesia

Occasions		Cartilage harvest		Tissue implant		No surgery	
Treatment		T	S	T	S	T	S
Pulse rate (beats/min)	Premedication	70 ± 8	69 ± 4	60 ± 4	70 ± 17	74 ± 3	74 ± 15
	Induction	100 ± 48	69 ± 8	77 ± 8	80 ± 23	89 ± 4	82 ± 12
	10 minutes	62 ± 12	74 ± 21	70 ± 6	74 ± 15	75 ± 6	71 ± 17
	20 minutes	69 ± 4	70 ± 8	67 ± 6	72 ± 12	92 ± 37	72 ± 20
Mean blood pressure (mmHg)	Premedication	83 ± 5	-	86 ± 2	71 ± 0	-	84 ± 12
	Induction	72 ± 9	71 ± 8	55 ± 20	62 ± 3	68 ± 40	70 ± 14
	10 minutes	50 ± 0	61 ± 2	42 ± 10	52 ± 0	59 ± 5	65 ± 30
	20 minutes	67 ± 0	50 ± 0	46 ± 8	55 ± 5	61 ± 10	65 ± 22
Duration of anaesthesia (minutes)		23 ± 2.7	21.3 ± 3.2	92.3 ± 30.4	98.7 ± 13.0	30.0 ± 0.0	30.0 ± 0.0
Recovery time (minutes)	Swallowing reflex	14.0 ± 4.4	15.0 ± 8.7	6.3 ± 1.5	14.0 ± 6.6	5. 7 ± 2.5	6.0 ± 4.0
	Chewing reflex	14.0 ± 4.4	15.0 ± 8.7	6.3 ± 1.5	14.0 ± 6.6	5.7 ± 2.5	6.0 ± 4.0
	Head movement	17.0 ± 1.4	19.0 ± 12.7	6.5 ± 2.1	16.0 ± 7.1	5.7 ± 2.5	8.0 ± 5.3
	Extubation	16.7 ± 6.7	28.5 ± 0.7	12.7 ± 4.0	20.3 ± 5.0	6.7 ± 3.2	10.3 ± 1.5

(n=6). Data are expressed as mean ± SD. There was no significant difference between treatment groups in all three occasions. Tramadol (T) at 2 mg/kg, IM or saline (S), at equal volume, IM.

Within each occasion, the duration of halothane anaesthesia for sheep that received tramadol and sheep that did not received tramadol was not different. Time from cessation of halothane to return to swallowing reflex, chewing reflex, head movement and extubation for all three occasions are summarized in Table 1. There was no significant difference in recovery time between tramadol-treated and nontreated sheep.

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SENSITIVITY AND SPECIFICITY OF A COMMERCIAL CANINE *DIROFILARIA IMMITIS* ANTIGEN TEST KIT IN STRAY DOGS

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The prevalence of dirofilariasis in stray dogs was determined by Knott's concentration test (KCT), necropsy and a commercial Anigen Rapid® *dirofilaria immitis* antigen test kit. One hundred and thirty-two local dogs of more than one-year old, scheduled for euthanasia were selected from Dewan Bandaraya Kuala Lumpur. Blood samples from 30 worm-free animals and 34 from those with worm burden were chosen to test the sensitivity and specificity of the commercial kit. Three millilitre blood samples were collected by cardiac puncture prior to euthanasia. At post-mortem, the heart and lungs were removed and examined grossly. The right atrium and ventricle, and pulmonary arteries were dissected and heartworms collected, preserved in 70% alcohol for worm count, identification and sex determination. The sensitivity and specificity of the Anigen Rapid® test for the 64 dogs were 33/34 (97%) and 29/30 (96.7%) respectively. The sensitivity to one worm infestation was 12/13 (92%). The sensitivity to one male and one female worm infestations was 7/8 (87.5%) and 5/5 (100%) respectively. With the KCT 44 dogs were positive for dirofilariasis. Of these, 38/44 (86%) dogs positive for dirofilariasis had adult worms only. Four dogs had adult worms and microfilaria and 2 had microfilaria only. The study showed that the Anigen Rapid@ test kit is highly sensitive and specific for the detection of *Dirofilaria immitis* in dogs.

Keywords: Dirofilaria, dogs, Anigen Rapid®.

Introduction

Heartworm infestation in dogs is a ubiquitous disease caused by the blood parasite *Dirofilaria immitis*. The presence of one of more species of vector-competent mosquitoes makes transmission possible wherever a reservoir of infection and favourable climatic conditions co-exists (McCall *et al*. 2004).

The common techniques for detection of circulating *Dirofilaria immitis* microfilariae include wet blood smear or direct wet mount, Knott's concentration test and filter test (Kittleson and Kienle, 1998). The modified Knott's concentration test is the preferred technique for differentiating *Dirofilaria immitis* from the nonpathogenic filarial species. These conventional methods are however inadequate since occult infestation can occur commonly (Reifur *et al.* 2004). The ELISA and immunochromatographic test systems are now available for detection of circulating heartworm antigen. The utility of ELISA test can be highly speculative because assessing the degree of parasitism by this method is limited by confounding complications such as transient increase in antigenaemia associated with recent worm death. This test often requires correlation with other relevant information (McCall *et al*. 2004).

This study was undertaken to determine the sensitivity and specificity of a commercial ELISA test kit in the detection of dirofilaria infection in stray dogs.

Materials and Methods

Animals
One hundred thirty-two mostly local dogs scheduled for euthanasia were selected from Dewan Bandaraya Kuala Lumpur. The dogs were more than one year old and of variable body weights. Blood was sampled into EDTA tubes from the dogs via cardiac puncture immediately prior to euthanasia to be used for the determination of sensitivity and specificity of the Anigen Rapid® test.

Necropsy
The dogs were euthanised with pentobarbital sodium. The heart and lungs were removed from the carcasses and examined grossly. The right atrium and ventricle and the pulmonary arteries were dissected to determine worm burden. All heartworms collected were preserved in 70% alcohol. The worms were counted, identified and their sex determined.

Modified Knott's Concentration Test (KCT)
Nine millilitres of 2% formalin was added to 1mL uncoagulated blood, mixed and centrifuged at 250 x *g* for 5 minutes. The supernantant was carefully decanted and a drop of 1% methylene blue added to the sediment and resuspended. Two drops of suspension were placed on a clean glass slide, covered with a glass slip and examined under a microscope.

Dirofilaria immitis antigen test kit (Anigen Rapid®)
At necropsy, 30 dogs free from worms and 34 with worm burden were chosen for the Anigen Rapid® sensitivity and specificity study. The kit consists of a sealed cartridge with a window for placement of blood samples. A drop of approximately 40µL whole blood followed by 4 drops of assay diluent was placed onto the window. The results were read after 5 to 10 minutes.

Results

At necropsy, 30 dogs were free from worms while 34 others showed worm burdens ranging from 1 - 14 worms/dog (Table 1).

Table 1. Anigen Rapid® *Dirofilaria immitis* antigen test on worm-infested dogs

No. of Dogs	**worm(s)/dog**	**sex of worm**	**Test-positive**
30	0	-	1
8	1	male only	7
5	1	female only	5
4	2	male only	4
1	2	female only	1
11	2 to 3	male + female	11
5	4 to 14	male + female	5
Total 64	**-**	**-**	**63**

In these dogs, the sensitivity and specificity of the test kit were 33/34 (97%) and 29/30 (96.7%) respectively (Table 2). The positive and negative predictive values were 97% and 96.7% respectively. In one worm infestation, the sensitivity of the test kit was 12/13

(92.3%). In dogs with one male worm infestation the sensitivity was 7/8 (87.5%) with one false-negative and in dogs with one female worm infestation, the sensitivity was 5/5 (100%).

Table2. Sensitivity and specificity of Anigen Rapid® *Dirofilaria immitis* antigen test kit

Anigen Rapid® Test	**worm-infested**	**worm-free**	**Total**
Positive	33	1	34
Negative	1	29	30
Total	**34**	**30**	**64**

The KCT showed that 44 of 132 dogs were positive for dirofilaria, giving a prevalence of 33.34%. These animals either had adult worms only, adult and microfilaria or microfilaria only (Table 3).

Table 3. Detection of dirofilariasis by modified Knott's concentration test

Dirofilaria immitis	**Number of carcasses**
Adults only	38 (89%)
Adults + microfilaria	4 (9%)
Microfilaria only	2 (5%)
Total	**44**

Discussion

The ELISA and immunochromatographic test systems are able to detect circulating heartworm antigens, thus providing using useful clinical data. The Anigen Rapid® dirofilaria test kit was claimed by the manufacturer to have 94.4% sensitivity and 100% specificity. Our results showing the sensitivity and specificity of the kit at 97.1% and 96.7% respectively seems to support this claim. With the present generation of heartworm antigen detection kit, most occult infections consisting of at least one mature female can be detected at almost 100% specificity (McCall *et al.* 2004). Circulating antigen concentration increases with increase in worm burden (Kittleson and Kienle, 1998). At low concentrations the antigen may be difficult to detect. The Anigen Rapid® dirofilaria test kit was developed to detect low circulating antigen concentrations regardless of sex of the worms.

The test also gave one false-negative and one false-positive result. False-negative results can commonly occur when the worm burden is low, female worms still immature and tests not done at room temperature. False-positive results on the other hand usually occur as a result of technical error or delay in reading results. Thus, it is important that when using the test kit, relevant clinical information is taken into account. If the validity of the result is doubtful, verification can be obtained by retesting with the same test kit or by using a different method.

The study showed that the prevalence of dirofilariasis in Selangor, Malaysia is 33.34%. This is within the range of 25.8% to 42% reported previously (Mullin 1970; Retnasabapathy *et al*. 1976, Dahliwal 1987). Occult infestation can occur in dogs and cats. These animals harbour adult worms but do not have circulating microfilaria. In Malaysia the prevalence of occult infestation has increased gradually from 21.4% in 1970 (Mullin 1970) to 57.89% in 2002 (Toh 2002).

The study has shown that Anigen Rapid® canine *Dirofilaria immitis* antigen test kit is a reliable diagnostic tool in dirofilariasis particularly in male worm infestations. This kit is highly sensitive and specific, easy to use and does not have special storage requirements. It should be used routinely in small animal practice for the diagnosis of dirofilariasis.

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RESTRICTION ENDONUCLEASE ANALYSIS OF HERPESVIRUSES ISOLATED FROM CAPTIVE WILDLIFE

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Herpesviruses were isolated from an oral swab of one adult Malayan bear and from plasma samples of two gaurs kept in captivity in a conservatory. Using restriction endonuclease (RE) analysis with eight RE enzymes: either single or double digestion with *Bam*HI, *Xho*1, *Eco*R1, *Kpn*1, *Hind*II, *Bam*HI/*Hind*III, *Bam*HI/*Eco*R1, *Bam*HI/*Kpn*1, the DNA profiling were analysed by Alpha Ease FC software and compared with reference herpesviruses. The RE pattern of the isolates from two wildlife species are neither similar to reference strains nor to each other. Isolates from both species show some similarities in a few fragments with the pseudorabies virus mutant and bovine herpesvirus 1.

Keywords: herpesvirus, restriction endonuclease, bear, gaur.

Introduction

Herpesviruses are highly disseminated in nature and most animal species have yielded at least one herpesvirus. The virus has been in humans, animals, birds, insect, reptiles, amphibians, and mollusks. Few herpesviruses are found to naturally infect more than one species. The highly adaptable nature of the herpesvirus makes the number of virus identified to date likely to exceed 130 (Roizman and Pellet, 2001). In this study, we report the isolation of the herpesviruses in gaurs and bear kept in captivity in Malaysia.

Materials and Methods

Specimens
Samples of plasma from two gaurs and oral swab from one adult Malaysian bear were obtained from the Malaysian Conservatory unit in Malacca.

The plasma and oral swab samples were centrifuged (1000 x *g* for 10 min) and the medium filtered through 0.45 filters (Sartorius Minisart, Germany). The filtered fluids were stored at –80 °C and inoculated onto cell cultures within 48 h.

Cell Culture and Virus Isolation
Fresh, 24-hr monolayers of African green monkey kidney (Vero) cells, grown in 75 cm^2 sterile disposable polystyrene cell culture flask was inoculated with 1ml of the filtered fluid samples. The inocula were adsorbed onto cells for 1 h at 37 °C and the medium was replaced with 60 to 70 ml RPMI 1640 maintenance medium containing 5% FCS and 100 unit/ml penicillin and streptomycin. The cell control consisted of an uninoculated flask of Vero cells.

The inoculated cell culture flasks were then further incubated at 37 ºC and were observed daily for cytopathic effect (CPE).

Negative-stained electron microscopy
Cell monolayer with 70% to 80% CPE was detached from the flask by using sterile glass beads. The detached cells and fluids were gathered from each of the infected flasks and centrifuged at 1800 x *g* for 15 minutes at 4 ºC to remove cellular debris. The resultant supernatant was then centrifuged at 18000 x *g* for 3 hours in a fixed-angled J2-21M Beckman rotor using a Beckman centrifuge (USA). The supernatant fluid was discarded and sterile 3 to 5 ml TNE buffer added to the pellet. The pellet suspension was sonicated for 30 sec to break-up the pellets and vortex gently. The virus suspension was layered gently onto 10% to 50% sucrose solution in TNE linear density gradient. The layered virus was ultracentrifuge at 41000 x *g* for 18 hours at 4 ºC. The clear white band that appeared was collected and dialysed against TNE buffer and then pelleted by ultracentrifugation at 41000 x *g* 1 hour at 4 ºC. Finally, the purified virus was resuspended in a small NTE buffer and stored at -70 ºC for future analysis. A drop of purified virus was placed on piece of wax paper and a 200-mesh carbon-formwar-coated grid was placed with the carbon side down onto the virus for 5 min, blot dry and then stained with 2% phosphotungstic acid. The grids were viewed under electron microscopy.

DNA preparation and analysis
A large flask containing 175 cm^2 of fresh Vero cells were inoculated with 1 ml of 10^{-2} virus dilution of each of the herpesviruses isolates, V4, V5 and V19. Inoculums are allowed to adsorb at 37ºC for 1 h. The medium was replaced with 60 to 70 ml RPMI 1640 maintenance medium supplemented with 5% FCS and 100 unit/ml of penicillin and streptomycin and cells were incubated at 37ºC. When 70 to 80 % CPE occurred, the medium was cleared by centrifuging at 1800 x *g* for 15 min at 4 ºC. The clarified medium was concentrated by centrifugation at 18000 x *g* for 3 h and resultant pellet was purified by sucrose gradient. A vial of purified virus was suspended with 500 µl extraction buffer containing 50 mM Tris HCl, pH 7.8, 1 mM EDTA, 27% sucrose and 0.5% SDS, 30 µl of 20% sarcosyl , and 15 µl Proteinase K for 1 h at 37 ºC. After extraction, viral DNA was precipitated with ethanol according to the method of Sambrook *et al.* (1989) and then dialyzed against two changes of TE buffer overnight. The DNA concentration was determined by spectrophotometer. Approximately 15 µl of DNA sample was digested at 37.5 °C with 2 µl of following enzymes: *Bam*H1, *Xho*1, *Kpn*1, *Eco*R1, and *Hind*III, *Bam*H1/*Eco*, *Bam*H1/ *Kpn*1, *Bam* H1/ *Hind*III. After about 15 h the mixture was deactivated at 65 ºC for 20 min and were stored at 4 ºC before the electrophoresis was done. The DNA preparations were electrophoresed through submerged 0.8% agarose gel in TAE buffer overnight at a constant 27 volts. The gel was stained with 1 µl/ml ethidium bromide solution for 15 min and photograph under ultraviolet illumination.

The acquired image of each RE pattern was used to estimate the molecular weight of viral DNA fragments by using the software program Alpha Ease FC (Manual of AlphaEase®FC Imaging System of FluorChem™).

Results and Discussion

Total genome size of herpesviruses in this study are estimated between 107 to 221 kb which is within the 100 to 250 kb estimated by Chanock *et al*. (1995), although other study found the range to be lower (Heinz *et al*., 1988). The higher molecular weight detected in this study

is probably could be attributed to the software program Alpha Ease FC that detects faint fragments by modifying the brightness and contrast of the image (Figure 1). The software enables precise estimation of molecular weight and eliminates technical error.

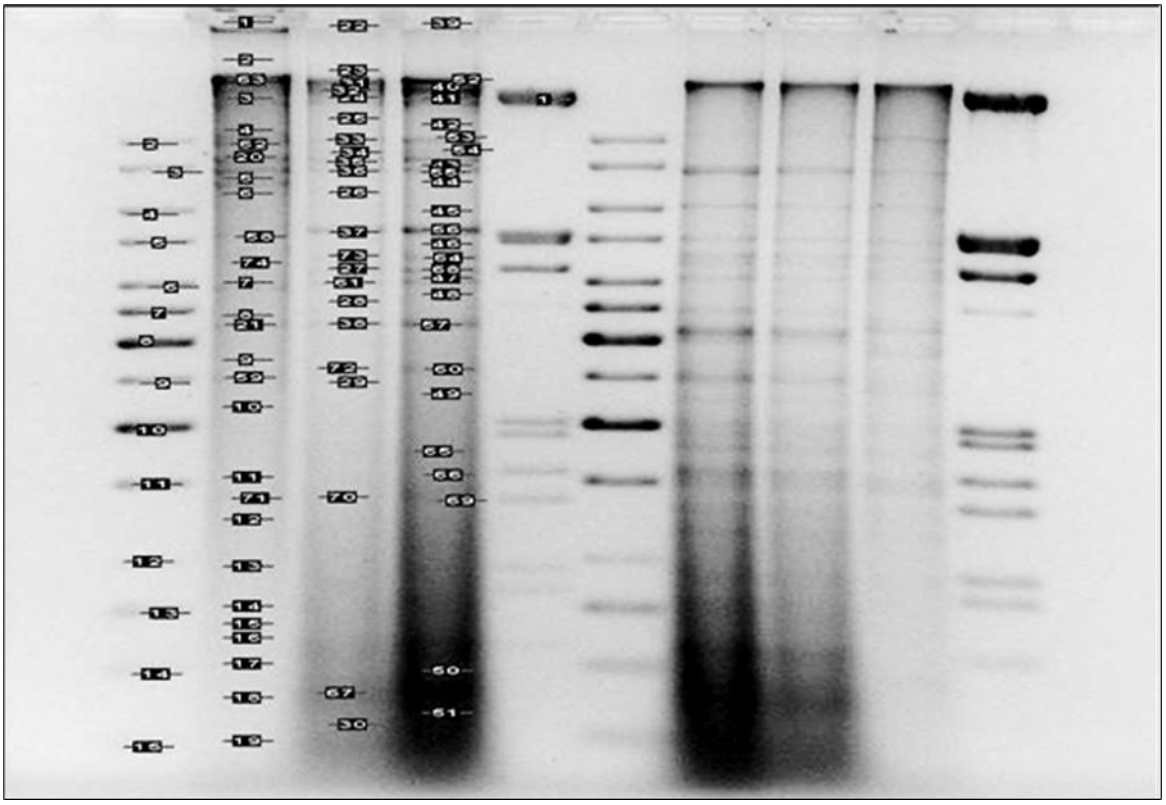

Figure 1. The number and molecular weight of RE fragments were estimated by the Alpha Ease FC software program.

Restriction endonuclease patterns, using eight enzymes either single or in combination, was not able to show either differences or similarities amongst them. However, there is a possibility that some similarity may exist between isolates V4 and V5. Since both isolates are derived from gaurs, they could originate from the same source. Most DNA profiles of these isolates from gaurs are not similar to V19 isolate from bear.

The genome size of V4 and V5 are almost similar, although some fragments cannot be detected in both isolates. However, V19 genomic size is lesser than V4 and V5. The RE pattern of these three isolates were compared to those of mutant pseudorabies virus and other published viruses. The *Bam*HI/*Hind*III double digested V4 and V5 DNA fragments revealed similarities to a mutant pseudorabies virus in their first 8 fragments, while V19 has similarity only in the first 3 fragments (Zeenathul, 1999). Single digestion with *Eco*R1 and *Hind*111 showed similarities to the first five fragments of bovine herpesvirus type 1.

None of the isolates are found to show similarities in fragments with the feline herpesvirus (Sigrid *et al*., 1984), pseudorabies virus (Porat *et al*., 1984), tupaia or tree shrew herpesvirus (Darai and Koch 1984), and equine herpesvirus 1, 4 and 3 (Studdert and Fitzpatrick, 1985).

This preliminary finding indicates that the isolates from wildlife did not originate from the same group of mutant pseudorabies virus, but may instead be a new mutant of herpesvirus since they possessed some pattern of the pseudorabies and bovine herpesvirus type 1 DNA profiles.

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TIBIAL DYSCHONDROPLASIA IN FLOCKS OF BROILER CHICKENS

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Tibial dyschondroplasia (TD) is a condition characterised by an abnormal mass of cartilage below the growth plate in proximal of tibia and tarsal bones. TD is of economic importance due to the increased susceptibility of the chicken especially to respiratory diseases and high carcass condemnation. Little is known on the occurrence of TD in broiler chickens in the country. It was the objective of the study to determine the incidence of TD in commercial broiler chickens. One hundred and thirty-two, 14- to 43-day old broiler chickens from 17 flocks were obtained from six broiler farms. On necropsy, the gross lesions were recorded and the thickness of the growth plate of the tibia and tarsal were measured and scored. The study showed that the overall percentage of chickens with TD was 17%. The occurrence of TD ranged from 10% to 83% with the highest occurrence recorded in the 22-day old chickens. The incidence of TD was higher in chickens reared in the closed house (31%) than open-housed system (13%), and in unhealthy (22%) than healthy chickens (3%). The incidence of CCRD in chickens with TD was 71%. The chickens with mild (score 1), moderate (score 2) and severe (score 3) TD lesions were 20%, 1% and 1%, respectively. It was concluded that the overall incidence of TD is relatively low and with mild lesion score. However, the potential threats of TD in the performance and health of chicken are high as some flock of chickens showed high incidence of TD (83%) and had severe lesion scoring (score 3).

Keywords*;* tibial dyschondroplasia, growth plate, broiler chickens, complicated chronic respiratory disease (CCRD), lesion scoring.

Introduction

Tibial dyschondroplasia (TD) is a condition where a mass of hypertrophic cartilage develops in the proximal end of the tibiotarsal bone in broilers and turkeys and it may cause lameness and limb deformities (Blood *et al*., 1999). This condition also known as osteochondrosis. This disease involved the growth plates situated at the end of the long bones (Farquharson and Jefferies, 2000), and it is characterised by the appearance of a plug of unvascularised, unmineralised, white opaque cartilage that dominates the proximal metaphysis of the tibiotarsus and sometimes the tarsometatarsus (Pines *et al*., 2005). The exact aetiology of TD is not established, although several potential causes have been suggested. TD can be associated with a major sex-linked gene (Sheridan *et al.,*1978). It is common in fast-growing birds, especially in male broilers in which the incidence can be as high as 30% to 49% (Leeson *et al.,* 1995). This high incidence may relate to the fact that the proximal tibiotarsus shows the fastest growth plate development in fast-growing birds (Riddel, 1975). TD may also be related to nutritional factors. Dietary electrolyte imbalance particularly high levels of chloride seem to be major factors in many field outbreaks of TD (Riddel, 1975). TD

incidence and severity were high in birds fed diets containing low calcium in combination with moderate levels of phosphorus (Edwards, 1984). TD is of economic importance to the poultry industry. Increased mortality, culling, downgrading of carcasses, and trimming of deformed legs at processing have been attributed to TD (Burton *et al*., 1981). TD may be prevented by reducing growth rate through light or feed restriction and diet modification (Lilburn *et al*., 1989). It was the objective of the study to determine the incidence of TD in commercial broiler chickens.

Materials and Methods

Chickens
One hundred and thirty-two commercial broiler chickens 14 to 43 days of age were obtained from 17 flocks of chickens in 6 different broiler farms. The chickens in Farm 2 and 5 were reared in a close-housed system, while the others in Farms 1, 3, 4 and 6 were reared in an open-housed system.

Necropsy
The gross lesion was recorded. The tibia bone was cut longitudinally at the end of proximal part of the bone and the presence of TD lesions was recorded. The bones were kept for further lesion scoring.

Measurement and Lesion Scoring
The shape of the growth plate was drawn onto trace paper and the length was measured (mm). The data was analysed and lesion scoring was conducted using a modified technique of Timms *et al*. (1986). Briefly, the lesion was scored as follows: normal (score 0 = 0 to 0.25mm), mild (score 1 = >0.25 to10mm), moderate (score 2 = >10 to 20mm) and severe (score 3 = >20mm).

Results

Clinical Signs
Chickens in the unhealthy groups were dull, depressed, and showed stunted growth. Many had CCRD with typical lesions of fibrinous perihepatitis, fibrinous pericarditis and airsaculitis.

Gross Lesions and Incidence of TD
A cartilaginous plug at the proximal end of the tibia was observed in chicken with TD (Figure 1). The overall percentage of TD from the 17 flocks of broiler chickens was 17% and was ranging from 10% to 83% with the highest recorded from 22-day-old chickens. Healthy chickens showed lower incidence of TD (3%) when compared to the unhealthy groups (22%). The incidence of CCRD was ranging from 0% to 60%. Seventy-one percent of chickens with TD had positive CCRD. Chickens raised in the close-housed system (Farms 2 and 5) had chickens with positive TD, ranging from 10% to 83%. In chicken flocks raised in the open-housed system (Farms 1, 3, 4, 6), only 64% of the flocks were with positive TD, ranging from 10% to 60%.

Lesion Scoring
The mean length of the tibia growth plate from all the samples without obvious gross lesion of TD was 1.59mm. The percentage of severity of TD from all the chicken samples based on

the lesion scoring were 20%, 1% and 1% for the mild, moderate and severe TD lesion, respectively.

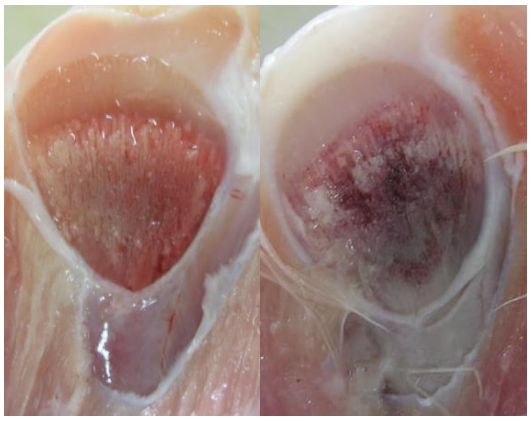

Figure 1: Abnormal mass of cartilage below the growth plate at the proximal end of the tibia of chickens in Farm 3 with lesion scoring of 1 or mild TD.

Discussion

The study demonstrated that TD is common condition in flocks of broiler chickens in the country with overall percentage of 17% and 22% based on gross lesion and measurement of the growth plate of the chickens, respectively. One percent of the chickens had severe TD lesion, whilst the 1% had moderate and 20% mild lesions. The impact of TD on the performance of broiler chickens in the country is unknown. However, the present study has shown that the incidence of TD was higher in unhealthy (22%) when compared to the healthy (3%) chickens. Furthermore, 71% of chickens with TD had positive CCRD. This suggests that chickens with TD are more susceptible to illness or secondary infection.

Broiler chickens with TD may have limb defect and lameness, and tend to be weaker and have lower feed intake. The chickens may not be able to reach the required body weight for market. In severe cases of TD, increased carcass condemnation will occur. The bones tend to break during processing due to the cartilaginous plugs in the bone. The prevalence of TD was between 14% and 35%, with the highest incidence showing a direct relationship with down-grading of carcasses due to leg abnormalities (Burton *et al*., 1981). Chickens with TD usually prefer to sit on the floor and thus are highly exposed to pathogenic agents such as *E. coli* and Staphylococcus spp and toxic materials such as ammonia. These could predispose the chickens to respiratory diseases and death.

The incidence of TD seems to be higher in flocks of chickens raised in the close-housed system compared to open-housed system. Reasons for these are unknown, although chickens in the close-housed system may be less exposed to sunlight and may lead to nutrient imbalance. Since the exact cause of TD is still not established, specific approach on the control and prevention of TD has not been recommended. Proper management practices such as feeding chickens with balanced diets and effective health monitoring programmes are some approaches to reduce the incidence of TD.

It is concluded that despite the overall incidence of TD being relatively low and with mild lesion score, the potential threat of TD in the performance and health of chicken is high. Some flock of chickens showed high incidence of TD (83%) and had a severe lesion score (score 3), whilst 70% of chicken with CCRD had TD.

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