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Faculty of Veterinary Medicine ***UPM***
5-8 January 2010

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Preface

The Doctor of Veterinary Medicine (DVM) final year projects were traditionally meant to be reports on the current status of the animal and veterinary industry. However, over the years the final projects have evolved exposing students to structured scientific research. These projects, under close supervision of lecturers, allow students to become familiar with the planning, conduct, data analysis and reporting of studies pertaining to veterinary and livestock issues. Many valuable information may be discerned from the reports of these studies, hence the compilation of these reports in a proceedings would enrich the repository of scientific knowledge in the field of livestock and veterinary sciences.

This proceedings is the fifth of its series. The proceedings were originally intended to be a reference for academic staff and students. The information gained from the proceedings could be used as a basis to design future studies. Because of the need to preserve the publishability of the data, the supervisors often opted to present their findings as abstracts, as are found in this proceedings. However, if the practice of presentation of research findings as abstracts persists, the proceedings will then cease to be good reference materials. We hope in future all reports in the proceedings will be in the form of extended abstracts so as to provide the readers with more information on the studies conducted. To achieve this goal, we will certainly need the cooperation of all parties involved in the research.

The editors wish to thank students and staff alike for their cooperation in facilitating the publication of the proceedings.

The Editors

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Wound Healing Potential of *Aloe vera* in Climbing Perch (*Anabas testudineus)*

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Abstract

Fresh gel portion of *Aloe vera* leaves was evaluated for the wound healing potential in climbing perch (*Anabas testudineus)*. Fifteen adult climbing perch were divided into three groups. Group 1 received *Aloe vera* gel, group 2 received Betadine® antiseptic ointment (positive control) and group 3 received no treatment (negative control). Wound was created at the flank area using a punch biopsy (8 mm in diameter). One topical application was applied on the wound. Assessment of healing progress was carried out on day 10. Gross observation and histological studies were carried out. There was no significant difference in wound reduction among the groups. However, group 2 showed highest rate of healing followed by group 1 and group 3, and groups 1 and 3 had similar rate of healing. Histological examination on cellular activity of healing process showed increased thickness of epithelium layer, infiltration of inflammatory cells, presence of fibroblast cell and rearrangement of the cell.

Keywords: *Aloe vera*, climbing perch (*Anabas testudineus),* wound healing

Introduction

Aloe vera leaves contain biologically active compounds such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and anthraquinones and various lectins (Wikipedia, 2009). *Aloe vera* (true aloe) has been recognized by many users as having the most effective healing power (Rasik et al., 1983). The Aloe's broad spectrum healing power lies in its purported ability to relieve pain; reduce swelling; penetrate deeply; stimulate cell growth (repair); kill bacteria, fungus, and viruses; stimulate circulation; balance nutrition and reduce inflammation. It has virtually no known toxicity (Coats and Aloha*,* 1980).

Climbing perch (*Anabas testudineus*), also known by its common name, *ikan puyu,* is a fresh water fish that can normally be found in paddy fields. This fish is air breathing type, that is, they can survive longer than other fish without water. It is carnivorous, a voracious eater, likes to eat insect, has hard scales and spiny fins. The superficial wound healing in fish differs greatly from those of mammals and other higher vertebrates. Contrary to mammals, epithelization in fish is a rapid process with movement of the epidermis, starting from the wound edge, within 1 to 4 hours of infliction of the wound (Banerjee and Mittal, 1999). This is achieved perhaps to prevent the heavy influx of water through the damaged skin.

There are not many studies on wound healing potential of *Aloe vera* in fish. Therefore this study was carried to establish the healing potential of *Aloe vera* in fish which included the gross observation, measurement of wound area and histological study.

Materials and Methods

The mature *Aloe vera* leaves were selected and skinned. The inner pulp and gel was collected and applied directly onto the wound. Betadine® antiseptic ointment was used as positive control and petroleum jelly (Vaselin®) was applied to protect the wound when fish was in the water.

Animals

Fifteen four-month-old climbing perch (*Anabas testudineus)* with an average weight of 150 g, were obtained from a commercial farm. They were divided into three groups. The fishes were acclimatized individually in plastic tanks (30 cm x 30 cm x 25 cm) for 3 days. They were fed with commercial pellets once a day.

Treatment schedules

Group 1 received *Aloe vera* gel, group 2 Betadine® antiseptic ointment and group 3 did not receive any treatment and acted as a negative control. All treatments were applied topically immediately after wound was inflicted at the right flank between the abdomen and the tail. Twelve scales were plucked manually with the help of a pair of forceps and the wound was made using punch biopsy of 8 mm (Figure1). The flesh was cut at the base and lifted with a pair of forceps. All surgical procedures were carried out under general anaesthesia using clove oil at the dosage of 0.5 mL/L. The fishes were allowed to recover from anaesthesia in the recovery tank with oxygen supply and removed to the individual tank after full recovery. Only one treatment was given. Vaselin® was then applied to protect the wound.

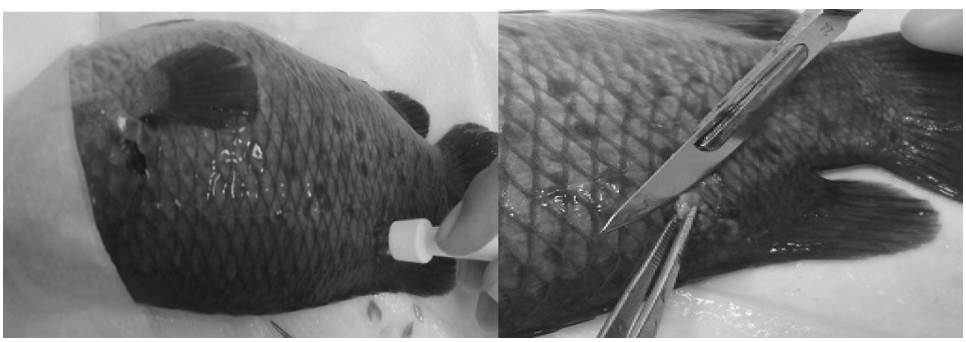


Figure 1. Skin biopsy

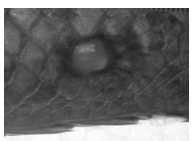
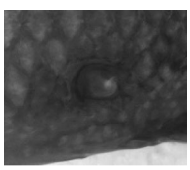
Measurement of wound area

Wound area was measured by drawing wound boundaries around it on a transparent plastic and the area within the boundaries was calculated using a graph paper. The values for each treatment were averaged and presented in mm².

Wound Healing Score

The wound healing scores are summarized as in Table 1.

Table 1. Wound healing scores in climbing perch

Score	Descriptions of lesion	Picture
1. No healing	Large area of wound (8mm) Red in colour	
2. Moderate	Dark shade at periphery of wound Slightly reduced Pale pink colour	
3. Good	Dark shade at the periphery towards centre of wound Whitish colour	
4. Excellent	Normal shade of skin	

Histological studies

Sections were qualitatively assessed under the light microscope for infiltration of inflammatory cells, fibroblastic proliferation and epithelization (Rasik et al., 1999).

Results and Discussion

On day 0, the wound was fresh and red and about 11 mm^2 area. On day 10, the areas surrounding the wound were dark with a small fresh area in the centre. The unhealed wound was pale pink covered by whitish shade and copious slime. There were some new scales growing around the periphery. They were small in size and softer than the normal scales. Reduction of wound area was highest in the positive control group followed by the negative control and *Aloe vera* group. Mean percentage of wound reduction area showed high variability among treatment. However, there was no significant difference in healing between all the groups. This may be due to only a single application of the treatment given, and the early dissolving of the protective gel which hindered further

healing. A more frequent treatment may produce better results. Another reason may be attributed to the fragility of the healing of cells in fish. The emulsion form of aloe vera probably has a better healing potential when made to optimum concentration suitable for healing.

Histology Evaluation

The epidermis appeared thicker especially in the centre and epithelial cells appeared irregularly arranged compared to the normal skin (Figure 2a). The epidermis appeared vacuolated and spongy due to the presence of prominent extracellular space between the epithelial cells which remained connected to each other by prominent intercellular cytoplasmic bridges (Figure 1b). The mucous cells also appeared thickened especially on the surface. The inflammatory cells and fibroblasts appeared prominently at the lesion. The inner layers of the granulation tissue at the level of *stratum compactum* appeared compactly arranged with the presence of blood capillary (Figure 1c).

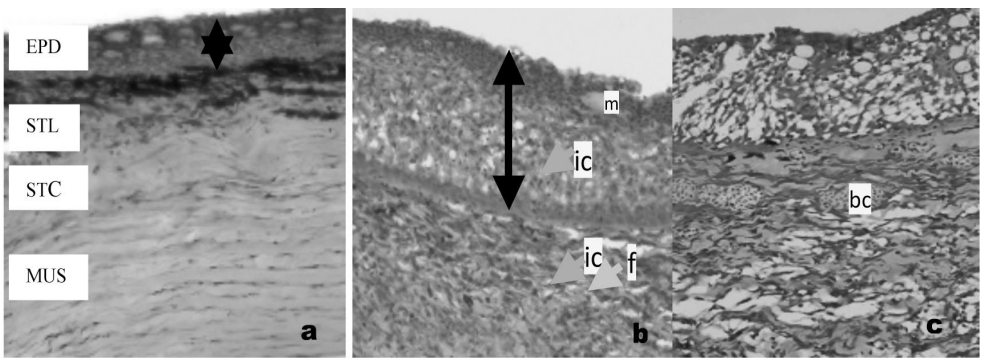


Figure 2. Cross-section of the skin of Climbing Perch

(EPD: epidermis; STL:stratum laxum; STC:stratum compactum; MUS:muscle; bc:blood vessel; f:fibroblast; ic:inflammatory cells)

Grossly, the wound area was not significantly different between all the groups. On day 10, the area surrounding the wound became dark and converging towards the centre. The unhealed wound was pale pink or whitish in color. This is in contrast to dry mammalian wound where the granulation tissue appears first and the epithelization takes place later. The new epithelial cells are produced due to mitotic division of the cells of the stratum germinativum of the epidermis (Fossum et al., 2002). The wound area was covered with copious amount of slime and regeneration of new scales was seen around the periphery of the wound. New scales were smaller in size and softer than normal scales. The mucus protected the body from bacterial or fungal infection and also has a role in the osmoregulatory processes (Hibiya, 1982). Grossly, it was difficult to distinguish between the lesions of the 3 groups because of their similarity. The results could have been different if the duration of study was longer. According to the previous study on *Channa striata* (Banerjee and Mittal, 1999), the normal shade was re-established after

24 hours but in this study the skin was not re-established to the normal shade even at day 10 where the unhealed area could still be observed in the centre of the wound. It can be concluded that the healing process occurs over time and can be shown by the characteristic of wound area reduction, dark shade migrating into centre of the wound and growth of the new scales at the periphery of the wound. In this study the percentage of wound reduction area in positive control (Betadine®) showed better result than negative control and *Aloe vera* treated. However, there was no significant difference in the healing process in all groups. If the frequency of application of the drug is increased, better results may be obtained. Alternatively due to the fragility of the healing cells in fish, *Aloe vera* may be administered as an emulsion in the water. Similarly the healing score should have been done more frequently to obtain more reliable results. The availability of *Aloe vera* after topical application is good as shown by fast healing in some fish in this study. The topical application in fish is a challenge as the drug is easily dissolved when placed in water. To prevent this, petroleum jelly (Vaselin®) was used to cover the wound area. However, there was no advantage of topical application of drug in fishes because of the limited time for the absorption of drug before it dissolved in water.

The cellular activity of the healing process in the climbing perch on day ten showed the middle layer of the epidermis appearing vacuolated and spongy. This characteristic was due to the presence of prominent extracellular space between the epithelial cells which remain connected to each other by prominent intercellular cytoplasmic bridges. This finding is similar to those of Banerjee and Mittal (1999). There was an increase in thickness of the epidermis and mucous cells especially on the surface. The structure of the epithelial layer appeared irregularly arranged. The layer of the epidermis became thicker at the centre of the lesion with high infiltration of inflammatory cells. The layer of the epidermis was thicker to give more protection against the environment. This finding is similar to that of Iwama and Nakanishi (1996) where the epidermal healing is very rapid and within hours a 2- to 3-cell-thick epidermis can cover the wound. In this study, the inner layers of the granulation tissue mostly at the level of *stratum compactum* became compactly arranged with the presence of blood capillaries and elongated fibroblasts. This was also similar to studies by Iwama and Nakanishi (1996) where the elongated fibroblasts appeared within the first week after inflammatory insult and undergo fibroplasis during the second week.

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Presence of Vancomycin Resistance among Enterococcus Isolates from Stray Cats in Universiti Putra Malaysia and Selected Neighbourhood in Sri Serdang, Selangor, Malaysia

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Abstract

The present study was undertaken to determine the distribution of *Enterococcus* species from stray cats in Universiti Putra Malaysia and Seri Serdang and the presence of vancomycin resistance among the isolates. Fifty-five rectal swabs were collected from stray cats found in UPM and around the area of Sri Serdang. The Enterococcus species isolated were inoculated onto vancomycin resistant enterococci (VRE) agar supplemented with 8 µg/mL of vancomycin. Biochemical tests such as catalase, bile-aesculin and 6.5% NaCI were conducted to further confirm VRE isolates. Multiplex polymerase chain reaction assay (PCR) were performed for *Enterococcus* genus and species identification and vancomycin-resistant gene detection. Presence of *Enterococcus* spp. were demonstrated in every rectal sample tested. Two species were identified: *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*). Among 55 isolates of *Enterococcus* tested, none was resistant to vancomycin at 8 µg/mL.

Keywords: stray cats, vancomycin-resistant enterococci (VRE), *Enterococcus* spp., PCR

Introduction

Enterococci have been recovered from the intestinal tract of mammals, soil, water, plants, insects, and food items (Witte et al., 1999; Giraffa, 2002). They are a leading cause of nosocomial infections and are intrinsically more resistant to antimicrobial agents commonly used in hospitals than other bacteria (Martone, 1998; Cetinkaya et al., 2000).

Vancomycin-resistant enterococci (VRE) are a group of bacteria that have developed resistance to many antibiotics, especially vancomycin (Poore, 2007). VRE was discovered in 1985 (Dixson et al., 1985) and was first reported in France and England in 1986 and in the US in 1987 (Farley, 1998). It has spread rapidly and became a major problem in many institutions both in Europe and US.

Some epidemiological studies suggested that animals carrying VRE in their gastrointestinal tract could be the source of VRE infections of humans (Stobberingh et al., 2000). These VRE of animal origin can colonize humans, thus, may transfer their resistance genes to humans (Griffiths et al., 1994, Sundsfjord et al., 1998). However, there are only a limited number of studies dealing with the occurrence of VRE in

companion animals, even though direct contact with such animals is a recognized source of pathogenic bacteria for humans (Simoons-Smit et al., 2000, Wolfs et al., 2001).

The present study aimed at establishing the presence of VRE in population of stray cats in two areas in Malaysia. The specific objective of this study is to determine the distribution of *Enterococcus* species among stray cats in colleges in Universiti Putra Malaysia and selected neighbourhood in Sri Serdang, and detect the occurence of vancomycin resistance among those isolates.

Materials and Methods

Sample and data collection

Fifty five rectal swabs were collected with 26 swabs from stray cats from colleges in UPM and 29 swabs from stray cats from selected location in Sri Serdang. The pre-package swabs contained the transport Amies medium (MEUS, Piove di Sacco, Italy) and were kept in ice during sample collection. Information was collected based on the location where each cat was found, estimated age and sex (intact, spayed or neutered).

Bacteriological Analysis

After collection, samples were labeled and kept in the refrigerator at 4˚C for 24 hours before being inserted into universal bottles containing 3 mL Brain Heart Infusion (BHI; Pronadisa™ Laboratorios Conda, Spain) broth for enrichment. Each sample was then incubated for 18 hours at 37˚C aerobically. A loopful of broth culture was streaked onto membrane filter *Enterococcus* selective agar Slanetz and Bartley (SBA; Merck Inc, Germany) and incubated aerobically for 24-48 hours at 37˚C. Red/maroon/pink colonies are presumptive enterococci colonies and 3-5 colonies of different type based on shape, appearance or size were collected and put into 1.5 PCR tubes containing 1 mL of BHI broth and mixed well. A loopful of the suspension was streaked on BHI agar using the diminishing sweep technique and incubated for 18-24 hours at 37˚C aerobically for purification. From BHI agar, single colony of different types were picked and streaked on BHI agar for purification and each subculture was labeled. These plates were then incubated for 24 hours at 37˚C aerobically.

In order to confirm the genus *Enterococcus,* biochemical tests were done on all pure colonies by doing Catalase test, Gram staining, growth in bile-aesculin, growth in 6.5% NaCI broth and growth on VRE agar supplemented with 8 µg/mL of vancomycin. The species van genes determination of VRE was performed using multiplex PCR assay method described by Kariyama et al. (2000), Ke et al. (1999) and Elsayed et al. (2001).

A drop of 3% of hydrogen peroxide was dispensed on a clean glass slide. A single colony was picked from a pure culture plate using a sterile inoculating loop and placed in the drop of 3% hydrogen peroxide, mixed well and observed for formation of bubbles. A positive result was indicated by formation of bubbles.

A drop of normal saline was placed on the glass slide and mixed with a loopful of presumptive enterococci colonies on the same slide. The slide was heat-fixed and allowed to dry for a few seconds. It was then drained with crystal violet for one minute. Then it was washed and drained with iodine for one minute. Next, the slide was washed again and drained with acetone for a few seconds. Finally, it was washed and drained with diluted carbol fuschin for 30 seconds. The slide was then observed under 100x oil immersion light microscope for Gram-positive cocci, single or short chain. A loopful of single colony were picked from pure cultures and streaked onto the bile aesculin slant. The inoculated tubes were then incubated for 24 hours at 37˚C. Positive growth on bile aesculin slant was indicated by changes of the colour from yellow to black.

A few pure single colonies were inoculated into 6.5% sodium chloride (NaCI) and mixed well. The tubes were then incubated for 24 hours at 37˚C. Changes of the broth colour indicate growth.

For confirmation of VRE colonies, representative enterococcal colonies were cultured onto VRE agar supplemented with 8 µg/mL of vancomycin. It was then incubated for 48 hours at 37˚C. On VRE agar, enterococci appear as round grey/pale brown colonies about 1mm in diameter surrounded by black zones indicates positive growth.

For DNA extraction a fresh cell suspension was made (McFarland's 2) in 500 µL of deionised sterile water and boiled in water bath at 100˚C for 10 minutes. It was then centifuged at 13,000 rpm for 5 minutes. The supernatant was transferred into clean 2 µL PCR tube for PCR.

PCR wase run for genus confirmation, species identification and van gene detection. Master mix constituents are prepared for single reaction of volume 25 µL. It contains 2.5 µL PCR buffer (1X), 0.5 µL dNTPs (200 mM each deoxynucleoside triphosphate), 0.5 µL of each primer from 2X stock solution (Primers at concentration given in Table 1), 0.5 µL Taq polymerase (1U) and water to make 25 µL. Twenty microlitre of the master mix were dispensed into 0.2 mL PCR tubes and 5µL of DNA from samples were added. The PCR tubes were then spin briefly and keep on ice. Positive and negative controls were also included. PCR will consists of initial denaturation for 4 minutes at 94˚C, 30 cycles of denaturation 94˚C for 1 minutes, annealing 54˚C for 1 minutes and extension 72˚C for 1 minutes. Followed by final extension for 10 minutes at 72˚C and holding temperature at 4˚C. 2% agarose gel was prepared with 0.5X TBE buffer for analysis. Electrophoresis was run by mixing 7 µL of PCR product with 1.5 µL of 6X loading die at 80V for 1 hour.

Results

Presence of *Enterococcus* spp. was demonstrated in every rectal sample tested. Two different species were identified: *E. faecalis* and *E. faecium*. There were 20 samples with unknown strains and 7 samples with more than one species isolated from the same cat. *E. faecalis* was the species most frequently isolated (39/55, 70.9%).

None of the *Enteroccous* isolated were resistant to vancomycin.

Discussion

In this study, all cats were positive for enterococci which may be attributed to their outdoor lifestyle and an increase in environmental contact. This is not uncommon as enterococci were commonly found in the environment (Ferguson et al., 2004). The importance of enterococci is largely attributable to their resistance to many antibiotics (Ossiprandi et al., 2006). As a result of the close contact between companion animals and humans, the ease at which bacteria can be shared is magnified (Jackson et al., 2008). Humans are exposed to various zoonotic bacteria that can be transferred from companion animals and in this case, even from stray cats due to their proximity with humans

In most studies on enterococci from dogs and cats, five or fewer species of enterococci have been reported with *E. faecalis* and / or *E. faecium* isolated most frequently (Rodrigues et al., 2002; Poeta et al., 2006). *E. faecalis* and *E. faecium* are also the predominant species indicated in human infections (Murray, 1990) and *E. faecalis* was the most prevalent in poultry (Poeta et al., 2005). From this study, *E. faecalis* was the predominant species isolated (70.9%) followed by *E. faecium* (5.5%). Other species were also isolated but could not be detected due to limited number of primers used in this study. *E. faecalis* and *E. faecium* were also the two most common species, comprising 80 to 90% and 5 to 10% of clinical isolates each (Flores et al., 1996) found in humans.

VRE were not detected from all *Enterococcus* isolates from stray cats found in colleges in UPM and around Sri Serdang area. The absence of VRE in these cats possibly suggests that the cats have not been exposed to the environment where selective pressure allows for the development of resistance to antimicrobes, or/and the stray cats had been exposed to prolong antimicrobial treatments or food with antibiotic residues. According to a study, colonisation of pets may be a consequence of eating raw meat contaminated with VRE (Van Belkum et al., 1996).

From this study, absence of VRE in stray cats may be associated with the environment the cats were found and where there is limited contact with humans. The stray cats do not have much contact with farm animals that may harbour VRE. Negative results for VRE from cats that had been neutered (i.e. have been exposed to hospital environments for neutering and spaying) may be due to short duration for hospital stays therefore denies the possibility of VRE from healthcare settings.

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Bacteriology of Vaccinated and Non-Vaccinated Eye of Cats

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Abstract

Thirty cats were divided into two groups: group 1 (owner-kept cats) and group 2 (stray cats). Group 1 consisted of 11 cats in which the vaccination status was up to date whereas group 2 consisted of 19 cats in which the vaccination status was unknown. Conjunctival swabs were taken from these cats for bacteria isolation and identification. Giemsa staining of the swabs was also conducted to identify *Chlamydophila* sp. Two cats in group 1, one male and one female Persian which had bilateral watery discharge, were positive (18.18%) for bacteria in their eyes. The male Persian cat was positive for *Enterobacter spp*. and *Moraxella nonliquefaciens* bilaterally. However, in both eyes of the female Persian cat, only *Enterobacter spp.* was isolated. In group 2, 11 domestic short hair stray cats had only *Staphylococcus intermedius* in their eyes which did not show any ocular discharges. Three cats in this group had bilateral isolation and four had unilateral isolation of this bacteria. Of the 30 cats studied, the most predominant bacteria isolated was *S. intermedius* (36.67%) followed by *Enterobacter spp.* (6.6%) and *M. nonliquefaciens* (3.3%). None of the 30 cats studied showed the presence of *Chlamydophila* sp. in their eyes and there was no evidence of eye lesions in these cats. It is believed that the *S. intermedius* isolated was normal conjunctival flora of the eyes of these cats. *Enterobacter spp.* and *M. nonliquefaciens* could also be the normal flora of the Persian cats, since these bacteria were isolated from their eyes in the absence of lesions but with ocular discharge that may promote the growth of these microorganisms.

Keywords: bacteriology, vaccinated cats, non-vaccinated cats, conjunctiva

Introduction

There are many studies on bacteriology of the eyes in many animal species such as in the Canadian beaver (Cullen, 2003), dogs (Marilena et al., 2005), elephants (Kodikara et al., 1999; Briksawan et al., 2004), horses (Andrew et al., 2003; Yagamata et al., 2005), opossum (Chantale et al., 2002), racoon (Chantale et al., 2002) and cat (Campbell et al., 1973; Shewen et al., 1980; Espinolaz et al., 2008).The most common bacteria isolated from variable animal species were *Staphylococcus spp.* and *Streptococci spp.* (Shewan et al., 1980; Kodikara et al., 1999; Chantale et al., 2002; Briksawan et al., 2004; Miller et al., 2005; Marilena et al., 2005;). Beta haemolytic *Streptococci* (Shewan et al., 1980; Kodikara et al., 1999), *Staphylococcus spp.*, *Pseudomonas spp.,* and *Streptococcus spp.* had been commonly associated with conjunctivitis or ulcerative keratitis. (Marilena et al., 2005).

Eye disease is one of the most common diseases diagnosed in domestic animals especially in cats and dogs. One of the most common eye diseases in cats and dogs is conjunctivitis (Martin et al., 1973; Peiffer et al., 1997; Mac Calla et al., 2001). Conjuctivitis is the inflammation of the mucous membranes of the eyes. Purebred cats are more prone to conjunctivitis. Conjunctivitis in cats usually proceeds to chronic stage. It usually occurs in young cats especially those below one year old. The most common agents causing conjunctivitis are Feline Herpesvirus, Chlamydophila felis, Feline Calicivirus, Mycoplasma sp, trauma and allergy (Mac Calla, 2001). Some cats may develop secondary bacterial infection after severe conjunctivitis. Besides eye pathogens, previous studies had showed the isolation and identification of conjunctival flora in various animals including cats. However, studies on the types of bacteria, either normal flora or pathogens, that can be isolated from the eyes of cats is still very limited. This study has the following objectives: to identify the species of bacteria that can be isolated from the eyes of owner kept cats (vaccinated) and stray cats (non-vaccinated) and, to identify *Chlamydophila* sp. in the eyes of cats (vaccinated and non-vaccinated cats) since *Chlamydophila felis* is one of the common agent causing conjunctivitis and has high prevalence in cats and also a zoonotic potential to humans (Hartley et al., 2001).

Materials and Methods

Conjunctival Swab Samples

The conjunctival swab samples were taken from both eyes of 30 cats from 16th until 24th November 2009. The samples were divided into two groups which consisted 19 of cats was Group 1 and 11 cats in Group 2. The vaccination status of Group 1 (owner kept cats) is up to date while for Group 2 (stray cats), the vaccination status in unknown. Conjunctival swabs from Group 1 were taken from cats kept by owners in Sri Serdang whereas Group 2 cats were taken from stray cats from residential colleges in UPM. The conjunctiva swabs were taken from cats in both groups, with or without eyes discharges in both eyes and regardless of age, sex, and breed using sterile cottons swabs. The cats were observed for eye lesions and details were recorded. After the conjunctival swabs were taken, the swabs were immediately transferred into transport media.

Bacterial Culture

The conjunctiva swabs from the transport media were streaked onto blood and Mc Conkey's agar and then smeared onto two slides for staining with Giemsa and Gram stain. The slides were observed under light microscope for bacteria (Gram-positive or Gram negative) and *Chlamydophila* identification. The plates that were streaked with the swabs were incubated at 37°C for 18-24 hours. After incubation, the bacteria colony morphology on the plates were observed and recorded. Then, the colony was stained with Gram stain and subcultured onto blood agar to obtain pure culture of the bacteria. The plates were then incubated for 18-24 hours at 37°C. After incubation, the colony morphology was recorded and the bacteria were Gram stained again to determine the cell morphology before biochemical tests were conducted.

Biochemical Test

Biochemical tests were carried out depending on the gram staining results. Gram-positive cocci bacteria were tested for catalase, coagulase, blood broth, acetoin production, maltose, mannitol and indole. Gram-positive rod was tested for catalase, urease, glucose, nitrate, sucrose, hemolysin and trehalose. Gram-negative bacteria were tested for oxidase and then inoculated into triple sugar iron, sulphide indole motility, urea and nitrate media. Then, the results of biochemical tests from each colony were referred to the Diagnostic Manual of Veterinary Clinical Bacteriology for bacteria identification.

Results and Discussion

No *Chlamydophila* sp. was identified from the conjunctiva swabs using the Giemsa staining technique (Table 1).

Two (male and female) out of 11 cats (18.18%) in Group 1 were positive for bacteria (Table 1). The male Persian cat was positive for both *Enterobacter spp*. and *Moraxella nonliquefaciens* in both eyes which were also watery whereas the female Persian cat was positive for *Enterobacter spp*. (pure growth) only and bilaterally present. The eyes of this female Persian were also watery. However, both Persian cats had no evidence of eye lesions.

In Group 2, 11 out of 13 cats (57.89%) were positive for *Staphylococcus intermedius* (Table 1). This bacteria was obtained in pure growth from the eyes of the 11 cats with no clinical signs of eye discharge or lesion (Table 2). Four out of these 11 domestic short hair stray cats (53.85%) had positive isolations of *S. intermedius* from both eyes (Table 2).

Overall, in the 30 cats studied, the most predominant bacteria isolated was *S. intermedius* (36.67%) followed by *Enterobacter spp.* (6.6%) and finally *Moraxella nonliquefaciens* (3.3%).

Table 1. Bacteriology of cats

Group	Vaccination	Total	*Chlamydophila*	No of cats with positive bacterial growth	Type of bacteria	Positive growth (%)
1	Up To Date	11	-	2	*Enterobacter sp* and *Moraxella nonliquefaciens*	18.18%
2	Unknown	19	-	11	*Staphylococcus intermedius*	57.89%

Table 2. Clinical signs and lesion in cats

Group	Vaccination status	Total	Breed	Eye Lesion	Eyes discharges	Unilateral (Right/Left)	Bilateral
Group 1							
Male	All Vaccinated	1	Persian	-	Watery	-	1
Female		1	Persian	-	Watery	-	1
Group 2							
Male	All	4	DSH	-	-	-	4
Female	Unknown	7	DSH	-	-	3R,1L	3

In the present study, 43.33% of the cats in both groups had positive bacterial growth. Unlike the previous study (Campbell et al*.,* 1973), where the species of bacteria was not documented, in this study, *Staphylococcus intermedius* was isolated from Group 2 cats only with unknown vaccination status.

Many factors such as sampling technique, geography, season and ambient temperature at the time of collection may influence the prevalence of the different species of bacteria from the eyes of cats (Gerding and Kokamo., 1990). However, this is debatable when studies reported that some of the factors did not have any influence statistically (Marilena et al*.,* 2005; Li Wang et al*.,* 2008). Breed, age and sex of dogs did not influence the species of bacteria that can be isolated from the conjunctiva, but which season of the year the samples were taken do have significance on the isolation of bacteria from dogs' eyes in Beijing (Li Wang et al*.,* 2008). A study of conjunctival flora in Thoroughbred mare in Florida showed that there was no significant difference in which season sampling was conducted but age of the mare showed significance where young horses had higher percentage of isolation of gram negative bacteria from the eyes (Marilena et al*.,* 2005)

Since *S. intermedi*us had been isolated from stray cats in Group 2 only, the absence of this microorganism from Persian cats in Group 1 could be due to the lacriminal fluid which contains antibacteria such as lysozymes and wandering macrophages or rate of washing of the conjuctival membrane which influence the effective colonisation of the eyes by microorganism (Shewen et al*.,* 1980). Also, possible previous treatment with antibiotic in Group 1 cats may explain why *S. intermedius* are present in stray cats only. However, in this study, we isolated *Enterobacter sp* and *Moraxella nonliquefaciens* in two Persian cats from Group 1 with watery eyes discharges. This can be due to the breed of the cats and also how the owner manages the hygiene practice of their cats especially litter tray management. Persian cats, especially purebred, are more prone to conjunctivitis. This is due to the anatomy of short tear duct that causes increase tear production. Tears do contain antibacterial substance but, it also can cause irritation to the conjunctiva. From the irritation, it can lead to mild inflammation of the mucous membrane and can further cause severe damage and even ulcerative keratitis due to

secondary bacteria infection. Tears also contain protein such as mucin which will favour the opportunistic bacteria to colonize the eyes (Michael et al., 2007)

In the present study, *Moraxella nonliquefaciens* was isolated from one Persian cat from Group 1. *M. nonliquefaciens* can be isolated from normal respiratory tract in humans. This bacteria rarely causes disease but there are two reports on *M. nonliquefaciens* in two immunocompromised human patients with endopthalmitis in Norway following posttrabeculectomy surgery (Laukeland et al*.,* 2002). However, there are no reported cases of *M. nonliquefaciens* causing lesions in animal such as cats.

In this present study, no *Chlamydophila* sp. was detected using Giemsa staining of conjunctival swabs. Since Chlamydia is an intracellular microorganism, adequate conjunctiva cells are needed to be obtained by scraping the conjunctiva with the animal under general anaesthesia to observe for inclusion body.

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The Effects of Commercial Flower Honey and Turmeric on Dermal Wound in Rats

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Abstract

Twenty healthy rats, ten adults (2-month-old) and ten young (1-month-old), were used in this study. Four skin biopsies were created at the dorsum of each rat under general anesthesia. The wound was each treated with honey, turmeric powder, turmeric-honey paste and a blank (control). The wounds were photographed on day 0, 1, 3, 5, 7 and 9. Wound area reduction was measured on day 9 after which the rats were euthanized. The skin samples were taken for histology. The results showed that there was no significant difference in the healing between treatments in young and adult rats. However, honey was the best treatment with the highest healing scores, followed by control, turmeric and honey-turmeric paste. Honey-turmeric paste resulted in a severe wound infection thus delayed healing.

Keywords: flower honey, turmeric, honey-turmeric paste

Introduction

Alternative medicine using herbs is another option in treating acute or chronic wound. The herbs are easily available in Malaysia and normally used by the Malay community for treatment.

Turmeric or *Curcuma longa,* a rhizomatous herbaceous perennial plant of the ginger family (Zingiberaceae), contains up to 5% essential oil and 3% curcumin, a polyphenol. curcumin or natural Yellow 3 is the active substance of turmeric and thought to have many medicinal properties (Goel et al., 2008; Ravindranath and Chandrasekaara, 1980). In South Asia, it is used as an antiseptic and antibacterial for cuts, burns and bruises also to treat skin, heart, liver and lung diseases, for epilepsy and bleeding disorders, and to purify the body-mind. Other properties of turmeric include as an analgesic, anti-inflammatory, anti-tumour, anti-allergic, antioxidant, antiseptic, antispasmodic, appetizer, astringent, and cardiovascular, carminative, digestive and diuretic stimulant (Ramdev, 2009).

Flower honey is a sweet element produced by honey bees and derived from the nectar of the flowers. For 2700 years, honey has been used to treat a variety of ailments mainly by topical application, but only recently the antiseptic and antibacterial properties of honey have been chemically explained (Wikipedia, 2009*)*. Honey is composed of sugars such as glucose and fructose and minerals such as magnesium, potassium, calcium, sodium

chlorine, sulphur, iron and phosphate. It contains vitamins B1, B2, B3, B5, B6 and C, each quantity of which determine the qualities of the nectar and pollen. There are also traces of copper, iodine and zinc.

Wound healing refers to the replacement of destroyed body tissues by living tissues (Walter and Israel, 1987) and comprises of two essential stages; regeneration and repair. In regeneration, specialized tissues are replaced by the proliferation of surrounding undamaged ones. In repair, lost tissue is replaced by granulation tissues which mature from scar tissue (Watson, 2003).

This study was conducted to determine the duration of wound healing using turmeric, honey and turmeric-honey combination, and secondly to study their effects on wound healing in different age groups of rats.

Materials and Methods

Twenty healthy rats were used and they were divided into two groups. Group 1 (G1) consisted of ten 5 weeks old rats weighing 100 g each and Group 2 (G2) 10 adults aged 9 weeks weighing 250 g each. Honey and turmeric used in this study were commercially available. Honey was 'Flower honey' containing energy, protein, fat, cholesterol, carbohydrate, fibre and sodium. Turmeric is in powder form that contains seventy percent pure turmeric.

The dorsal area of each rat was shaved and cleaned with 50% alcohol, followed by povidone iodine. Skin was stretched and four circular wells were made using a biopsy punch (Figure 1) 8 mm in diameter. Skin biopsy was carried out under anaesthesia using Ketamine HCl 35 mg/kg and Xylazine HCl 5 mg/kg intramuscularly. The rats were allowed to recover in a cage, fed with pellet and water *ad-libitum*. Treatments were applied immediately after inducing the wound as follows: well no. 1= Control, 2= honey, 3=turmeric and 4=honey-turmeric paste. The wounds were observed and photographed on day 0, 1, 3, 5, 7 and 9. The healing progress of the wounds was scored as 0=no healing, 1=slight, 2=moderate, 3=good, 4=very good and 5=excellent.

Raw wound areas were measured by drawing their boundaries on a transparent plastic and the area squares within the boundaries were counted using graph paper. The values for each treatment were averaged and presented in mm^2:

The percentage of wound reduction (healing) was calculated using the formula as follows:

$$\% = \frac{\text{wound area day 0} - \text{wound area day 9}}{\text{wound area day 0}} \times 100$$

For histology studies, wound tissues were preserved in 10% formalin, and later subjected through different grades of alcohol to ensure complete dehydration before embedded in paraffin wax. Serial sections of paraffin embedded tissues of 3 mm thickness were cut

using a microtome and stained with haemotoxylin and eosin. Sections were qualitatively assessed under the light microscope for infiltration of inflammatory cells, fibroblastic proliferation and epithelisation.

Data obtained were analysed using a two-way ANOVA. A value of $P \leq 0.05$ was considered as significant.

Results

Results showed on days 5, 7 and 8 that there was no significant difference in wound healing between young and adult group. On day 9 the results showed that control and honey healed significantly faster than other treatments.

Honey was the best treatment with highest healing scores, followed by control, turmeric and honey turmeric paste (Figure 2).

In adult, results showed that control group healed fastest compared to other treatments (Figure 3). Two of ten adults in control group showed complete healing where the skin regained most of its tensile strength.

In young rats, wound treated with honey had the fastest rate of healing compared to other groups (Figure 3). Three of ten treated with honey showed complete healing on day 9 when the skin surface has completely apposed.

Figure 1. Circular wells were made using a biopsy punch

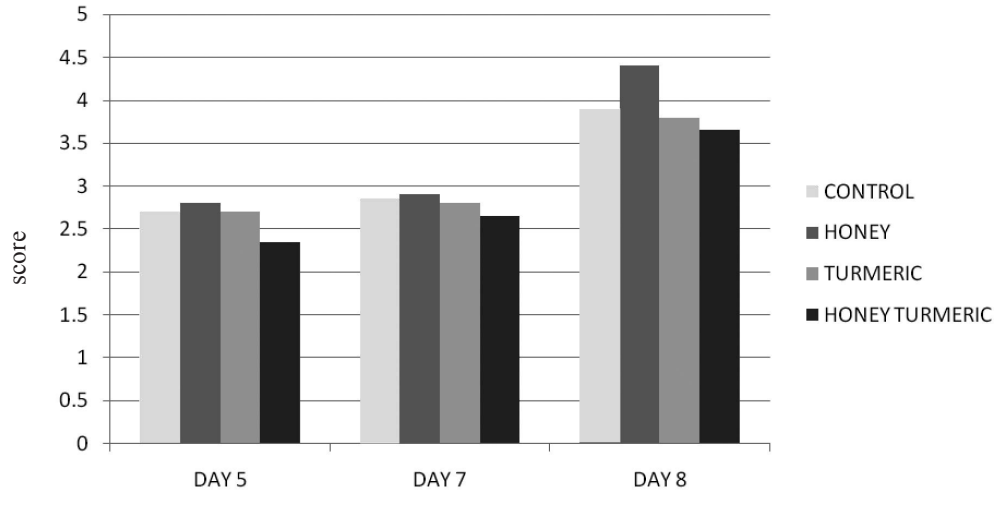

Figure 2. Wound healing score

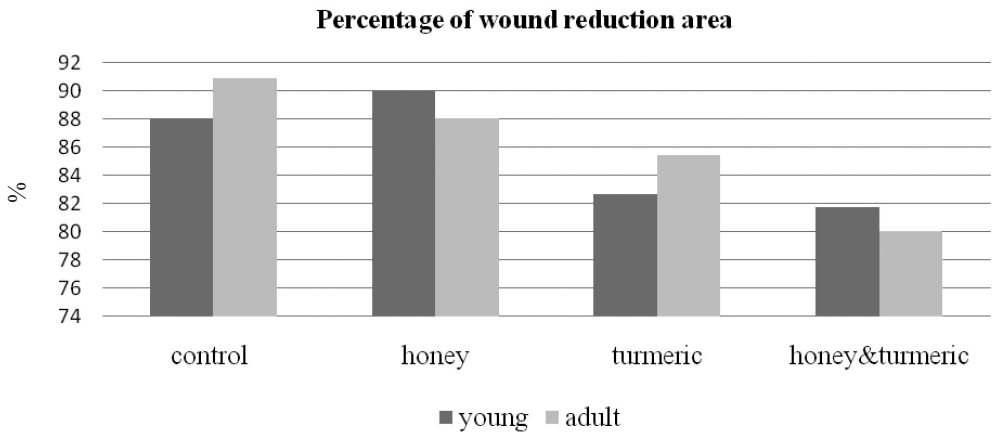

Figure 3. Mean percentage of wound reduction area 9 days post-treatment

Discussion

In this study, topical application of powdered honey has significantly better wound healing activities. These results were similarly found by Rasik and Raghubir (1999) and Efem (1988) although they used the extract form. This may be attributed to the angiogenic and mitogenic properties of honey. This study also showed that there was no significant difference in the duration of wound healing for turmeric and honey. Honey and turmeric in combination (paste), was found to be not suitable to heal wound as they produced severe reaction making the wound raw and puss-filled. However, infection could not be ruled out as the rats were placed in an open cage. Another factor was the

two items may be not chemically compatible and there was not much work done to study the chemical reaction of the two in healing wound.

According to Stashak and Theorel (1991), generally, wound in young animals healed more rapidly than wound in adults which showed sluggish inflammatory response to injury and delayed wound contraction. Conversely was true in this study which showed no significant difference in wound healing in both young and adult groups. One reason probably be due to a small difference in the ages of the two groups.

The study showed that in the young rats, the wound treated with honey-turmeric paste initially allowed infection to set in which was evident by the presence of pus which eventually healed. The non-sterile honey and turmeric may have a latent period before the bacteria succumbed to treatment. This finding was also reported by Lusby et al. (2002) which stated that the suppression and resolution of infections that were found under the dressing of honey may be the result of bactericidal action from prolonged exposure or as a result of the natural defense system being more successful with multiplication of bacterial cells held in check. According to Efem (1988) following topical application of honey, 51 out of 59 wounds were infected at the beginning of the treatment, and became sterile after 1 week of treatment. This study found that there was no significant difference in healing between control and honey as application was done only once but according to Efem (1988) the results may be significantly different if treatments were applied daily for 7days.

The results obtained from the study indicated that treatment using flower honey healed the fastest followed by turmeric and honey-turmeric paste. There was no significantly difference in wound healing between young and adult rats.

For future studies, it is recommended to use one treatment per rat. This facilitates monitoring of the progress of the wound. Frequent application of treatments such as daily or on alternate days may give a significant outcome. 'Three-week' age difference was not sufficient to compare age-group relation in rats, ideally they should be at least 6 months apart.

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Isolation and Identification of Pathogenic Bacteria from Red Tilapia in Cage-Cultured System and its Environment

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Abstract

Bacteria were isolated from the brain, eye and kidney of red tilapia, as well as water and debris samples. The weight and length of red tilapia were measured and the water quality as well. API test were done to identify the type of bacteria from the isolates. In Kenyir Lake, bacterial isolates that predominated in the fish were *Micrococcus* spp. and *Aeromonas hydrophila* at 13.64 %, in water samples it was *Staphylococcus xylosus* at 40% and in the debris samples, *Pseudomonas aeruginosa* and *Enterobacter cloacae* at 50%. In the Semantan River, the predominant bacteria in fish and debris samples were *Aeromonas hydrophila* at 23.53 % and 90 % respectively. In the water samples, *Staphylococcus lentus* and *Staphylococcus xylosus* were the predominant bacteria with 30 and 20%, respectively. The ammonia, sulphide, iron and nitrite-nitrogen levels in the Semantan River were over the acceptable limits and this may lead to high fish mortality. This study concluded that *Aeromonas hydrophila* and *Staphylococcus spp.* were the most predominant bacteria in red tilapia and poor water quality played a major role in red tilapia succumbing to infections by pathogenic bacteria.

Keywords: Bacteria, red tilapia, API test, water quality.

Introduction

Red tilapia (*Oreochromis niloticus* hybrid) was first introduced into Malaysia in the mid 1980's. It was initially considered to be hardy and resistant to diseases (Siti-Zahrah, 2004). The incidence of microbial pathogens in fish, especially those of bacterial origin is one of the most significant factors affecting fish culture (Post, 1989; Zorrilla et al., 2003).

Sugita et al. (1982) mentioned that *Aeromonas* spp. and *Pseudomonas* spp. were the predominant bacterial genera in the tilapia fish. In contrast, Chowdhury et al. (1989) found that the predominant bacteria recovered from tilapia are *Micrococcus*.

Atwood et al. (2001) found that small-sized weighted Nile tilapia were more tolerant to nitrite than larger fish. LaDon (1992) cited that more ammonia was excreted by heavy fish as waste.

One of the factors affecting fish culture is the quality of water, which in turn, determines the incidence of microbial pathogens particularly those of bacterial origin (Austin and Austin, 1999; Owens, 2003).

The objectives of the present study are to isolate and identify pathogenic bacteria from red tilapia, water and feces or debris and to determine the water parameters from cage-cultured tilapia in Kenyir Lake, Terengganu and Semantan River, Pahang.

Materials and Methods

Fish, water and debris sampling and water quality measurement

At each site, 30 cage-cultured red tilapia fish were randomly caught, 10 water and 10 debris samples were randomly taken from fish cages. The temperature, pH and dissolved oxygen and conductivity were measured using YSI 556. The ammonia, iron, sulphate and nitrites were determined using a Hach Spectrophotometer (DR 2800 Portable Spectrophotometer). All fish were measured and weighed. External observations on fish were also recorded.

Bacterial isolation from fish and debris at sampling site

Incision was made on the eye surface using sterile scalpel blade and then, a sterile wire loop was used to obtain a sample from the eye. The samples were cultured onto the blood agar (BA). The same procedures were also applied to other organs such as brain and kidney. Any organ abnormalities were recorded. Debris or fecal sample were picked using sterile wire loop and cultured onto blood agar. The inoculated BA media were incubated at room temperature, ~22°C.

Subculture of fish and debris bacterial colonies from BA medium

Inoculated BA medium were observed for the presence of bacterial growth after 24 hours incubation. The bacterial colonies present were sub-cultured onto BA medium and incubated at 30°C in an incubator for 18-24 hours.

Isolation of bacteria from water samples into BHI broth

One milliliter of water sample taken using a sterile pipette was inoculated into 9 mL of brain heart infusion broth (BHIB) in a tube media and mix well using a rotator. The inoculated broth was later placed in an incubator shaker at 30°C for 18 hours. Growth in the tubes was indicated by the presence of turbidity of the broth.

Subculture of bacteria from water samples in BHI broth onto BA medium

The broth samples were mixed using vortex mixer. One hundred microliters of the broth was then dropped onto BA medium and streaked with a sterile wire loop. The inoculated medium was then incubated at 30°C for 18-24 hours.

Second sub-culture of pure bacterial colonies onto BA and TSA medium

The bacterial colonies were sub-cultured onto Trypticase Soya Agar (TSA) medium for Gram-stain, catalase and oxidase tests. The bacterial colonies were sub-cultured onto BA medium for second time to get pure colonies of bacteria for API (Analytical Profile Index) test.

Gram staining, catalase and API test

Gram staining was done using the pure culture of bacterial colonies grown on TSA medium. For Gram-negative bacteria, API 20E was used. For Gram-positive bacteria, catalase test was done. Catalase positive bacteria were subjected to API 20 Staph test. Catalase negative bacteria were tested with API 20 Strep test. Oxidase tests were done on Gram-negative bacteria colonies grown on TSA medium. All the API test kits used were incubated in normal incubator at 30°C for 24 hours. Identification of bacteria was made by using API test software.

Results and Discussion

Kenyir Lake

The bacterial isolates from tilapia of Kenyir Lake are mostly predominated by *Micrococcus* spp. and *Aeromonas hydrophila* at 13.64 % of both bacteria and followed by non-fermenter sp. at 9.09%. In water samples, the most predominant bacteria isolates are *Staphylococcus xylosus* with percentage of 40%, followed by *Staphylococcus lentus*, *Klebsiella terrigena* and *Kocuria varians* at 20%. In debris samples, there were 2 species of bacterial isolates have been isolated there, which are *Pseudomonas aeruginosa* and *Enterobacter cloacae* at 50%.

Semantan River

The most predominant bacterial isolates in the tilapia there are *Aeromonas hydrophila* with 23.53%, followed by *Staphylococcus xylosus* and *Staphylococcus caprae* both with 11.76%. In water samples, the most predominant bacteria isolates are *Staphylococcus xylosus* with percentage of 40%, followed by *Staphylococcus lentus*, *Klebsiella terrigena* and *Kocuria varians* with percentage of 20% of all bacteria, respectively. While, the 90% of bacterial isolates in debris samples of Semantan River are *Aeromonas hydrophila* and the remaining isolates which are 10% are *Staphylococcus lentus.*

Water quality

Only pH and conductivity between Kenyir Lake and Semantan River have no significant difference. All of the parameters of water quality in Semantan River were higher than in Kenyir Lake except for temperature, DO and pH References

The findings of *A. hydrophila* being the predominant bacterial genera in tilapia fish in the current study are in agreement with the report of Sugita et al. (1982).

The internal organ of *A. hydrophila* infected fish were found congestion or swollen of the kidneys, softening of brain tissues and enlargement of liver. These findings are in agreement with Huizinga et al. (1979).

*Micrococcu*s spp. is the most predominant of *Staphylococcus* spp. that being isolated from other studies conducted by Siti-Zahrah et al. (2008) and Chowdhury et al. (1989). This is in agreement with the result at Kenyir Lake.

No abnormal findings on external organs were noted. This is in agreement with the findings from previous study by Siti-Zahrah et al. (2008), who noted that grossly sampled tilapia affected by *Micrococcus* spp. did not show little or no clinical signs.

Ammonia level in Kenyir Lake and Semantan River were exceeded the acceptable limit (0.02 ppm) for fish. The high level of ammonia in the present study is due to the high level of ammonia in the feed as found in Semantan River where goat faeces and visceral organs of chickens used as a source of feed.

The tolerance of tilapia to nitrite may also influenced by the fish size. Atwood et al. (2001) found that small-sized weighted Nile tilapia were more tolerant to nitrite than larger fish. From this study, the weight of the fish in Semantan River is more than Kenyir Lake fish, indicative of low tolerance to nitrite. This may also explained why high mortality occurred at Semantan River fish.

Thus, the environment also contributes to the existence of some pathogenic bacteria in red tilapia especially contaminants from feed, water and debris. The poor water quality may also cause fish more succumb to diseases or infection eventually death due to environmental stress and attacked by opportunistic pathogens such as *A. hydrophila*.

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Effect of Medium-Chain Triglycerides on Piglets in Three Farms in Selangor and Penang, Malaysia

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Abstract

The aims of this study were to evaluate effect of medium-chain triglycerides (MCT) between treatment and control groups on growth performance, mortality rate and fecal coliform count in piglets. In addition, the effect of MCT on weaning body weight was evaluated. This study was separated into Phase I and Phase II. In phase I, 18 litters were selected from three farms in Selangor and Penang. Piglets in each litter was equally divided into control group and treatment group where only treatment group piglets were fed twice with 2 ml MCT on Day 1. Body weight was taken on day one, three, five and seven. Phase II involved 12 litters from a farm. The same procedure as in phase I was done, except the body weight was taken on day one, fourteen and twenty eight. MCT treatment group piglets were found to have higher growth performance and lower mortality rate thAn control group piglets. No difference in fecal coliform count was observed between treatment and control group. MCT showed more prominent effect on growth performance during weaning period of the piglets. In conclusion, MCT supplementation had positive effect on the growth performance of piglets as a result of increased body weight and average daily gain. Besides, MCT were able to reduce mortality in piglets in all the farms.

Keywords: medium-chain triglycerides (MCT), average daily gain, body weight gain, fecal coliform count, mortality

Introduction

Pre-weaning piglets are subjected to nutritional and environmental stress, thus resulting in reduced feed intake, low weight gain, diarrhea and death. Pre-weaning mortality varies considerably among production units, ranging from 5 to 39% of piglets born alive (Wieland et al*.,* 1993). In addition, energy insufficiency was identified as one of the major causes of mortality, given that energy reserves at birth are low (Noblet et al*.*, 1997). The neonatal piglets are prone to develop hypoglycemia due to the limited liver glycogen that can supply the energy requirement and the insufficient gluconeogenesis in newborn piglet (Too, 1997). The runt piglets would not be able to compete with others for milk, thus lead to starvation and mortality.

Due to the unique digestive and metabolic properties of medium chain triglycerid (MCT) supplementation, it was claimed as a remedy for this energy insufficiency (Wieland et al., 1993). With medium-chain triglycerides, the piglets become energetic and alert, which enable them to suckle milk more efficiently.

The MCT were also reported to be naturally occurring antimicrobial agents that could be used as growth promoter, preventive and curative treatment to promote health (Dierick et al., 2002). Thus these MCT might have effect on coliform bacteria in pigs such as *Escherichia coli*, which is a common bacterium causing piglet enterotoxigenic colibacillosis in Malaysia. Neonatal piglets are susceptible to enterotoxigenic colibacillosis which would lead to digestive disturbances and gastrointestinal disease. This study was conducted to evaluate effect of MCT on body weight gain, weaning body weight, mortality rate, and fecal coliform count of piglets in MCT treated and control group piglets.

Materials and Methods

Experimental Animals

In phase I experiment, 18 litters were selected from three farms, each farm contributed 6 litters. Eight piglets in a litter were chosen and randomly distributed into two groups, which are the control and treatment groups. This was to reduce bias due to the different sows, the disturbance of maternal antibody and environments. Prior to treatment, all piglets were allowed to suckle colostrum. Daily routine farm activities such as iron supplements, needle teeth removal were undisturbed and equally perform on both the control and treatment groups. Meanwhile, another twelve litters were selected in phase II experiment. Each litter was randomly and equally distributed into control and treatment group. Routine farm activities were as in phase I study.

Treatments

In phase I and phase II study, each piglet in the treatment group were given MCT within 6 hours after farrowing and the second dose after 6 hours whereas the control group were not be given any MCT. Body weight of both groups piglets in phase I were recorded on day 1, 3, 5 and 7, also in phase II where body weight of piglets were recorded on day 1, 14 and 28 with a body weighing scale.

Growth Performance and Mortality Rate

In both phases, cumulative weight of siblings, litter size, sex and mortalities of the piglets were recorded on every visit. In addition, average daily gain, body weight difference and body weight gain in percentage on day 3, 5 and 7 were calculated from the records. The same parameters were also recorded for phase II study on day 1, 14 and 28.

Coliform Plate Count

Eighteen fecal samples were taken randomly for coliform count, which consist of 9 fecal samples respectively from treatment and control group. The count was done by adding 1 g of fecal sample to 9 ml peptone broth, followed by ten-fold serial dilutions with peptone broth. One mililitre aliquots of each dilution were then transferred to their respective petri dishes, to which 15 ml of sterile molten Violet Red Bile Agar (VRBA) was added. The petri dishes were incubated at 35°C for 24 hours before performing bacterial colony counts.

Statistical Analysis

The data was analyzed using independent T test to compare the differences in growth performance of piglets and fecal coliform count due to the effects from the treatment. The statistical test was conducted at 95 % confidence level using SPSS program.

Results and Discussion

Weight Gain

In phase I, MCT group had higher mean body weight, ADG and percentage body weight gain (Figure 1). MCT can be utilized as a fuel by the newborn piglet and are able to spare critical fuels, glycogen and protein that stored in the piglets prior to birth (Benevenga et al., 1989). The animals supplemented with MCT in diet showed a higher mucosal mass and protein content and increased villus length and crypt depth in the proximal part of the small intestine (Galluser et al., 1993). It is also reported that the MCT might enhance calcium and amino acid uptake and also have the positive effect of intracellular protein synthesis (Galluser et al., 1993). These effects of MCT will finally contribute to better growth performance of piglets in MCT treatment group.

Limited significant difference was detected within the seven day period trial, so another study was being conducted to monitor the growth rate from day one until weaning (28th day). In phase II of the study, the growth performance showed prominent improvement on 14th till 28th day (Figure 2). This was further supported by (Dierick et al., 2002) where the MCT produced the most pronounced daily growth rate in the first two weeks after weaning at twenty one days, where the MCT produced up to 30% better growth performance over the soybean oil as a control.

Mortality Rate

Overall, there were more mortality in the control group, sixteen control group piglets and six MCT group piglets died. Mortality of piglets in the control group were mainly due to failure to consume milk with other piglets leading to poor body weight, starvation, death or crushed by sows. Meanwhile, treatment group piglets died of diarrhea or crushed by sows. The mortality rate in control group was higher than treatment group which were 11.6 and 4.1% respectively. This can be due to improved energy status of treatment group by orally dosing MCT where MCT was reported to be an energy source with high digestibility and oxidation rates (Lee and Chiang, 1994).

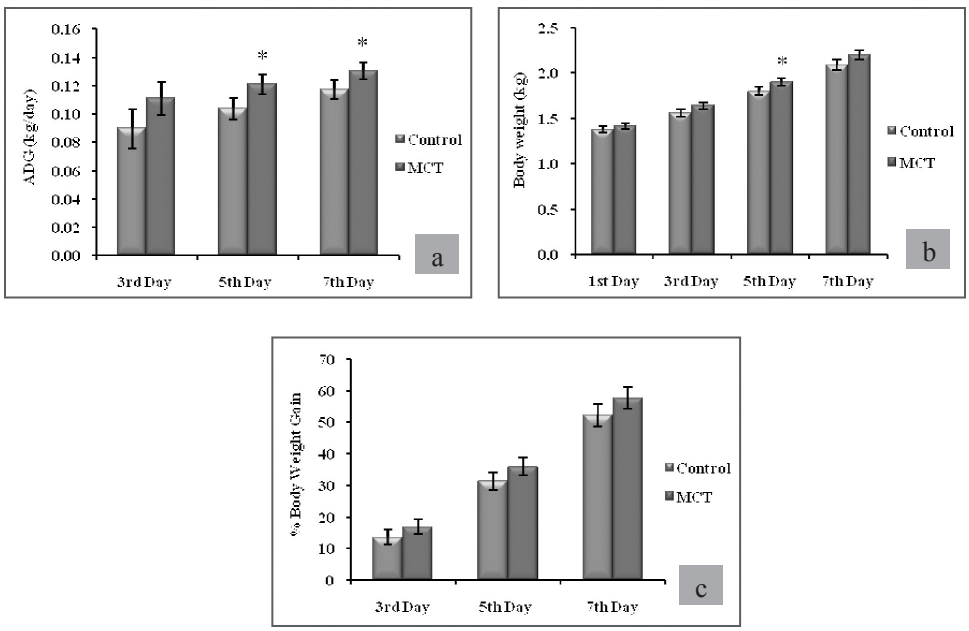

Figure 1. Combined result of three farms in Phase I. (a) Body weight of piglets. The MCT group had higher body weight than control group and was significantly higher on fifth day. (b) Average daily gain of piglets. MCT group had higher ADG than control group and was statistically significant on fifth and seventh day. (c) Percentage of body weight gain compared to first day. MCT had higher percentage than control group. Error bar indicates standard error of mean. Error bar indicates standard error of mean. Bar with * on the top is significantly different in values ($p<0.05$).

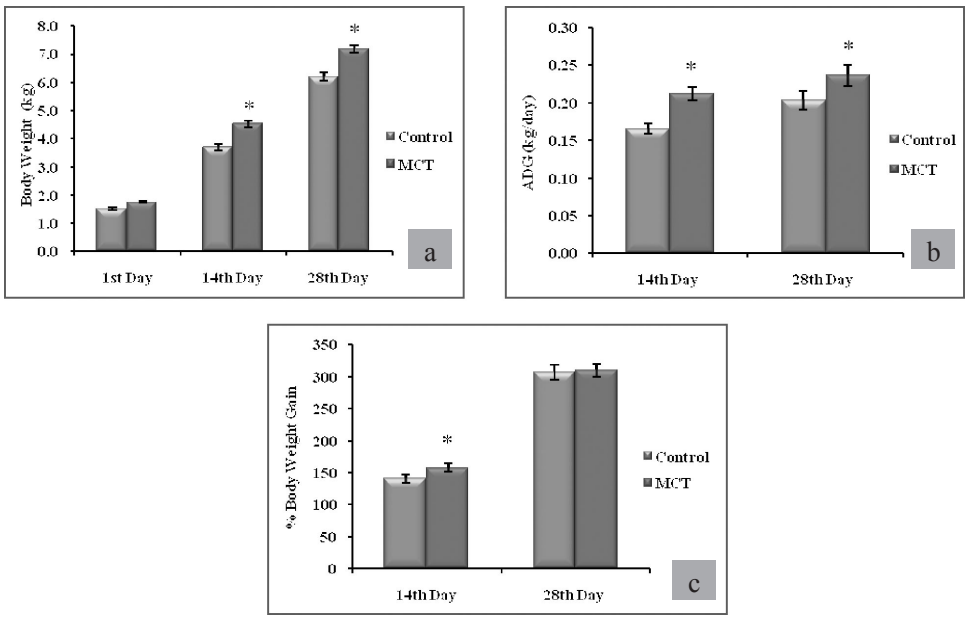

Figure 2. Results for Phase II. (a) Body weight of piglets. The MCT group had higher body weight and was statistically significant on the 14th and 28th day. (b) Average daily gain of piglets. The MCT group had higher ADG and was statistically significant on 14th and 28th day. (c) Percentage of body weight gain of piglets compared to the first day. The MCT group was higher than control group and statistically significant on 14th day. Error bar indicates standard error of mean. Bar with * on the top is significantly different in values ($p<0.05$).

Fecal Coliform Count

There were no significant differences ($p>0.05$) in fecal coliform count between MCT treatment group and the control piglets. MCT treated piglets were observed to have lower fecal coliform count (Figure 3). The findings are inconsistence with a previous experiment of MCFA efficacy against colibacillosis, which indicated that MCFA are able to inhibit bacterial growth in vitro (Gallois et al., 2008). Although no significant difference in fecal coliform count, but the farmers had mentioned that MCT treated piglets had less diarrhea problem as compared to control group piglets. This findings could be supported by a previous research which had proved that pathogenic bacteria including *Pseudomonas spp.*, *Campylobacter spp.*, *Vibrio cholera*, *Salmonella typhimurium*, *Shigella sonnei*, *Hemophilus influenza*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Helicobacter pylori* and enterotoxigenic *E.coli* could be inactivated by MCFA or their monoglycerides (Petschow et al., 1998), where the MCT have suppression effect on bacterial load in gut and thus reducing the diarrhea problem in piglets. Although MCT was hypothesized to be able to suppress growth of fecal coliform in gut, but the advantage was not noticed in this research, as this could be due to samples size was too small. In addition, the piglet might not completely consume the MCT given to them which may also lead to failure of MCT to suppress the coliform

growth in the gut. Stressful condition and sample contamination during fecal sample collection also may be the factors that lead to this observation.

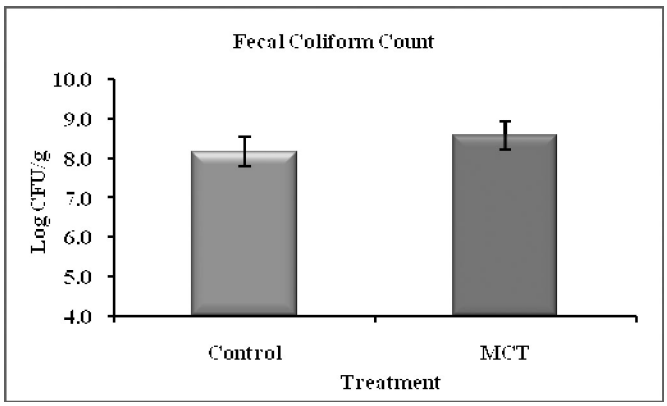

Figure 4. Fecal coliform count of piglets from farm X, farm Y and farm Z. MCT group had higher fecal coliform count than the control group. Error bar indicates standard error of mean.

Conclusion

Medium Chain Triglyceride supplementation in piglets showed higher growth performance than the untreated piglets as shown by the higher body weight, average daily gain and percentage of body weight gain. The MCT showed more prominent effect during weaning age of piglets. Less mortality was observed in MCT treated piglets as compared to control group piglets. No difference in fecal coliform count was observed between the MCT treated piglets and the untreated piglets. In conclusion, MCT is a good supplement for piglets due to its growth benefit.

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Changes in Blood Parameters of Endurance Horses in 30-km Training

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Abstract

Eight endurance horses registered for competition in different categories of Sultan Cup Endurance Ride, in November 2009 were selected for an evaluation of the soundness of the horses and an examination of the changes of blood parameters in training prior to the competition. Three blood samples were taken from each horse: pre-ride, immediate post ride and 24 hours post-ride. All horses were trained at 30 km and departed at the same time and tested on the same track. Blood samples were evaluated for both haematology and biochemistry components: red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), segmented neutrophil, lymphocyte counts, electrolytes concentration, total protein (TP), aspartate aminotransferase (AST), creatine kinase (CK) and lactate. One horse was diagnosed with exertional rhabdomyolysis post-training and was removed from statistical analysis. In this study, the significant changes in most blood parameters indicated that the 30-km endurance training induced some physiological responses in horses with minimal stress and loss of water and electrolytes as reflected in the changes of blood parameters. Although elevation in serum muscle enzymes and lactate was significant, it was believed to be a normal physiological response of horses towards training without noticeable muscle injuries and/or metabolic acidosis.

Keywords: training, endurance horses, haematology, biochemistry, adaptive physiological response

Introduction

Through evolution and artificial selection, horses have become extraordinary athletes compared to other species of animals of the same size. With high maximal aerobic capacity, large intramuscular storage of energy substrates especially glycogen, high mitochondrial volume in muscles, the ability to increase oxygen carrying capacity via splenic contraction, and the efficiency in thermoregulation as well as in their gaits, have produced horses with excellent athletic ability (Hinchcliff, 2005).

Training is defined as the induction of anatomical, physiological and functional adaptations in response to stresses and strains through repetitive exercise (Hinchcliff, 2005). Hence, appropriate training program induces favorable adaptation, which increases the fitness and improves the performance of horses. A fit endurance horse, on the completion of an endurance ride, must still be sound in the aspect of metabolism and gait. In terms of metabolic evaluation, it is the determination of physiological

soundness, which can be reflected in the changes of blood parameters in both components of haematology and biochemistry. This study is to determine the trend of changes of blood parameters in endurance horses trained under tropical condition and to evaluate the fitness and soundness of endurance horses by examining different blood indicators before entering the competition.

Materials and Methods

Eight clinical healthy Arabian horses registered to participate in Sultan Cup Endurance Rides, in November 2009, were selected for the study. Training at 30 km was organized two weeks prior to the competition, though horses were regularly trained before this, but at a shorter distance of 10 to 20 km. The training started in the morning, with all horses starting simultaneously on the same track, and progressed at the speed of 16 km/h. Water points were provided at every 10 km so all horses had access to water. It ended within 2 hours, when all horses returned at the same time for veterinary check.

Three blood samples were taken from the jugular vein of each horse, i.e. pre-ride, immediate post-ride and 24 hours post-ride samples. Each sample of blood was collected into a plain blood tube and an EDTA-containing tube. Blood in plain tubes was centrifuged and serum obtained was kept in serum tubes for biochemistry testing. Haematology component was evaluated for differential leukocyte count, microhematocrit for PCV while others were tested using a haematology automatic analyzer. All serum samples were processed using a Hitachi 902® biochemistry automatic analyzer.

Results

All horses which returned from the 30 km training were fit with only 5 minutes of recovery period for heart rate measurement, except for a horse that had an extended heart rate recovery period of 30 minutes. During veterinary check, all horses were sound and fit without noticeable dehydration, and muscle fatigue, except for the horse with a prolonged recovery period which was detected to have muscle stiffness and lameness and had persistent and exceptionally high serum asparfate aminotran sterase (AST) and creatine kinase (CK) post training in the blood. Thus a final diagnosis of exertional rhabdomyolysis was made on the horse and it was excluded from the statistical analysis as the AST and CK values post training were considered to be outliers.

Haematology

Data are presented as mean ± standard error for haematology parameters studied, as shown in Table 1.

The mean values for immediate post training were the highest for all parameters studied except for lymphocyte count, which was the lowest compared to that of pre-training and 24 hours post training. Significant ($P < 0.05$) differences were observed between pre-training and immediate post-training in total WBC count, segmented neutrophil count, and lymphocyte count, while significant differences between immediate post training and 24 hours post-training were detected in all parameters except for lymphocyte

count. While expecting many of the blood parameters would return to pre-rides values, however, significant differences were noticed between pre-training and 24 hours post training in RBC count, PCV, and segmented neutrophils. Therefore, the hypothesis of no differences in the above parameters were rejected.

Table 1. Haematology parameters of horses in training

Blood Parameter	Pre-Training	Immediate Post	24 Hours Post
RBC ($x10^{12}$/L)	$8.07^{a} \pm 0.35$	$8.35^{a} \pm 0.31$	$7.17^{b} \pm 0.27$
PCV (L/L)	$0.41^{a} \pm 0.01$	$0.42^{a} \pm 0.01$	$0.36^{b} \pm 0.01$
WBC ($x10^{9}$/L)	$7.74^{a} \pm 0.37$	$10.08^{b} \pm 0.67$	$8.03^{a} \pm 0.18$
Segmented Neutrophil ($x10^{9}$/L)	$4.36^{a} \pm 0.21$	$7.33^{b} \pm 0.71$	$5.22^{c} \pm 0.22$
Lymphocyte ($x10^{9}$/L)	$2.53^{a} \pm 0.16$	$1.93^{b} \pm 0.09$	$2.10^{ab} \pm 0.19$

All values are expressed as mean ± se.

^{a,b,c}Means with different superscripts, within the same row are significantly different at $P < 0.05$.

Table 2. Biochemistry parameters of horses in training

Blood Parameter	Pre-Training	Immediate Post	24 Hours Post
Na^{+} (mmol/L)	136.87 ± 0.39	136.77 ± 1.34	137.74 ± 0.41
K^{+} (mmol/L)	$3.94^{a} \pm 0.08$	$2.69^{b} \pm 0.08$	$3.96^{a} \pm 0.09$
Cl^{-} (mmol/L)	$98.93^{a} \pm 0.38$	$94.09^{b} \pm 0.92$	$100.46^{c} \pm 0.48$
TP (g/L)	$71.27^{a} \pm 1.15$	$78.51^{b} \pm 0.92$	$72.46^{a} \pm 1.54$
AST (U/L)	$336.09^{a} \pm 25.40$	$391.94^{b} \pm 30.30$	$368.46^{c} \pm 25.89$
CK (U/L)	$176.00^{a} \pm 13.42$	$316.86^{ab} \pm 62.64$	$241.86^{b} \pm 30.76$
Lactate (mmol/L)	$0.97^{a} \pm 0.05$	$2.33^{b} \pm 0.23$	$0.76^{c} \pm 0.07$

All values are expressed as mean ± se.

^{a,b,c}Means with different superscripts, within the same row are significantly different at

Biochemistry

Table 2 presents the data expressed in mean ± standard error. No significant changes ($P > 0.05$) were observed for serum Na concentration of all samples. Apart from that, it was obvious that the samples taken instantly post training demonstrated the lowest means for serum K^{+} and Cl^{-} concentration, while it showed the highest means for serum toral Protein (TP), AST, CK and lactate compared to the other two samples. Significant difference was found in serum K^{+} concentration between the means of pre-ride and immediate post ride and also between immediate post ride and 24 hours post ride, but there is no significant change between pre-ride and 24 hours post-ride samples, indicating the recovery to pre-ride values. Similar finding was observed for TP. On the other hand, serum Cl^{-}, AST, and lactate had significant changes in all levels, i.e. pre-training, immediate post and 24 hours post training. While changes occurred in serum

CK level, significant changes of mean was only observed between pre-training and 24 hours post training.
P < 0.05.

Discussion

In comparison to previous studies that involved longer distances in temperate condition, endurance horses training at 30 km under tropical condition had consistent findings, with significant changes in blood parameters immediate post-training and reversion of these changes 24 hours later, except for RBC count, PCV and CK parameters.

Significant increase of segmented neutrophil count was also reflected in a significant increase of leukocyte count immediately post training, and with a significant reduction of lymphocyte count immediate post training, which indicated that the training at 30 km had caused stress to the endurance horses. Stress leukogram of neutrophilia and lymphopenia was typically observed in two horses immediately post training, though the other 5 horses only showed mild to modest changes in segmented neutrophil and lymphocyte count as was also reported by Carlson (1987) and Snow (1983).

As the Na^+ concentration was closely associated with the volume of water intake during and after the training, 3 out of 7 horses showed reduction immediately post training while 4 horses showed elevation. Therefore, there was no significant changes in Na^+ concentration immediately post training, consistent with earlier findings but a net loss of Na^+ through sweating was expected (Topliff, 2006; Grosskopf, 1983). The loss of K^+ and Cl^- ions through sweating was significant immediately post training, but the magnitude of loss was estimated to be lesser than that of Na loss. (Topliff, 2006; Carlson, 1987). At the same time, the loss of water through sweating was high immediately post training, which was reflected in the significant elevation of TP.

All AST values exceeded normal range (120 – 160 U/L) for all three samples, which was most likely caused by regular training, causing minor muscle injuries or muscular leakage. With prolonged removal half life of AST, there was not much decrease 24 hours post training (Valberg, 2006). Increase energy demand had caused a significant elevation of lactate values immediately post training, that there was an increase in glycolysis over the threshold of oxygen-dependent citric acid cycle and electron transport chain leading to the increase of conversion of pyruvate to lactate. However, with all values of serum lactate less than 4 mmol/L, it is estimated that all horses worked aerobically (Islas, 2006; Topliff, 2006).

Blood parameters that were not congruent with earlier findings were RBC count, PCV and CK. As PCV is closely related to RBC count, similar statistical results were obtained. There was no significant change between pre ride and immediate post ride for both RBC count and PCV. Through individual interpretation, there were two horses with elevated pre-training values as indicated in Figures 1 and 2, which was highly suggestive of the cause of excitement during blood sampling as they were unfamiliar with the procedures, or due to the transportation of these horses to the track for the preparation for training

(Carlson, 1987; Persson, 1983). Besides, it could be due to higher water intake during and after the training. As for CK, though the value of immediately post training was the highest among all, it was not a significant increase from its pre-training sample. From individual interpretation, there were two horses with exceptionally elevated CK readings immediate post training, that caused noticeable individual variation (Figure 3).

All changes in blood parameters recovered 24 hours later, although segmented neutrophil count, lymphocyte count, and AST did not return to the pre-ride values due to their prolonged correction due to disruption of the homeostasis caused by the training (Valberg, 2006; McWilliams, 1995).

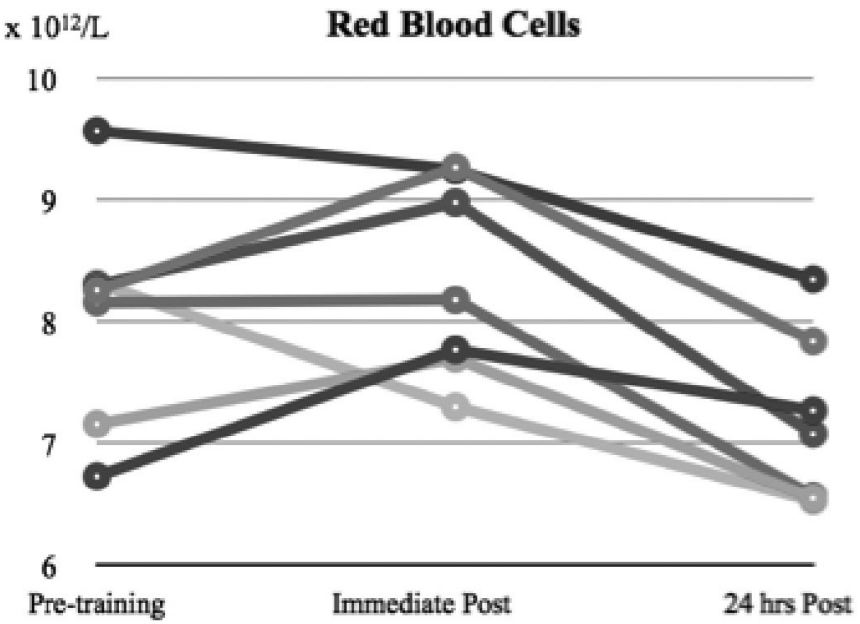

Figure 1. Changes of RBC count in individual horses

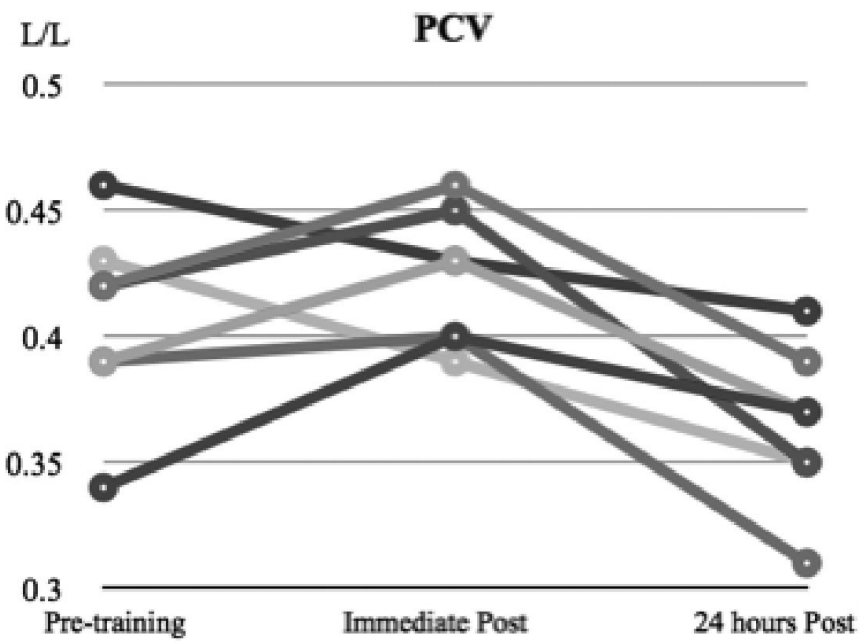

Figure 2. Changes of PCV in individual horses

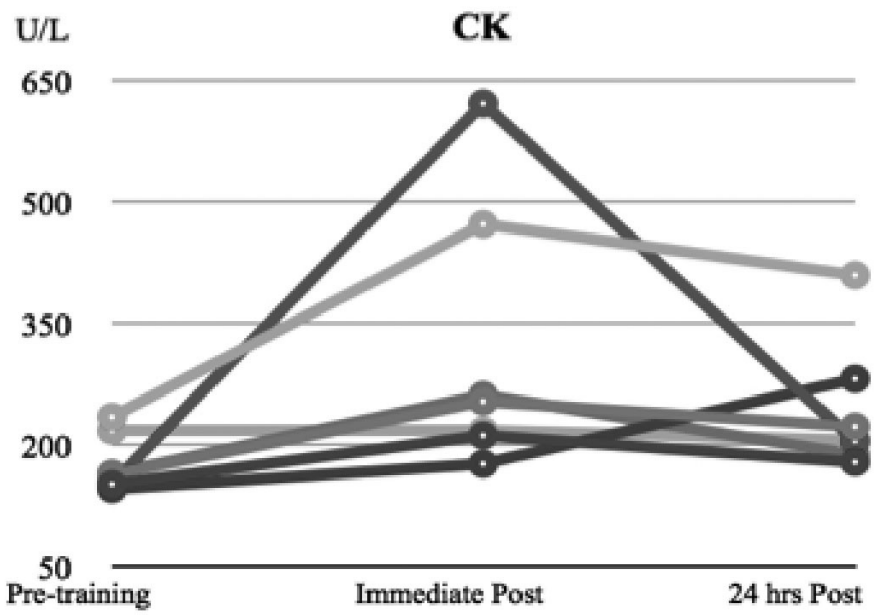

Figure 3. Changes of serum CK in individual horses

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Screening of Chinese Medicinal Herbs for the Inhibition of *Brucella melitensis*

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Abstract

The antimicrobial activities of extracts from Chinese herbs commonly available in the local Malaysian Chinese medicine halls against three field isolates and one reference strain of *Brucella melitensis* were evaluated. A total of ten herb extracts were obtained via ethanol extraction. Antibacterial screenings were done using disc diffusion method. Herb extracts with inhibitory zones of 10 mm or more in diameter were further subjected to the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination. Of the 10 herbs, which were *Lunicera japonica, Flos Lonicera, Coptis chinensis, Adrographis paniculata, Isatis indigotica, Radix paeoniae rubra, Polygonum orientale, Galla chinensis, Semen plantaginis, Fructus forsythia* and *Cortex phellodendrim,* four were found to possess inhibitory effect against *Brucella melitensis* strains. The herbs are *Coptis chinensis, Radix paeoniae rubra, Galla chinensis,* and *Cortex phellodendrin.* The MIC ranged from 3.75 to 30 mg/mL. It was suggested that these four herbs are potential alternatives for the treatment or prevention of brucellosis caused by *Brucella melitensis*.

Keywords: *Brucella melitensis,* minimum inhibitory concentration (MIC), herbal extract.

Introduction

Brucellosis is a zoonotic disease caused by the bacterium from the *Brucella spp*. This disease is endemic in many areas of the world including Malaysia. It is characterized by chronic infection in animals involving many species such as cattle, swine, sheep, goat and even horses leading to abortion, infertility, genital infection and the formation of localized lesions in many parts of the body tissues (Galloway J.H,1972). Human beings are susceptible to several biotypes of this agent namely *Brucella abortus, Brucella canis, Brucella melitensis and Brucella suis* but are resistant to infection by *Brucella neotomae and Brucella ovis.* The organism is of public health importance as it can cause debilitating disease (Malta fever) like fever and chills with frequent relapses which can persist for months if left untreated. Current control measures in farms are disease surveillance and investigative studies, vaccination, test and slaughter programs to contain and eradicate the disease. With these measures, brucellosis is no longer a threat in Western Europe but is still prevalent in Malaysia and other parts of the world (Swabe, 1999). The wide practice of the application of *B. abortus* S19 vaccination of susceptible young female animals either with the full dose or the reduced dose together with the slaughter of positive animals has resulted in the significant decrease of the

overall rate of infection in countries like Egypt (Refai et al., 1990). For conventional human treatment, doxycycline and rifampin combination are used for six weeks to combat the infection. Streptomycin is included in severe cases. The tedious treatment regime and ease of transmission of this disease highly suggest a quest for alternatives or improvement on ways to manage this disease.

Ethnopharmacology is the scientific study correlating ethnic groups, their health, and how it relates to their physical habits and methodology in creating and using medicines. Since global eradication of animal brucellosis will not be realistic in the near future due to socio-economic and political factors, and since the development of a satisfactory human vaccine currently is also not achieved yet, there exists a need for optimal antibiotic treatment schedules. These would ideally minimize the percentage of treatment failures and relapses whilst simultaneously being affordable for populations of low socio-economic status as well as being convenient in order to ensure adequate patient adherence. Ethnopharmacology is a very good alternative to explore in the sense that historically both human and animal medicine have relied heavily on plant materials. Major pharmaceutical companies had started off by selling plant extracts whilst a quarter of all prescription drugs currently sold in the western world still use active ingredients derived from plants (Cox and Balick, 1997). The first stage in a drug development program using plants as the starting material is the collection and analysis of data on the uses of plants by various native cultures. Ethnobotany, ethnomedicine, folk medicine and traditional medicine can provide information that is useful as a 'pre-screen' to select plants for experimental pharmacological studies. There are a number of Chinese herbs available in Malaysia that has potential to treat brucellosis. However, the potential of the herbs has not been evaluated yet. In this study, 10 types of commonly available Chinese herbs were tested in bioassay systems that are believed to predict the action of these drugs in humans. The goal of this ethnopharmacology study is to identify drugs to alleviate human illness via analysis of plants assumed to be useful in Chinese culture throughout the world.

The objectives of this study are to determine the antibacterial effects and minimal inhibitory concentrations of Chinese crude herbs against *Brucella melitensis.*

Materials and Methods

Dried Herbs

A total of ten dried herbs were obtained from Chinese medicinal shop. The herbs were *Lunicera japonica, Flos lonicera, Coptis chinensis, Adrographis paniculata, Isatis indigotica, Radix paeoniae rubra, Polygonum orientale, Galla chinensis, Semen plantaginis, Fructus forsythia* and *Cortex phellodendrim.* The origins of these herbs are from various parts of China.

Herb Extract Preparation

Thirty grams of herbs were macerated with 80% ethanol (500 mL) using a blender and left for five days at room temperature. The mixture was filtered and evaporated at 60ºC to obtain the dried herb extracts.

Brucella Isolates

One reference strain (*Brucella melitensis* 16M), one isolate from goat (*Brucella melitensis* 293) and two isolates from sheep (*Brucella melitensis* 183 and *Brucella melitensis* 4611) were used.

Antibacterial Screening (Disc Diffusion Method)

Extract concentration of 30 mg/mL was made by mixing 30 mg crude extract with one millilitre (1 mL) of 3% DMSO. Seventy microlitres (70 µL) of each extract was added to blank discs. The herb extract infused discs were dried at 60ºC in an oven overnight. Negative control were prepared by adding 70 µL of 3% DMSO to blank discs and dried at 60ºC overnight while Clavumox disc was used as positive control. Twenty mililitre Mueller Hinton Agar (MHA) plate were seeded with 20 ul suspension of test microorganism in 0.5 McFarland concentrations. One herb extract infused disc, one positive control disc and one negative control disc were put in each MHA plate. Triplicates were done. Inhibition zones diameters were read using the Aura Image (Oxoid). Extract with inhibition zone diameter of ten millimetres (10 mm) or more were subjected to MIC determination.

Determination of MIC of herb extracts on Brucella melitensis

Two-fold serial dilution methods were used. Briefly, 30 mg/mL crude extracts were filtered through 0.25 µm millipore filter for sterilization. The extracts were serially diluted starting from 30 mg/mL up to 0.015 mg/mL. Dilutions were made in 96-well microplates. Two hundred microlitres (200 µL) of 30 mg/mL extracts were dispense in the 1st column and 100 µL *Brucella* broth medium in the 2nd to 12th well. Two-fold serial dilution was done by pipetting 100 of extract from the 1st well into the 2nd well and so on. Twenty microliter (20 µL) of *Brucella* inoculum was added to every well. Streptomycin (0.05 mg/mL) was used as positive control while the negative control was made up of medium and inoculums. Triplicates were done for all four isolates. The plates were wrapped in parafilm and incubated at 37 ºC for three days. The growth of inoculum was determined by turbidity. Clear wells indicate absence of bacteria growth. The MIC of herbs was the lowest concentration in the medium that completely inhibit visible growth.

Determination of MBC of herb extracts on Brucella melitensis

The MBC of herb extract was carried out by inoculating ten microlitres (10 µL) of mixture from each well on MHA plate. Inoculated MHA plates were incubated at 37ºC for three days. The lowest concentration that yielded no growth after subculturing was taken as MBC.

Results and Discussion

The antimicrobial screening results of the herb extracts are shown in Table 1. Four herbs showed inhibitory effects on the organism namely, *Coptis chinensis, Radix paeoniae rubra, Galla chinensis and Cortex phellodendrim. Galla chinensis* showed the largest inhibition zone which were, 39 mm for *Brucella melitensis* 16M, 36 mm for *Brucella melitensis* 293, 33 mm for *Brucella melitensis* 183 and 40 mm for *Brucella melitensis* 4611. *Cortex phellodendrim* showed the smallest inhibition zone which is, 13 mm for *Brucella melitensis* 16M, 12 mm for *Brucella melitensis* 293, 12 mm for *Brucella melitensis* 83 and 15 mm for *Brucella melitensis* 4611. The larger the inhibition zone the stronger the inhibitory effect of the tested sample. As *Galla chinensis* has the largest inhibitory zone, it means that it has the strongest inhibitory effect on *Brucella melitensis* and has high potential to be used as an antimicrobial agent against this microorganism. In fact, the inhibitory zone of *Galla chinensis* is almost similar to Clavumox which has the highest zone of 45 mm thus, making it a potential alternative drug for management of infections. The other six herbs did not show any inhibitory zone thus, they do not have the potential to be used as anti *Brucella* agents. Among the four *Brucella i*solates, *Brucella melitensis* 293 is the most resistant to the inhibitory effect of the herbs because the inhibitory zones are the smallest which are 14 mm for *Coptis chinensis*, 14 mm for *Radix paeoniae rubra,* 32 mm for *Galla chinensis* and 12 mm for *Cortex phellodendrin.*

Table 1. Inhibition Zone Diameter in mm of Herb Extracts on Four Strains of *Brucella melitensis*

Herbs	***Brucella melitensis* 16M**	***Brucella melitensis* 293**	***Brucella melitensis* 183**	***Brucella melitensis* 4611**
Lunicera japonica	0	0	0	0
Flos lonicera	0	0	0	0
Coptis chinensis	22	14	29	22
Andrographis paniculata	0	0	0	0
Isatis indigotica	0	0	0	0
Radix paeoniae rubra	18	14	18	16
Galla chinensis	39	32	33	40
Semen plantaginis	0	0	0	0
Fructus forsythia	0	0	0	0
Cortex phellodendrim	13	12	12	15
Clavumox	44	42	45	45
3% DMSO Infused Blank Disc	0	0	0	0

The antimicrobial activities of different extracts tested are shown in Tables 2 to 5. Bactericidal activity was confirmed by determining the MBC:MIC ratio. The MBC:MIC ratio describes a relationship between the minimum *in vitro* bactericidal concentration and the MIC of an antibiotic. If the MBC:MIC ratio of a pathogen is between 1:1 to 2:1, the drug is considered to be bactericidal against that pathogen.

For *Brucella melitensis* 16, all herbs fulfill the ratio except for *Coptis chinensis* which has a ratio of 4:1 and *Cortex phellodendrin* which did not show any effects at the tested concentration.

Table 2. MIC and MBC of herb extracts on *Brucella melitensis*16

Herbs	***Brucella melitensis 16M***		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	7.5	30	1: 4
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis 293*, all herbs fulfill the ratio except for *Cortex phellodendrin* which did not show any inhibitory effects at the tested concentration.

Table 3. MIC and MBC of herb extracts on *Brucella melitensis* 293

Herbs	***Brucella melitensis 293***		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	3.75	7.5	1: 2
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis* 183, the results were the same as for *Brucella melitensis* 293.

Table 4. MIC and MBC of herb extracts on *Brucella melitensis* 183

Herbs	*Brucella melitensis 183*		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	3.75	7.5	1: 2
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis* 4611, the MIC-to-MBC ratio was the same with the previous 2 strains. However, MIC and MBC values were higher.

Table 5. MIC and MBC of herb extracts on *Brucella melitensis* 4611

Herbs	*Brucella melitensis 4611*		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	7.5	15	1: 2
Radix paeoniae rubia	3.75	3.75	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

From the results, 2 herbs showed bactericidal activity which are, *Radix paeoniae rubia* and *Galla chinensis*. *Cortex phellodendrin* did not show bactericidal activity at the highest concentration tested while *Coptis chinensis* an MBC-to-MIC ratio that exceeded the bactericidal ratio of 1:2.

In comparison to previous work by Zhu et al*.,* (2009) who assessed the herbs' effects on *Ureaplasma urealyticum*, the findings are similar where *Coptis chinensis, Radix paeoniae rubra, Galla chinensis and Cortex phellodendrim* also yielded potential inhibitory effect on *Brucella melitensis.* However, the MICs of these herbs on the two different bacteria are different. Taking *Brucella melitensis* 16M as a comparison to *Ureaplasma urealyticum,* the MICs of *Coptis chinensis* on *Brucella melitensis* 16M is 7.5 mg/mL while on *Ureaplasma* is 20 mg/mL, MICs of *Radix paeoniae rubia* on *Brucella melitensis* 16M is 7.5 mg/mL while on *Ureaplasma* is 2.5 mg/mL, MICs of *Galla chinensis* on *Brucella melitensis* 16M is 3.75 mg/mL while on *Ureaplasma* is 1.25 mg/mL. *Cortex phellodendrin* did not yield any detectable MIC on *Brucella melitensis* at the highest tested concentration of 30 mg/mL while it yielded an MIC of

5 mg/mL on *Ureaplasma urealyticum*. From these comparisons and studies, it shows that these herbs can be developed into useful antimicrobials that can act against more than one type of bacteria.

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Bacteriological Analysis of Commercial Cat Canned Food

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Abstract

In any attempt to produce safe food products for animal consumption, canned food should be processed with regards to canned food safety. Commercial cat canned food of three different brands were analyzed for bacterial contamination. Bacteriology results showed that all canned foods tested were free of contamination. However when the cat canned food were exposed to room temperature, *Proteus mirabilis, Staphylococcus intermedius, Bacillus spp. and Arcanobacterium spp.* were isolated as early as 24 hours post exposure. Nevertheless, the isolation of bacteria from contaminated processed foods in this study in cat canned food differed from previous study of contamination in dog foods and human canned food. 40% of cat owners who reported their cats developed diarrhea especially when their cats consumed cat canned food of ocean fish flavor could be due to improper food handling of opened unfinished canned food. This study highlights the importance of creating awareness of proper food handling to pet owners by veterinarian and animal health care personnel.

Keywords: cat canned food, bacteriology, contamination, diarrhoea

Introduction

Canning process aims to prevent food spoilage and preserve the quality of the foods, so that the food can be kept for an extended period of time without refrigeration and without the loss of nutrition values (Blementhal, 1990). Commercial canned foods are not invariably sterile. They can be spoiled by bacteria, yeasts or moulds. These microorganisms can be acquired during handling and processing, surviving any preservation treatment, and contaminating food in storage. The principal pathogenic microorganisms that had been documented associated with spoilage of human canned foods were *Clostridium botulinum*, *Salmonella spp.* and enterotoxin producing *Staphylococcus aureus*. Among them, *Staphylococcus spp.* rank first in terms of frequency followed by *C. botulinum* and *Salmonella spp.* (Hersom and Hulland, 1980). Microorganisms that contaminate processed pet food are responsible for digestive tract diseases such as diarrhea, vomiting, nausea and abdominal pain. Improper storage of opened canned food is another factor contributing to spoilage of canned food. Environmental temperature and oxygen availability influence the bacterial growth in opened canned food. The objective of this study therefore is to detect possible bacterial contamination in selected commercial cat canned food as this will help in establishing the effect of environment where the food was taken, type of handling and type of storage condition the microbial quality of the product.

Materials and Methods

Commercial cat canned food

One hundred and fifty commercial cat canned food of ocean flavor from three different brands were obtained from seller. Fifty cat canned food were used to represent each brand (Brand A, B and C).

Isolation of microorganisms

Sterile swabs were used to get sample from inside the can. Each swab sample was rolled onto a clean slide and heat fixed for three seconds and gram staining was performed to observe the staining characteristics of microorganism. The first swabs were taken from cans that were opened for the first time. Then, 10 cans from each brand were used for further bacterial culture. Five cans were placed at room temperature and another five cans were placed in a fridge. Using similar method as described before, swabs were taken after 24 hours from cans left at room temperature and after 24, 72, 120 and 168 hours from cans stored in a fridge. Then, the swabs were inoculated onto blood agar and Mac Conkey agar. All the plates were incubated at 37°C for 24 hours to obtain primary culture. Pure cultures of the isolates were obtained by subsequent sub-culturing on fresh agar plates.

Identification of microorganisms isolated

This was done based on cultural, morphological and biochemical characteristics of the isolates using standard methods (Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology by Bacteriology Laboratory, Faculty of Veterinary Medicine).

Questionnaire

Thirty copies of questionnaire regarding cat food survey were distributed among staffs and students.

Results

The result from the first bacterial culture of 150 cat canned food showed that none of the canned food were positive for the presence of any bacteria. Analysis of questionnaire revealed 12 out of 30 respondents stated that their cats had diarrhea (40%) after eating canned food (Table 1). Seven out of 12 cat owners (47%) stated that ocean fish flavor caused diarrhea in their cats. This is followed by tuna (13%), mackerel (13%) and assorted flavor (13%). Chicken and seafood showed 7% occurrence of diarrhea respectively. However, there was bacterial growth in all 15 canned food that were placed at room temperature. The bacteria that was frequently isolated from those canned food was *Proteus mirabilis*, (73%) followed by *Bacillus spp.* (13%), *Staphylococcus intermedius* and *Arcanobacterium spp.* with 7% respectively (Table 2). Canned foods stored in a fridge showed no bacterial growth after one until seven days.

Table 1. Occurrence of diarrhea among cats given canned food

Owner response	Frequency	Relative Frequency
Diarrhea	12	40%
No diarrhea	18	60%
TOTAL	30	100%

Table 2. Type of bacteria isolated from opened cat canned food

Bacteria	**Frequency**	**Relative Frequency**
Proteus mirabilis	11	73%
Bacillus spp.	2	13%
**S,intermedius*	1	7%
Arcanobacterium spp.	1	7%
	15	**100%**

**Staphylococcus intermedius*

Discussion

The cat canned food was free of bacterial growth which suggests that the processing method were carried out under hygienic condition. The canned foods were sealed in tin coated steel can and were subjected to heat treatment to kill food spoilage organisms. On the other hand, pet foods and manufacturing plants are subjected to inspection and regulations by Food and Drug Administration (FDA) to ensure the good quality and safe food products are produced. Food preserved by canning is at immediate risk of spoilage once the can has been opened. Poor handling and storage conditions may also result, over time to cause contents spoilage of food contents inside the can. Four different types of bacteria which are *Proteus mirabilis, Bacillus spp., Staphylcoccus intermedius and Arcanobacterium spp.* were isolated from exposed cat canned food. *Proteus mirabilis* was found to be the cause of skipjack tuna spoilage (Yoshinaga, 1982). *Bacillus cereus* is thermophilic bacteria of public health significance as it can cause food poisoning. Diarrhea can occur in cats that had been fed with contaminated canned food. Optimum temperature and availability of oxygen and nutrients can support bacterial growth in opened canned food. Canned food is a shelf stable product by which they can last longer if it is stored in clean, dry, cool cabinets to keep them at their best quality (Boyer, 2001). The need for good hygienic practices, proper handling, storage and retail of raw ingredients in clean environment and at refrigeration temperature should be emphasized to ensure good quality and safe canned food.

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Reliability of Total Haemocyte Count as Stress Indicator in Giant Tiger Shrimp (*Penaeus monodon*)

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Abstract

Stress is the main factor causing losses to the aquaculture industry including shrimp farming. It depresses the immunity of shrimp, increasing susceptability to disease and thus causing high mortality and morbidity. Stress in shrimp basically come from the management and farming activity such as transportation, handling, ablation of eye stalk for reproduction, anesthesia for mild to painful procedure and other practice. Previous reports have shown the reduction of total hemocyte count (THC) when shrimp was challenged with stress. In the present study, THC of giant tiger shrimp *(Penaeus monodon)* were compared between control group and 3 treatments i.e., subjected to unilateral eyestalk ablation, post 10% ethanol anesthesia stress and transport stress respectively. Statistical analysis showed that there was significant ($P<0.01$) difference for THC between control group and those with unilateral eyestalk ablation. Significant mean difference ($P<0.01$) was also seen in control group and those with post-anesthesia stress using 10% ethanol. However, there was no significant mean difference between control group and those subjected to transport stress.

Keywords: Penaeus monodon Total hemocyte count, transport stress, eyestalk ablation, stress indicator.

Introduction

Penaeus monodon is a marine crustacean that is popular for its firm meat texture. With two-thirds of *P. monodon* production coming from farming, mainly in South-East Asia, with over 900,000 tons of tiger shrimp consumed annually. However, despite the efforts and attention given to shrimp farming, the production and performance of *P. monodon* is still not satisfactory. Therefore, there is a need to reevaluate current practices and production management.

Animal health status is a very vital factor in the production and profit of aquaculture industry. However, the use of practical and reliable methods for a rapid analysis of shrimp health status is still uncommon among shrimp farmers. In this industry, the shrimp health is normally evaluated through reduction in growth, abnormal behavior or even widespread mortalities. Apart from infection, people always overlook the effect of stress when considering the shrimp health status. Certain culture practices frequently cause stress to the animals by means of physical (transport and eye ablation) or chemical (post anesthesia stress) means, which can result in a reduction of the shrimp immunological resistance and, thus, facilitate infections.

Eye ablation is a culture practice normally used in ovarian development in brood stock female shrimp. A complete ovarian maturation often ensues 3 to 10 days after unilateral eyestalk ablation, provided that other stress factors such as transport stress, temperature factor does not interfere. The latency period between the eye ablation and ovarian maturation depends on the readiness of the population and also the other concurrent stress factors. The theory behind this practice is that the gonad inhibitory hormone (GIH) which is produced in the neurosecretory complexes in the eyestalk is reduced through removal of the eyestalk. However, in addition to ovarian hypertrophy, other physiological function such as molting, osmotic and ionic balance and pigmentation in crustaceans are also affected and may contribute to major mortalities follow improper eyestalk ablation technique. Increase of susceptibility to infection following these stressful practices is also of great concern. Thus, the stress caused by the eye ablation needs to be evaluated for its negative effects.

Transport stress factor raises more industry concern nowadays as it is always blamed to be the root of other potential problem such as handling stress, heat stress, mechanical shock through vibration, and deterioration of water chemistry. Indicator of stress due to transport would be an interesting area to study.

Chemical stress factor, such as post anesthesia stress has always been neglected. However, it is one of the problems that should not be ignored. Anesthesia for crustacean can be induced either through cooling or use of chemical. Shrimp anesthesia is used prior to transportation or minor invasive procedure such as sample taking or eyestalk ablation. This practice is intended to reduce the movement of animals and also to calm down the animals. However, it is important to understand the stress that the anesthesia procedure imposes on the shrimp. For the post-anesthesia stress physiological mechanism is assumed to be similar to those in small animal anesthesia and no research has been done in this area.

The objective of this study was to evaluate whether total hemocyte count is a reliable and rapid stress indicator for shrimp that has been subjected to eye ablation, transportation and anesthesia.

Materials and Methods

Adult male and female tiger shrimps were purchased from Prawn Planet at Equine Park, and transferred to the Hatchery Unit of Aquatic Animal Health Laboratory, UPM. The shrimp were kept in seawater with salinity of 23.5% at stocking rate of 10 shrimps/ 20 L. The animals were kept in the laboratory for at least 12 hours prior to their use. Only apparently healthy animals with body length around 17cm were used in these experiments.

The shrimps were divided into four groups of 20 shrimp in each group: the first group (G1) was subjected to unilateral right eye ablation. The eye ablation technique commonly practiced by farmer was used whereby slitting one eye with a razor blade was done. Then crushing eyestalk, with thumb and index fingernail, beginning one-half to two-thirds

down the eyestalk and moving distally until the content of eyes was removed. Animals were evaluated one hour post treatment.

The second group of animals (G2) underwent 3 hours transport stress from UPM to Klang and back to UPM again. Animals were packed at high stocking rate (10 shrimps /10 L). Portable aeration was supplied along the journey to meet the high demand of oxygen for these animals. Animals were evaluated immediately post treatment.

The third group (G3) was anesthetized using ethanol 10% and was evaluated after their recovery from lateral recumbency. Absolute 100% ethanol was added into the seawater to obtain a solution at 10% concentration of ethanol. This concentration was chosen based on the results from pretreatment trial.

Group four (G4) composed of animals which were not treated and showed no stress signs. They were kept as the control for this experiment. Animals for G4 were selected randomly from the same stock with the 3 group animals stated above.

Shrimp hemolymph was collected by inserting a 25-gauge needle attached to a 1 mL syringe into the ventral sinus of animals, transferred to hematocrit tubes which contain sodium heparin to prevent clotting. The hemolymph was later transferred into a Neubauer Hemocytometer for examination under light microscope. This procedure was done with extra care to prevent debris or clot from entering the Neubauer chamber.

Total hemocyte count was determined using a Neubauer Hemocytometer under light microscope. The depth of the Neubauer chamber was 0.1 mm with area of 0.0025 mm^2 per square. The THC from two partition of Neubauer chambers were counted as A and B respectively to obtain the average value. From each partition, five chambers were counted to represent the whole partition. This is similar to the procedure for sperm count. The unit used was cell/mL. The formula derived was 50000 × average values from microscopic count.

Results and Discussion

Statistical analysis showed that there was a significant difference ($P<0.01$) for THC between control group and those from unilateral eyestalk ablation. Between control group and the group that underwent post anesthesia stress with 10% ethanol, there was also significant mean difference ($P<0.01$). There was no significant difference in mean THC between the group exposed to transport stress and the control.

From the results of this experiment, there was a significant mean difference in both eyestalk ablation and post anesthesia group compared to the control group. For the eyestalk ablation, the procedure as described by Perazzolo et al. (2002) was adapted. The significant mean differences for unilateral eyestalk ablation and post anesthesia group shows that THC is very suitable to evaluate stress conditions resulting from the two treatments. However, different individual may respond differently to stress in terms of THC. This can be explained by the different stage of growth (especially molting stage),

stress tolerance, surrounding factors, and health status of the shrimp. So, to set a certain standard mean value for stress-free shrimp as a guideline is tedious. Probably the THC as an indicator of stress can only be used for the same stock of shrimp. Whether the THC value is representative for the degree of stress is questionable as the actual physiological pathway that causes the reduction in THC has not being studied.

The results of the present study showed that eyestalk ablation and post anesthesia effect caused significant stress to the animals. There is a need to improve the induce spawning technique and anaesthesing the shrimp so as to reduce the stress factor carried by current practices.

For the third treatment group, which was exposure to transport stress did not show significant difference probably due to the following reasons. Firstly, there are no clear procedure on how to induce transport stress. In the present study, a car was used instead of a lorry. Maybe the shrimp should have been exposed in conditions with a vibrator or shaker at temperature similar to what the animals undergo during transport. In the present study the animals were transported in the morning to avoid heat stress and prior to transport they were not fed 12 hours to avoid deterioration of the water quality as what is being practiced by the farmer. Maybe these steps help to minimize the stress during transportation. Therefore, it is important for farmers to control the temperature and fast the animals prior to transport.

In the present study, the total volume of hemolymph was withdrawn from the ventral sinus. The hemolymph volume collected was found to be higher in the treatment group compared to control. This was a consistent finding for all the treatment groups. From this observation, the assumptive theory will be that the THC reduction was more on hemolymph volume rather than to the actual reduction in cell numbers. According to study by Guadagnoli et al. (2005) on changes in cardiac output and hemolymph flow during hypoxic exposure in the gravid grass shrimp, there was a redistribution of hemolymph flow away from main vessels that results in an enhanced hemolymph flow to the sternal artery that supplies the ventral segmental system, the gills, the buccal apparatus and the ventral nerve cord. According to Guadagnoli et al. (2005), this mechanism is more like a survival method for crustacean to cope with stress and life threatening situations. This finding raises the thought and consideration of using hemolymph volume as stress indicator which is less labour consuming and cheap.

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Histological Assessment of Blood Cockles (*Anadara granosa*) using Different Stains and Fixatives

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Abstract

Blood cockles (*Anadara granosa*) or locally known as 'kerang' are mollusc belonging to Bivalvia class. It is an important fisheries commodity and a delicacy in Malaysia. In RMK-9, one of the objectives of Malaysia's aquaculture industries is to maximize the production of bivalves up to 130,000 MT per year, which includes cockles, green mussels, clams and oysters. Cockles become one of the important bivalves cultured in Malaysia as it had very good market value in Malaysia, Thailand and Singapore. According to Phillips and Muttarasin (1985), 80% of the cockles marketed in Thailand were imported from Malaysia. In spite of the diversity and its economic importance, the knowledge of bivalves specifically cockle medicine should be developed in order to meet the requirement of diagnostic work which is necessary in determining the cause of diseases infecting the animals. Up to date, there is no comprehensive information of the histology of cockles. Thus, this study was undertaken to provide basic histological descriptions of normal and anomalies microstructures in *Anadara granosa* by using different fixatures and stains. Forty live cockles were divided into two groups; one group was fixed with Davidson solution and the other fixed with 10% buffered formalin. The tissues were then processed for 18 hours, and later embedded with paraffin wax and sectioned at 5 µm thickness. There were stained with four stains namely, Haematoxylin and Eosin, Masson's Trichome, Periodic Acid Schiff's and Van Gieson's stains. The sections were evaluated under a computer attached-compound light microscope at low and high magnification. The organs were assessed for microscopic structures staining affinity (Cox *et al.,* 2006). Haematoxylin and Eosin stain was considered the better stain to be used for mantle, foot complex and haemocytes tissues. Combination of Haematoxylin and Eosin and Periodic Acid Schiff's stains can be used to stain digestive system and palp. Gills could be stained with Masson's Trichome and Van Gieson's, while for gonads Masson's Trichome would be the preferred choice. Van Gieson's on the other hand, was the choice for foreign body detection. Gills of cockles could be divided into three parts: frontal, intermediate and abfrontal zone similar to *Mytella falcate* (David *et al*., 2008). The intermediate zone have a homogenous densely stained structure embedded in the filament which highly indicative that it provided supportive frame to the frontal zone.

The palp (Figure 2) consisted of ciliated cuboidal and columnar epithelia, which was postulated that this organ collected food particles from the gills, and helped in transferring to the mouth opening as in *Mercenaria mercenaria* (Eble, 2001). The haemocytes could be divided into agranular and granular types, similar to as reported by Cheng (1981). The mantle was postulated to play an important role in helping to move particles into the shell cavity due to the present of cilia along the epithelium cells

at the edges. The digestive gland appeared to be ciliated, as in *Mercenaria mercenaria*, thus its function might not as secretion gland solely, but its cilia helped to move food particles forward. The tall columnar epithelium of intestine was secured on thin basement membrane which was then directly connected to thick layer of connective tissue provided a "channel" for the hemocytes to reach the epithelium.

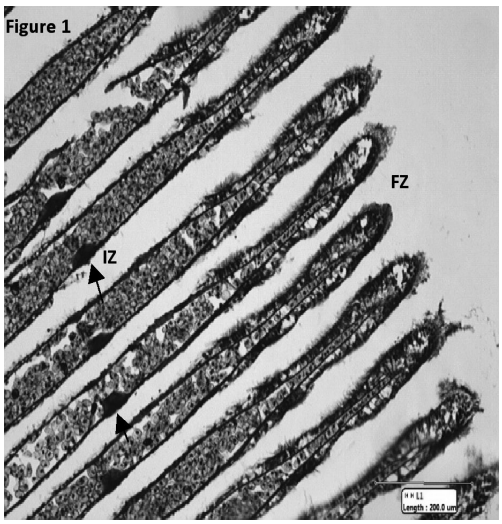

Fig. 1 The intermediate and frontal zone of gills filaments. The intermediate zone (IZ) with embedded structure (arrow). The frontal zone (FZ) consisted of tall columnar epithelium with cilia. Masson's Trichome, (×200).

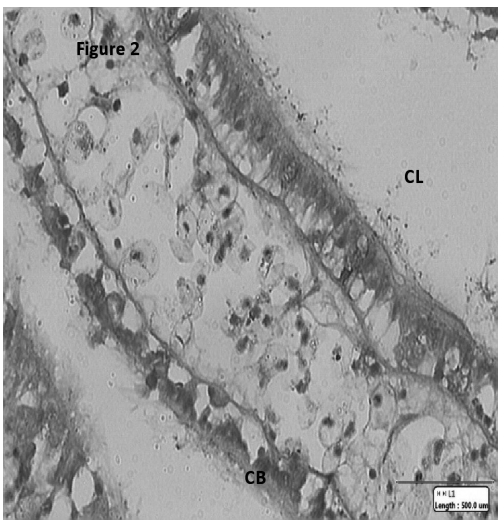

Fig.2 The palp filament which consisted of ciliated tall columnar epithelia with apex-located nuclei (CL) and ciliated cuboidal epithelia at the opposite side (CB). PAS, (×400).

The acini of male gonads were bulb-shaped, constructed of thick connective tissue and the gametes were seemed to fill up the lumen. This condition was similar seen in *Placopecten magellanicus* (Beninger and Pennec, 2006). In comparison to a study done by Broom (1983), the female gonads seen in this study were considered normal, healthy and not in spawning stage. The foot tissues were covered with tall columnar epithelium with large nucleus located at the base of the cells and rested on dense connective tissue. The muscle fibers were arranged multi directional which is suggestively due its important function in locomotion and burrowing.

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Muscle Fibre Typing, Collagen Composition Analysis of Breast and Thigh Meats in Two Breeds of Chicken of Different Growth Performance

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Abstract

The present study was done to evaluate the type of muscle fibre composition in thigh and breast muscle of Red Jungle fowl and Ross. It was also to evaluate the collagen composition in thigh and breast muscle of Red Jungle fowl and Ross. The histological appearance and size of the muscle fibres of Red Jungle fowl and Ross were evaluated. Four wild adult Red Jungle fowl and four market adult Ross chicken were used in this study. Breast muscle (pectoral) and thigh muscle (gastrocnemius) were examined. Three stains were used in this study and there were Myosin ATPase stain, Masson Trichrome stain and Haematoxylin and Eosin stain. Myosin ATPase stain was used to evaluate the muscle fibres type composition, Masson Trichrome stain was used to evaluate the collagen content of the muscle and Haematoxylin and Eosin stain was used to evaluate the histological appearance and size of the muscle fibres. Histologically, the thigh muscle of the Red Jungle fowl has higher proportion of muscle fibres type I to type II than Ross. There were no difference in proportion of muscle fibres type I to type II of breast muscle in Red Jungle fowl and Ross. The thigh and breast muscle of Red Jungle fowl has high collagen content while Ross chicken's thigh and breast muscle has less collagen content. The size of muscle fibre of Red Jungle fowl was small while that of Ross was big.

Keywords: Red Jungle fowl, Ross, myosin ATPase stain, Masson trichrome stain, histology

Introduction

Jungle fowl is considered to be the ancestor of all the domestic chicken (Collias and Saichuae, 1967). Ross is one of the domestic breed of commercial broiler chickens and known for its broad chests, yellow feet, and a ferocious appetite that enables them to grow at rapid paces. Modern broilers are typically fed with high quality formulated diet to increase muscle growth hence increase the body weight.

Different types of system production and feed may attribute to the differences in meat quality between the Red Jungle fowl and the commercial broiler which may be related with the development states of the collagen content and muscle fibres type composition. The muscular structure of the Red Jungle fowl is believed to have different structures in term of collagen content, types of muscle fibre and muscle fibre size microscopically compared to the Ross chicken.

Materials and Methods

Four wild adult Red Jungle fowls and four commercial broiler chicken (Ross) 56 days old were used in this study. Upon euthanasia, the breast muscle (pectoral) and thigh muscle (gastrocnemius) were taken for examination. Three stains were used in this study namely the myosin ATPase stain, Masson's trichrome stain and haematoxylin and eosin (H&E) stain. myosin ATPase stain was used to evaluate the muscle fibres type composition, and helped to differentiate the Type I muscle fibre from Type II. Masson's trichrome stain was used to evaluate the collagen content of the muscle, while the H&E stain was used to evaluate the histological appearance and size of the muscle fibres. For myosin ATPase stain, the muscle sample was snap frozen and then sectioned into 15 µm. The sample was then stained using myosin ATPase. For Masson's trichrome and H&E stain, the sample was first fixed in 10% formalin and then sent for tissue processing. Next, the sample was sectioned using microtome at 3 µm thick and lastly they were stained with Masson's trichrome and H&E stains. For myosin ATPase, type I muscle fibre was stained dark while type II fibre stained light in colour. Ten muscle bundles from one section of each muscle were randomly selected. Mean of the proportion of Type I to Type II muscle fibres were calculated. For Masson's trichrome stain, the muscle fibre stained red while collagen stained green in colour. The distribution of collagen fibers was scored. For H&E, muscle fibre was stained pink in colour. The diameter of six muscle fibers from 5 muscle bundles were chosen randomly and the measurement was taken. Mean of the diameter of one muscle bundle was calculated.

Results and discussion

Histologically, the thigh muscle of the red jungle fowl has higher proportion of muscle fibres type I to Type II than Ross. There were no different in proportion of muscle fibres type I to type II of breast muscle in red jungle fowl and Ross. The proportion of type I to type II muscle fibres of thigh muscle was higher than breast muscle in Red Jungle fowl. This suggests that Red Jungle fowl tend to walk rather than flying.

The thigh and breast muscle of Red Jungle fowl has higher collagen than Ross chicken. This may be due to the differences in muscle fibres size of the chicken. Ross chicken has bigger muscle fibres size than Red Jungle fowl for breast and thigh muscles.

Ross chickens were kept under the intensive system such as close house system or caged system while Red Jungle fowl is free range chicken (Shaik Mohd Amin Babjee, 2009). Under close house system, the activity of the Ross chicken was controlled and they have lesser movement under this system. In addition, their activity was controlled by the lighting system. Red Jungle fowl has better movement under free range system, and thus they have more collagen content in the muscle.

Unlike Red Jungle fowl, Ross chickens do not need to travel to find for the food, and they are normally given high energy feed to improve the meat production. This leads to the increase of the body weight and also the increase of fat deposition.

Ross chicken has better performance than Red Jungle fowl because its performance has been upgraded by crossing with stocks with higher production. The presence of more giant fibres in the muscles of fast growing chicken meat could be considered as one of the side-effects of genetic selection.

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Assessment of 1-month Conditioning Program Practised by Equine Establishment in Conditioning Endurance Horses

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Abstract

Fitness indicators in horses are physiological variables such as V_{140}, V_{200}, V_{LA2} and V_{LA4}. Endurance conditioning increases fitness level of horses. Fitness levels of 16 endurance horses from 3 different establishments practicing a similar conditioning regime were evaluated after a 1-month of conditioning. A standardized exercise test was carried out for all horses prior and after conditioning. Heart rates were monitored and blood samples were obtained throughout the exercise test and blood samples were then analyzed for lactate, cortisol, and muscle enzymes. Paired sample T-tests between pre and post conditioning were performed to evaluate the effects of 1-month conditioning program on these physiological variables. Results showed that there were no improvement in V_{140}, V_{200}, V_{LA2} and V_{LA4} after a 1-month conditioning program, however exercise velocity in Run 1 and AST in Run 2 were significantly different. Therefore, it was concluded that the 1-month conditioning program practiced was insufficient to increase the fitness levels of horses due to its short period and ineffective conditioning program.

Keywords: endurance horses, standardized exercise test, fitness indicators

Introduction

Equine exercise physiology is defined as the study of equine's body system to adjust, adapt and respond to exercise (Marlin, et al., 2002). Principles of endurance conditioning program consist of long slow distance (LSD) conditioning to build up the aerobic capacity, strength conditioning consisting of low velocity but high intensity exercise nearly anaerobic threshold such as hill work to improve both aerobic and anaerobic capacities, and fartlek or interval conditioning as recommended in fast exercise conditioning (Ridgeway, 1994) to reduce muscular injuries and less stress to horses due to continuous exercise of long distance.

This study was conducted to compare fitness level of endurance horses after a 1-month conditioning program and to evaluate the conditioning program by comparing the responses of exercise velocity, heart rate, blood lactate, cortisol and muscle enzymes of endurance horses.

Materials and Methods

Animals

Sixteen clinically healthy horses were used in this trial. Horses were from three different establishments practicing a similar conditioning program.

Standardized Exercise Test

Standardized Exersice Test (SET) was conducted using a 500-m flat straight track consisting of four incremental exercise velocities. Heart rate was continuously recorded during SET by a POLAR Equine S610i heart rate monitor. Blood samples were obtained through jugular venipuncture, then centrifuged and analyzed for lactate, cortisol and muscle enzymes. Fitness parameters of heart rate (V_{140}, V_{200}) and lactate (V_{LA2}, V_{LA4}) were determined by a graph of heart rate and lactate over velocity.

Statistical Analysis

Paired sample T-test was used to compare velocity, heart rate, lactate, cortisol, muscle enzymes and fitness parameters prior and after 1-month conditioning.

Results and Discussion

Velocity

There was only significant difference at Run 1 during SET, where the mean post-conditioning value (6.37 ± 0.82 m/s) was significantly higher than the pre-conditioning value (5.77 ± 0.94 m/s); however there were high mean values in post-conditioning observed. This was due to physiological adaptations, leading to a decrease in cortisol secretion due to a decrease in responsiveness to adrenal cortex (Marc, et al., 2000) and improvement of gait velocity, thus resulting in larger step.

Heart Rate

There was no significant difference in heart rate, however the mean values of post-conditioning were observed to be lower at rest (36 ± 6 bpm), Run 1 (130 ± 15 bpm) and recovery (50 ± 6 bpm). Rapid recovery heart rate is a good indicator of fitness in endurance horses (Marlin, et al., 2002); however the conditioning program was insufficient to produce significant difference

Lactate

Mean value of lactate was higher in post-conditioning (8.73 ± 5.51 mmol/L) compared to pre-conditioning (6.46 ± 4.27 mmol/L). Furthermore there was a significant difference during recovery. This is due to positive correlation of lactate to velocity, resulting in increased post-conditioning lactate values.

Cortisol

There was no significant difference in cortisol level, however a trend of lower mean values in post-conditioning was observed during SET. Cortisol showed similar trends as heart rate and lactate, where it was found to be positively correlated with velocity (Masahiko et al., 1998).

Muscle Enzymes

There was significant difference observed in Run 2, where pre-conditioning AST value (333.7 ± 89.1 U/L) was higher than post-conditioning AST value (286.4 ± 37.3 U/L). CK showed a similar pattern as lactate, with the only significant difference observed was during recovery phase. It seemed that the conditioning program practiced was formulated with strenuous conditioning and insufficient duration to improve aerobic capacity.

Fitness Parameters

There was no significant difference among the four fitness parameters; furthermore the mean values of these parameters were lower in post-conditioning compared to pre-conditioning.

Conclusion

It is concluded that conditioning program practiced by these equine establishments was insufficient and ineffective to improve fitness levels of endurance horses.

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A Retrospective Study on Equine Cases Referred to University Veterinary Hospital, UPM from 2005-2009

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Abstract

A retrospective study was carried out to determine the prevalence and total number of equine cases reported to University Veterinary Hospital (UVH), UPM from year 2005 to 2009 and to determine the relationship between case occurrence and type of purpose of horses. Data were gathered from UVH records. A total of 1,521 equine cases were recorded from 22 horse establishments, involving eight different purposes of horses with eight and 30 categories of general and specific clinical problems, respectively. The occurrence of equine cases in year 2005 was 228 (15.0%), 2006 was 216 (14.2%), 2007 was 372 (24.5%), 2008 was 340 (22.4%) and 2009 was 365 (24.0%). The most common general clinical problems were musculoskeletal problems, traumatic injuries and gastrointestinal problems. However, based on specific conditions, traumatic injuries showed the highest percentage of occurrence within the 5 years.

Keywords: retrospective study, equine cases

Introduction

The equine industry in Malaysia is expanding due to an increase in the number of imported and local horses throughout the country as well as to an increased number of equestrian sports. Most horses owned by individual owners are used for leisure rides with occasional participation in equestrian events such as show jumping, dressage, eventing and endurance. Horses owned by private clubs are commonly used for riding schools and equestrian sports while horses owned by government bodies are mainly used for patrolling and ceremonial events. This project was carried out to determine the prevalence of equine cases referred to University Veterinary Hospital (UVH), UPM from year 2005 to 2009, to determine factors contributing to the number of new cases referred and to identify the most common clinical problems in horses under local conditions.

Materials and Methods

Data were obtained from case records of UVH, UPM from year 2005 to 2009. The information includes case number, horse identification, horse establishment and clinical problems. Descriptive analysis was carried out on all data obtained. Additional information on the availability of clinicians on duty and dates of horse competitions and events from year 2005 to 2009 were gathered through verbal communication with equine clinicians.

Results and Discussion

Total Number of Cases 2005 to 2009

A total of 1,521 of equine cases were referred to UVH, UPM from year 2005 to 2009. The highest number of cases was in year 2007 with 372 (24.5%) cases followed by 2009 with 365 (24%) cases and 2008 with 340 (22.35%) cases. The prevalence of equine cases from year 2005 to 2009 showed no significant pattern between the years. However, there was an increased number of cases coming into UVH due to additional number of equine clinicians in UVH.

Cases Based on Horse Establishment

A total of 22 equine establishments were registered as UVH clients from year 2005 to 2009. Twelve horse establishments were owned by the government bodies, such as Dewan Bandaraya Kuala Lumpur (DBKL) at Cheras, Titiwangsa, and Kiara, Polis Di-Raja Malaysia (PDRM) at Cheras, Putrajaya and Kiara, Majlis Perbadanan Klang (MPK), Institut Sukan Negara (ISN), Angkatan Tentera Malaysia (ATM), MARDI, Penjara Kajang and Universiti Utara Malaysia (UUM), Kedah. There were a total of eight horse establishments that were owned by private bodies: El-Mina Sg Buloh, Templer Park, ARL Putrajaya, Ar-Raudhah, Penn Endurance, Ranhill, Tun Mahathir and individual owners (horse owners with less than five horses). Universiti Putra Malaysia (UPM) owned two other establishments, which were the UPM Equine Centre and UVH stables. The highest number of cases admitted were horses owned by individual owners with 256 (16.83%) cases and from PDRM Cheras with 186 (12.23%) cases, followed by DBKL Titiwangsa, Ar-Raudhah and UPM Equine Centre with 131 (8.61%), 127 (8.35%) 121 (7.96%) cases, respectively. Although there was a positive relationship between total population of horses and the number of cases attended at a particular establishment, it was observed that few establishments with smaller horse population had a high number of clinical cases indicative of improper management either in taking care of the horses or the horses were overtrained.

Cases Based on Types of Work

The purposes of the horses were categorized into eight groups. There were patrolling, endurance, polo, riding school, dressage, show jumping, leisure and multipurpose (horses used for more than one purpose). The highest number of cases involved the patrolling horses with 495 (32.54%) cases, followed by multipurpose horses with 329 (21.63%) cases and endurance horses with 233 (15.32%) of the total number of cases. The percentage of cases was actually positively correlated with the number of horses involved based on different purposes.

Cases Based on General Clinical Conditions

There were 11 groups of general clinical conditions based on body systems. The highest number of cases was musculoskeletal problem with 551 (36.2%) cases, traumatic injury with 298 (19.8%) cases, followed by gastrointestinal problem with 166 (11.7%) cases. Urinary and reproductive problems were groups with the least number of cases with the

total of 3 (0.2%) and 7 (0.5%) cases, respectively. Other cases with frequency of less than five cases throughout the five years were categorized in the miscellaneous group, representing 13 (0.9%) cases. Similar findings were reported by other studies (Kaneene et al., 1997; Egenvall et al., 2006) and lameness was the most common diagnosis in these equine cases.

Cases Based on Specific Clinical Conditions

There were 30 specific clinical conditions identified. The highest percentage were traumatic injury with 298 (19.6%) cases, non-specific musculoskeletal problems with 195 (12.8%) cases, followed by colic with 166 (10.9%) cases. Musculoskeletal problem was subdivided into nine groups. The highest number of cases was represented by the non-specific musculoskeletal problem with 195 (36.04%) cases, followed by foot problem 79 (14.60%) cases, join problem with 68 (12.57%) cases, tendinitis with 66 (12.20%) cases, lymphangitis with 50 (9.24%) cases, back pain with 37(6.84%) cases and myositis, saddle sore and fracture with 29(5.36%), 13(2.40%) and 4(0.74%) cases, respectively. The second major clinical problem occurred in horses was skin problem that was subdivided into five groups, which were dermatophilosis with 39 (2.56%), habronemiasis with 10 (0.66%), hypersensitivity with 68 (4.47%), proud flesh with 21 (1.38%) and Queensland itch with 17 (1.12%) cases.

Conclusion

Percentage of cases referred to UVH, UPM increased in year 2007 (24.5%), 2008 (22.8%) and 2009 (24.0%) compared to year 2005 (15.0%) and 2006 (14.2%). Increased number of new cases was associated with the increased population of horses, resulting in increased number of UVH clients and subsequently due to the availability of clinicians on duty. There was a positive relationship between the number of cases referred to UVH and the occurrence of horse competitions and events resulting to an increase incidence of clinical problems. The major clinical problems in horses under local conditions were associated with musculoskeletal problems (35.63%), traumatic injuries (19.59%) and gastrointestinal problems (11.70%).

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Histopathology of Goldfish (*Carassius auratus*) exposed to Chlorine Toxicant

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Abstract

Chlorine is widely used as disinfectant in Malaysian fish farms. As we know chlorination as a method for water disinfection is practiced by most water municipal treatment in Malaysia (Abdullah et al., 2003) due to its efficiency and cost effectiveness. Like many toxins in water, chlorine is more toxic to fish than humans (Mahjoor and Loh, 2008). Thus, the objectives of the current study were to assess pathological changes in organs, to ascertain behavioral response of goldfish when exposed to acute chlorine toxicity and to determine the Median Lethal Concentration (LC_{50}) of free chlorine. Juveniles of Oranda goldfish, *Carassius auratus,* were exposed to chlorine in a 96 hour static renewal test to determine the LC_{50} of free chlorine. The goldfish behavioral responses were recorded. Moribund fish were promptly sacrificed and processed for histopathology. Using SPSS 16.0 probit analysis, the LC_{50} of free chlorine in juvenile goldfish was determined to be at 0.3 ppm. Goldfish showed signs of dyspnea, lethargy and increased mucus production. Histopathologically, the gills indicated secondary lamellar edema, exfoliation of interlamellar cells' membrane and congestion in the capillary lumen. Kidney parenchyma manifested generalised degeneration, but with marked desquamation of tubules and dilated tubular lumen in the posterior kidney. Spleen showed congested blood vessels and hemosiderosis while liver showed some vacuolative changes in the parenchyma suggestive of liver necrosis. Even though the findings in gills, liver and kidney were not specific for chlorine toxicity, hemosiderosis in the spleen could be used to differentiate with other toxicities and diseases. Current findings were in agreement with earlier report by Mahjoor and Loh (2008) and also supported Zeitoun (1977) observation that the cause of death in chlorine toxicity was due to hemolytic anemia.

Keywords: Goldfish, LC_{50} free chlorine, behavioral response, histopathology

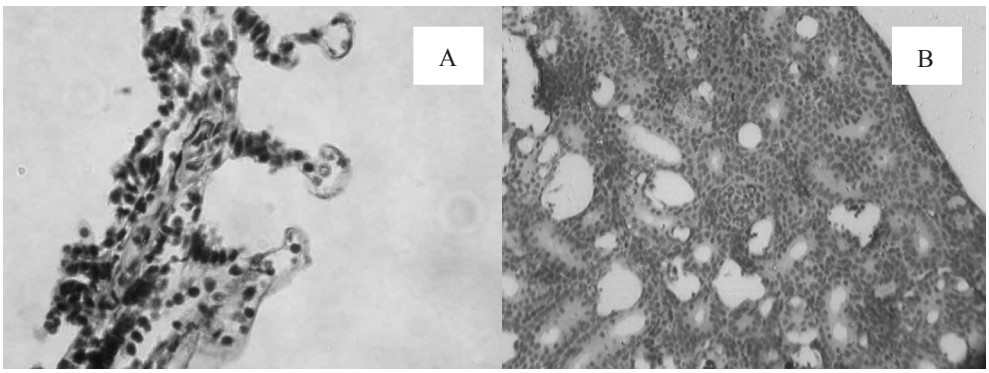

Figure 1. (A) Gills of goldfish bathed in 1ppm free chlorine showing edema at the tip of primary gills filament. (B) Posterior kidney from fish exposed to 1ppm showing formation of vacuoles of various shapes and sizes. (×400), H&E.

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Quality of Salvaged Epididymal Spermatozoa in Local Dogs

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Abstract

The quality and quantity of epididymal spermatozoa and their relationship to scrotal circumference and testicular weight for local dogs in Malaysia were successfully determined. The longevity of spermatozoa in an alternative storage medium was also determined. The salvage of epididymal spermatozoa was done in twenty-two humanely euthanised adult local dogs. The overall mean ± SEM of the total number, general motility, progressive motility, and percentage live and abnormal spermatozoa of cauda epididymal spermatozoa salvaged per dog were 408 ± 55.31 x 10^6, 80.91 ± 1.84%, 46.25 ± 3.34%, 72.43 ± 1.47% and 6.80 ± 0.95%, respectively. The scrotal circumference and testicular weight were not appropriate indicators of the number of salvageable cauda epididymal spermatozoa as indicated by moderate correlation coefficients of 0.545 and 0.546, respectively. There was a significant difference ($P<0.05$) in general motility, progressive motility and live percentage of spermatozoa during storage (4-8ºC) between an extender based on 0.9% NaCl and a commercial canine semen extender over time. However, for the first 2 days, these parameters were similar in value. Therefore, 0.9% NaCl should be further investigated as a potential medium for short term storage of epididymal spermatozoa.

Keywords: canine, epididymal spermatozoa, scrotal circumference, testicular weight, extender

Effect of Hypoxia on the Response of Canine Mammary Gland Tumor Cells to Bovine Lactoferrin, Doxorubucin and Recombinant Human Erythropoietin

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Abstract

The exact role of hypoxia in tumor biology remains controversial because there is no conclusive evident on its effect on tumors. There is concern that tumor hypoxia is one of the causes of chemoresistance in cancer cells. Recently, erythropoietin receptors have been found in human breast cancer cells indicating that recombinant human erythropoietin (rHuEPO) treatment of cancer-related anemia can influence the functions of the cells. Bovine lactoferrin (bLF) was also shown to have antiproliferative effects on cancer cells. The aim of this study was to determine the *in vitro* effects of rHuEPO, bLF and Doxorubicin (DOX) on a canine mammary gland tumor cell line, CMT-stylo cells, under hypoxic condition. The cells were treated with bLF, rHuEPO, DOX rHuEPO and DOX and bLF, rHuEPO combinations. These treated cells were subjected to MTT assay. The results showed that hypoxia lowered the proliferation rate of the CMT-stylo cells while combination treatments showed improved killing. Flow cytometry analysis showed that DOX had cytotoxic while bLF had antiproliferative effects on the CMT-stylo cells.

Keywords: hypoxia, canine mammary gland tumor, MTT assay, flow cytometry, Doxorubicin, bovine lactoferrin, recombinant human erythropoietin.

Effect of Recombinant Human Erythropoietin and Bovine Lactoferrin on Canine Mammary Gland Tumor Cell

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Abstract

Adjuvant chemotherapy is recommended for metastatic canine mammary gland tumor. Erythropoietin receptors (EPOR) was once thought to be only expressed on the surfaces of the erythroid progenitor cells. Recently, EPOR have been identified in several neoplastic cell lines and solid tumors including human and canine mammary gland tumors. Bovine lactoferrin (bLF) has several biological activities, including anti-tumor effect on some human and animal tumors. Clinical trials have been carried out in human medicine based on these effects. In this *in vitro* study, doxorubicin, recombinant human erythropoietin (rHuEPO) and bLF were used separately and in combination in order to determine the effect of different drugs on canine mammary gland tumor. Recombinant human erythropoietin was found to have a non-significant effect on the canine mammary gland tumor cell line. Doxorubicin alone gave a more promising result in cytotoxic effect of the cells in a dose-dependent manner. Bovine lactoferrin however did not show a clear anti-proliferative pattern on the tumor cells. The drug combination treatment did not show better anti-proliferative or cytotoxic effect on the cells than doxorubicin alone. The combination of these drugs induced growth arrest at G_2/M phase.

Keywords: canine mammary gland tumor, erythropoietin, bovine lactoferrin, doxorubicin

Prevalence of Canine Babesiosis among Stray Dogs in Kuala Lumpur and Risk Factors of Hypoglycemia in Canine Babesiosis

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Abstract

A study was conducted to review the importance of measuring blood glucose levels in canine babesiosis as hypoglycaemia is a sign of profound metabolic derangement and its occurrence and possible risk factors can aid in determining the severity of the disease. A total of sixty stray dogs at Dewan Bandaraya Kuala Lumpur, were included in this study. Thin blood films from the marginal ear vein of each dog were made and stained with Giemsa stain and observed under the light microscope to detect the *Babesia* organisms. Four out of the 60 stray dogs (6.7%) were found to be positive for canine *Babesia;* 5.0% (3/60) were *Babesia canis* positive and 1.7% (1/60) *Babesia gibsoni* positive. Blood was collected from the infected dogs and packed cell volume was measured. Serum was obtained and serum glucose levels were determined. Two of the *Babesia* positive dogs were anemic and two of the positive dogs were hypoglycemic. Risk factors of hypoglycemia could not be evaluated as the number of positive dogs was too low and the results would have been inconclusive.

Keywords: canine babesiosis, *Babesia canis, Babesia gibsoni*, thin blood films, hypoglycaemia

A Retrospective Study of Feline Lower Urinary Tract Disease at University Veterinary Hospital, Universiti Putra Malaysia

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Abstract

Being one of the most commonly presented diseases in University Veterinary Hospital, the study of the common criteria that predispose a cat to feline lower urinary tract disease (FLUTD) is essential. Although there were two retrospective studies carried out previously at the University Veterinary Hospital on FLUTD, this study however had a value-added aspect whereby the common urinalysis findings of those affected cats were studied. Additionally, comparisons of the common predisposing criteria between this study and the previous studies were also done. In this study, medical records of FLUTD cases presented to University Veterinary Hospital, UPM from 2005 to 2008 were selected. The collected data were analysed using descriptive analysis. From this study, there was no specific trend of the occurrence of FLUTD. As the cases were tabulated according to their months of occurrence there was no specific trend as well. Intact male cats had the highest percentage being affected. Domestic Short Hair and indoor lifestyle cats were the most commonly affected. Middle-aged cats with ideal body weight commonly succumbed to the disease. Basically, most of the presented cats were fed with commercial dry food. Stranguria/dysuria was the most commonly observed clinical signs suggestive of obstructed FLUTD. As for the urinalysis findings, most cats had abnormal urine colour, neutral urine pH, 4+ RBC and 2+ WBC. The most commonly observed crystal was triple phosphate followed by amorphous urates and calcium oxalate. With this knowledge, the owners should be well informed whether their cats are at risk of getting FLUTD or not.

Keywords: amorphous urates, calcium oxalate, descriptive analysis, FLUTD, stranguria, dysuria, urinalysis and triple phosphate.

Neem (*Azadirachta indica)* Oil as an Anthelmintic in Goats

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Abstract

Helminth infection is a major factor for reduced productivity in many small ruminant industries. In an attempt to control the helminth problem, chemical anthelmintics are often used widely for the purpose of prophylaxis and treatment of helminthes. Development of resistance towards common chemical anthelmintics due to frequent or improper usage necessitates the study of various plants for their potential anthelmintic properties. Further, there is a high demand for herbal products as an alternative to chemical anthelmintics. *In vivo* study was conducted to evaluate the efficacy of *Azadirachta indica* (Neem) oil which was obtained commercially against *Haemonchus contortus.* Twelve female Boer goats from a private farm were flushed with a commercial anthelmintic a month before the study and then they were equally divided into control (n=6) and treatment (n=6) groups. Faecal egg counts using the Modified McMaster technique and the FAMACHA score for assessing anemic signs were carried out weekly for six weeks. The result of faecal egg count showed that there was no significant reduction in the treated group indicating no difference between the control and treatment group. The present study indicated that giving neem oil orally had no effect on adult worms in the abomasums as reflected in the faecal egg counts. Further studies need to be done to reconfirm the effect of neem oil as an alternative anthelmintic in goats.

Keywords: anthelmintic, *Azadirachta indica*, *Haemonchus contortus,* goats

Induction of Immunosuppression by Benzo (a) Pyrene in Broilers

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Abstract

Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon which has shown carcinogenic, teratogenic, mutagenic and claimed immune-suppressive potentials. However, the exact mechanism whereby BaP induces such immunotoxicity is not fully explored. The effect of BaP on the immune system and morphology of selected lymphoid organs of broilers were investigated. Forty day-old chicks were divided into control and BaP groups compromising of 20 birds each. The control group was instilled with tricaprylin only while the other received 15 mg BaP/kg intra-tracheally for 5 consecutive days. Live ND vaccine (La Sota strain) was given on Days 7 and 21 to all chickens. Five chickens from each group were sacrificed via cervical dislocation and the liver, lung and lymphoid organs were collected at Days 7, 14, 21 and 35. Tissues were subjected to assay for cytochrome P450 (CYP1), SOD and MDA. In this study, marked expression of CYP1 in the broilers denoted the sensitivity of broilers to BaP exposure. Such expression has in turn led to oxidative stress which led to immune-supression via damage of lymphoid organs. This warrants judicious assessment of poultry health status with respect to the occurrence of haze or air pollution episodes. Effective immune-modulatory strategies will render adequate flock health and translate maximum profit to the farm.

Keywords: broiler, benzo(a)pyrene (BaP), immunosuppression, oxidative stress, lymphoid organ morphology

Immunosuppressive Effects of Benzo (a) Pyrene on Newcastle Disease Vaccination in Broilers

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Abstract

Newcastle disease (ND) vaccination was used as a model in assessing the role of benzo(a)pyrene (BaP) in inducing immunosuppression. Forty day-old chicks were divided into a control and BaP group comprising of 20 birds each. The control group was instilled with tricaprylin alone while that of BaP received 15 mg BaP/kg intratracheally for 5 consecutive days. Live ND vaccine was given on Day 7 and repeated again on Day 21 to all chickens. Prior to post mortem, blood was collected from five chickens from each group at Days 0, 7, 14, 21 and 35 for determination of HI titer. At necropsy, liver and lung samples were procured for the determination of glutathione transferase (GST), glutathione peroxidase (GPx) and malionaldehyde (MDA) activities. The BaP group gained a slower immune protective level compared to that of the control (21 versus 7 days). The hepatic and pulmonary GST activity and MDA level of the BaP group demonstrated an increment until Day 14 p.i. which then tapered towards the end of study. However, the GP_X activity was only invoked towards that later stage of the experiment. Likewise, it was also shown that the GST and GP_X activities were negatively correlated. Thus, this study unveiled that the metabolism of intra-tracheally instilled BaP brings about systemic oxidative stress which induces immunosuppression in broilers.

Keywords: broiler, ND titer, GST, GP_X, MDA level

A Retrospective Study of Acquired Canine Thrombocytopaenia

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Abstract

Acquired canine thrombocytopaenia is a common canine haemostatic disorder in veterinary medicine. The purposes of this study were to investigate patterns of thrombocytopaenia in 2007 and 2008 on monthly and yearly basis, respectively, in the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM), to study the aetiology of acquired thrombocytopaenia, to describe signalment, management, and disease preventive measure of the thrombocytopaenic dog, and lastly to relate significant findings of the haematological and biochemical parameters to the thrombocytopaenia. The data were obtained from Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, UPM and the patient medical records were obtained from UVH. All data were reported based on a descriptive analysis. Results showed the numbers of dogs with acquired thrombocytopenia were 67 and 69 in 2007 and 2008, respectively. Most of the cases were reported in January 2007, and May and December 2008. Acquired canine thrombocytopenia occurred in any sex or breed. The most significant haematological and biochemical findings of the thrombocytopaenic dogs were regenerative left shift, regenerative anaemia, and hyperglobulinaemia. The aetiology of acquired thrombocytopaenia in dog was mostly from infection-and inflammation-associated diseases. In conclusion, thrombocytopaenia is a prevalent and potentially important diagnostic finding in a variety of disease states.

Keywords: acquired, aetiology canine, retrospective and thrombocytopaenia.

Reproductive Performance of Kedah-Kelantan Cattle at Pusat Ternakan Haiwan Pantai Timur, Malaysia

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Abstract

Data between 1996 and 2009 at Pusat Ternakan Haiwan Pantai Timur, Malaysia were used to analyse the age at first calving, age at conception, calving interval and mean birth weight of Kedah-Kelantan (KK) cattle. Monthly rainfall from 2006 to 2008 were also analysed to determine the correlation between rainfall and calving rate and calf mortality rate. The results indicated that the mean age at first calving was 1146 ± 148 days, the mean age at conception was 858 ± 148 days, the mean for first, second, and third calving interval were 403 ± 137, 389 ± 116, and 376 ± 90 days, respectively. The result also showed that the calving interval subsequently decreased with increasing parity. No significant differences were found between the three calving intervals ($P > 0.05$). The mean birth weight was significantly different between female (12.93 kg) and male (13.97 kg) calves ($P < 0.05$). There was a weak correlation between the calving rate and monthly rainfall pattern ($r = 0.26$) and between mortality of calves below 3 months of age with rainfall pattern ($r = 0.17$). The results also showed that there was low negative correlation between mortality rate of calves below 6 months of age ($r = -0.28$) and total mortality ($r = -0.04$) with rainfall. In conclusion, Kedah-Kelantan cattle showed very good reproductive performance and are suitable for commercial beef production in Malaysia.

Keywords: Kedah-Kelantan, age at first calving, age at conception, calving interval, calving rate, mortality rate

Prevalence of Endoparasites in Village Chicken (*Gallus gallus domesticus*) and Wild Jungle Fowl (*Gallus Gallus Spadiceus*)

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Abstract

Thirty apparently healthy chickens comprising 15 adult wild jungle fowl (Gallus gallus spadiceus) and 15 free-ranged village chickens (Gallus gallus domesticus) were obtained from Jenderam Hulu in the district of Sepang, Selangor, Malaysia. From these samples, 3 of the village chickens were kept in a closed house system whereas the other village chickens were free ranged. All the wild red jungle fowls were caught from the Jinjarum in the district of Banting, Selangor. The nematodes species recovered from the necropsied 15 wild jungle fowls were *Oxyspirura manson* (32.6%) with mean intensity of 13.7 and *Lemdana sp*.(1.5%) with mean intensity of 3. *Capillaria sp* (0.5%) with mean intensity of 1.5. *Tetrameres sp.*(4.8%) with mean intensity of 6.5 and *Heterakis gallinarum* (10.85%) with mean intensity of 4.6. The cestodes recovered were *Raillietina sp.*(20.4%) with mean intensity of 11.1 and *Hyemenolepis sp.* (1.84%) with mean intensity of 1.03. *Tanaisia sp.*(26.5%) is the trematodes recovered with mean intensity of 26.5 and there is also the presence of *Acanthocephala sp.*(1.5%) with mean intensity of 2.25 in this group of chicken. In 15 village chicken, nematodes found were *Oxyspirura mansoni* (55.7%) mean intensity of 3.5*, Lendana sp.* (1.47%) with mean intensity of 31.3. *Syngamus trachea* (0.9%) with mean intensity of 2.5*, Capillaria sp* (0.7%) mean intensity of 1*, Tetrameres sp.* (4.8) mean intensity of 8.7*, Ascaridia sp.*(2.6%) mean intensity of 3.5 *and Heterakis gallinarum* (15.6%) with mean intensity of 8.5. The cestodes that were recovered were only *Raillietina sp.* (15.3%) with mean intensity of 8.7 and the trematodes were *Tanaisia sp.*(1.8%) with mean intensity of 10. From the results obtained, there was no significant difference in the endoparasites seen in village chicken and wild jungle fowl based on the T-test (P>0.05). The most common parasites in these two groups of chickens were the nematodes species *Heterakis gallinarum.*

Keywords: wild Jungle Fowl, village chicken, nematodes, cestodes, trematodes.

Semen Evaluation in Jungle Fowl, Domestic Chicken and Ayam Serama

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Abstract

This research was conducted to investigate variation in semen quality of three chicken breeds. Nine cocks comprising 3 cockerels each of jungle fowl, domestic chicken, and ayam serama were used in this project. Semen was collected once a week by abdominal massage method. The semen was evaluated for volume, colour, wave pattern, general and individual motility, concentration, live and, abnormal percentage and sperm size. No significant differences were observed in volume of semen among all three breeds ($P>0.05$). Creamy and milky colour of semen were observed for jungle fowl and domestic chicken, whereas for ayam serama the color was watery. There were significant differences ($P< 0.05$) between semen concentration in jungle fowl ($94.44 \times 10^9 \pm 905.3$ sperms/mL) and ayam serama ($1.83 \times 10^9 \pm 743$ sperms/mL). For general motility, no significant ($P >0.05$) differences were observed among these three breeds. The types of individual motility observed and analyzed were forward, rotating, vibrating, and backward. Jungle fowl had highest forward motility and lowest for rotating motility. There were no significant ($P >0.05$) differences in term of sperm size between jungle fowl and the other two breeds. All three breeds had total live spermatozoa of more than 90%. Six sperm defects were observed: macrocephalic, mid piece knotting, bend head, plasma droplet, spermatid and bend tail. It was concluded that there were significant ($P >0.05$) differences between the three cock breeds in semen color, concentration, individual sperm motility particularly forward and rotating motility, size of sperm and total abnormalities. Jungle fowl appeared to have higher quality semen compared to domestic chicken and ayam serama. Although ayam serama appeared to have lower semen quality than domestic chicken and jungle fowl, it still had sufficient quality for use in artificial insemination.

Keyword: Semen evaluation, jungle fowl, domestic fowl, ayam serama, and sperm morphometric,

Correlation between Serological Tests and Identification of *Brucella Melitensis* in Goats

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Abstract

Brucellosis is a specific contagious disease of humans and animals caused by bacteria of the *Brucella* group, and caprine brucellosis is primarily caused by *Brucella melitensis*. In Malaysia, serological tests such as Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) are most widely used in diagnosis of brucellosis in small ruminants, while isolation and Polymerase Chain Reaction (PCR) provide valuable information regarding the presence of *Brucella* organism. This research was performed to evaluate and determine the correlation between serological tests (RBPT and CFT) and presence of *Brucella melitensis* in seropositive goats. Ninety-nine goat sera were collected from five goat farms and subjected to RBPT. Out of ninety-nine goat sera tested, 11(11%) were positive. They were then subjected to CFT. All RBPT seropositive goats were then killed and tissues such as supramammary lymph nodes, uterus, mammary glands and spleen were taken for isolation and identification using culture and PCR technique. The results showed that there was no correlation between the serological tests and the presence of *B.melitensis* in goats. However, there was strong correlation between RBPT and CFT ($r = 0.83$, $P < 0.01$). Therefore, neither RBPT nor CFT is suitable to be used as confirmatory diagnostic tool for control of caprine brucellosis.

Keywords: *Brucella melitensis*, serology, isolation and identification, PCR

Breeding Soundness Examination in Kedah-Kelantan Bulls

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Abstract

Breeding soundness examination (BSE) is a tool to identify and select good and potential breeder bulls. The bull is an important aspect in natural breeding in which the bull supplies semen to fertilize the cow. A study was carried out at Pusat Ternakan Haiwan Pantai Timur Kelantan using BSE as a tool to evaluate, identify and prove the claim that the Kedah-Kelantan (KK) bulls at the farm are good in their reproductive performance. The parameters evaluated in BSE included physical examination parameters of feet, legs, eyes, body score, and scrotal circumference and also internal and external examination parameters of reproductive organ. Parameters for semen evaluation which included general motility, live percentage, volume, sperm morphology and concentration, were also determined. The bulls were then classified either as satisfactory potential breeders, unsatisfactory potential breeders or differed bulls, according to the standard parameters of BSE. Ten KK bulls were selected from an active breeding herd. All of the bulls passed the minimum standards of BSE which include the bull must be free from disease and abnormality of reproductive organ, has achieved the minimal scrotal circumference size of 22.5 cm at two years of age and at least has 30% of general motility and 70% of normal sperm morphology. From the results, it is concluded that all of the bulls can be classified as satisfactory potential breeders.

Keywords: breeding soundness examination, Kedah-Kelantan, scrotal circumference, sperm, semen evaluation

Parasite and Virus Infracommunity of Malayan Water Monitor Lizard (*Varanus salvator*)

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Abstract

Water monitor lizards are one of the most unique creatures in the world.The objectives of this preliminary study is to investigate the prevalence of ecto-, endo-, haemoparasites and virus in the Malayan Water Monitor Lizards and to determine whether these lizards harbour zoonotic parasites. Fifteen wild monitor lizards consisting of fourteen *Varanus salvator* and one *Varanus bengalenses* mainly from Universiti Putra Malaysia, Perkampungan Orang Asli Pulau Carey Klang and National Zoo Kuala Lumpur were examined for ecto-, endo, blood parasites, and the presence of virus. Giemsa-stained thin blood film was examined for blood parasites. Samples of blood, tissues and swabs from oral and cloacal regions were inoculated into Vero cell culture to detect the presence of virus. Ectoparasites on the skin and endoparasites in the internal organs were harvested and identified. This study revealed one species of haemoprotozoa (*Haemoproteous sp*.), one species of ectoparasite (*Amblyomma helvolum*), and five endoparasites including cestodes (*Duthiersia expansa*), trematodes (*Monogenae* fluke) and nematodes (*Hastospiculum macrophallos, Kalicephalus sp*., and *Tanqua tiara*). Two species of the endoparasites have never been previously reported. The cytopathic effect can be detected in the cell culture with the highest prevalence in cloacal swabs. Both *V. salvator* and *V. bengalenses* carry almost the same parasites. The result from this study is very interesting and further studies are needed to identify the virus obtained and to study other species of varanids family as limited study was done in Malaysia.

Keywords: monitor lizards, ectoparasite, endoparasite, haemoparasite, virus

Molecular and Morphological Detection of Plasmodium Species in Wild Macaques in Selangor, Malaysia

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Abstract

Malaria is a serious global health problem, and rapid, accurate diagnosis is required to control the disease. A number of methods have been developed in recent years for diagnosing this disease, including polymerase chain reaction (PCR)-based technique that detect specific nucleic acid sequences, and microscopic examination of thin blood films that remains the most widely and commonly used method. In this study, blood samples were collected from 125 wild macaques consisting of 18 *Macaca nemestrina* and 107 *M. fasicularis* from various areas of Selangor. Giemsa-stained thin blood films (TBF) were prepared, and PCR using *Plasmodium* genus-specific primers for initial amplification and nested species-specific primers for *Plasmodium knowlesi* was conducted on all the blood samples. The prevalence of *Plasmodium* by TBF was 1.9% in *M. fasicularis* and 27.8% in *M. nemestrina*. The molecular prevalence of *Plasmodium* was 64.5% in *M. fasicularis* and 100% in *M. nemestrina.* When *P. knowlesi*-specific PCR was carried out, the prevalence in *M. nemestrina* was 5.6%, whereas in *M. fasicularis* it was 23.3%. These results indicate that the local wild macaques harbor a high rate of infection of *Plasmodium*. In addition, the prevalence of *P. knowlesi*, the zoonotic malaria parasite is higher than previously assumed. This warrants further investigation as these macaques may be potential reservoirs of human malaria in Malaysia.

Keywords: polymerase chain reaction (PCR), thin blood films, *Plasmodium knowlesi, Macaca nemestrina*, *Macaca fasicularis*.

Pathogenicity of a Malaysian Infectious Bronchitis Virus Isolate in Specific Pathogen Free Chickens

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Abstract

Infectious bronchitis (IB) is one of very important diseases in chicken. It is caused by IB virus (IBV) from the Coronaviridae family. This disease is a worldwide, acute and highly contagious disease. The virus easily changes in nature and emerges as a new strain and causes problems. In Malaysia, the nephrogenic strain was detected in 1995 and the new variant QX strain was reported recently in 2009. These 2 strains caused high mortality and loss of production. Hence, the objectives of this study were to isolate and identify IBV from chickens during field outbreaks of the disease in 2009 and determine the clinical signs, gross and histological lesions of specific pathogen free (SPF) chicken infected with the IBV isolate. Samples of lungs, kidneys and trachea from suspected IB birds were tested by reverse transcriptase polymerase chain reaction (RT-PCR) assay and then inoculated to 10-day old SPF embryonated chicken eggs via allantoic route for virus isolation, propagation and preparation of the IBV inoculum. The allantoic fluid and chorioallantoic membranes (CAM) from the inoculated eggs were then tested for the presence of IBV before inoculated into SPF chicken. Seventy-two day old SPF chicken were divided into 3 groups known as Groups A, B and C. Groups A and B were inoculated with IBV inocula 0.1 mL via intranasal route while group C remained as uninoculated and served as control group. Four chickens were sacrificed prior to IBV inoculation. At days 1, 3, 5, 7, 14 and 21 post-inoculation (pi), four chickens from group A and C were sacrificed. The bodyweight was noted prior to sacrifice. On necropsy, gross lesions were recorded and samples of trachea and kidneys were collected and fixed in 10% buffered formalin for histological examination. Mortality and any abnormal clinical signs were recorded at least twice a day. Feed and drink were given *ad libitum*. The results showed that IBV was successfully detected from the trachea, lung and kidney samples of the chickens by RT-PCR. Infectious Bronchitis Virus was successfully isolated and propagated in SPF embryonated chicken eggs and detected in the allantoic fluids and CAM. The pathogenicity study showed that the IBV isolate caused severe respiratory signs and high mortality (60%) up to day 14 pi. Mild to severe gross and histological lesions were recorded in the trachea and kidneys up to day 14 pi. However, signs of recovery with mild respiratory signs, absence of mortality and mild kidney lesions were recorded at day 21 pi. It was concluded that the IBV isolate is highly pathogenic and might be a new or variant strain of IBV.

Keywords: IBV isolate, new strain, SPF chicken, RT-PCR.

Pathogenicity of the Malaysian *Salmonella enteriditis* Phage Type 6a Isolate in Specific Pathogen Free Chickens

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Abstract

Salmonella enteritidis (SE) remains an important cause of zoonotic diseases. Humans are commonly infected with SE from chickens through food borne origin. It is suspected that different phage types of SE could cause different severity of infections in chickens. It was the objective of this study to determine the pathogenicity of SE phage type 6a in specific pathogen free (SPF) chickens. Seventy-two, day-old SPF chicks were divided into three groups namely, SE, mortality and control groups. All chicks were fed with an antibiotic free diet and fresh water *ad libitum* throughout the study period. The SE and mortality groups were inoculated orally with 0.1 mL of 10^7 cfu of SE phage type 6a. Four chicks were sacrificed prior to SE inoculation. At days 1, 3, 5, 7, 14 and 21 post-inoculation (pi), four chicks each from the SE and control groups were sacrificed. The study showed that there was no significant difference ($p>0.05$) between the SE and control groups in weight gain throughout the trial. The SE group showed clinical signs of listlessness, ruffled feathers and mild diarrhoea from day 3 pi until day 14 pi: slightly pinkish diarrhoea during the first 7 days pi but watery to pasty dark brownish thereafter. The gross and histological changes of the liver, spleen, ileum, caecum and caecal tonsil were only mild ranging from mild congestion, degeneration and necrosis to mild inflammation. No mortality was recorded. The study indicates that SE phage type 6a isolate of Malaysia is of low pathogenicity in one-day old SPF chicks.

Keywords: specific pathogen free chicks, *Salmonella enteriditis* phage type 6a, pathogenicity

Effects of Phase Feeding on Carcass Characteristics and Meat Composition of Kampung Chickens

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Abstract

A study on kampung chickens was undertaken to investigate the influence of scheduling of feeds by reducing protein levels during the various phases of growth of the birds until market age, on carcass characteristic and meat quality. A total of 62 3-week-old female kampung chickens were reared in two-tier wire floor cages with two chicks per cage for eight weeks. The chicks were randomly divided into four treatment groups (single-phase, 2-phase, 3-phase and 4-phase) and fed with step-down dietary protein levels (19, 17, 15 and 13%) accordingly. At the end of the study period, ten birds per treatment were randomly sampled for analysis. Carcass yield and component parts (breast, thigh, drumstick, wing, abdominal fat, back and neck) of the birds were determined as percentages of live weights. Meat chemical composition from breast muscles was determined for dry matter, crude protein and crude fat content. No significant differences were found in the percentages of carcass yield, component parts, dry matter, crude protein and crude fat contents between the four phases. It is concluded that phase feeding did not adversely affect carcass yield and meat chemical composition. This feeding strategy could therefore be an economical practice for rearing kampung chickens without sacrificing carcass yield and meat quality.

Keywords: phase feeding, carcass characteristic, meat composition, kampung chickens

Abiotic and Biotic Control of *Argulus sp.* among Goldfish (*Carassius auratus*)

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Abstract

Argulus spp. are branchiuran crustaceans with a direct life cycle, therefore current aquaculture practice with a high density of potential hosts provides ideal conditions for effective transmission of this obligate ectoparasite. Chemicals are usually used to treat parasite infestation of fishes. However chemical treatments result in side-effects including environmental pollution, parasite resistance, alteration of zooplankton ecosystem, changes in certain hematological parameters and contamination of host organs. Thus there is a need for alternative measures to control parasitic diseases in the aquaculture industry. To date, there are no studies reported on biological control of fish lice, in particular the use of Thai silver barb (TSB) (*Pontius gonionotus)* to control *Argulus* population. In this study, the effectiveness of abiotic and biotic control methods for an ectoparasite infestation was examined. Therefore, an abiotic study was designed firstly to examine the effect of water current on parasite abundance and incidence of the fish lice, *Argulus sp.* among captive goldfish (*Carassius auratus)*. Secondly, in a biotic study, the effectiveness of using TSB to reduce the parasite number was investigated and thirdly, to compare both the abiotic and biotic control methods in their effectiveness to reduce parasite number. The mean abundance and incidence of the ectoparasite, *Argulus sp.,* on goldfish was measured. In the abiotic study goldfish infected with *Argulus sp.* was mixed with other susceptible goldfish hosts and kept in tanks with fast flowing or stagnant water. There was no difference in the parasite mean abundance and incidence between fast flowing and stagnant water. Thai Silver Barb which is a predator of the *Argulus* was introduced to the parasite infected goldfish for the biotic study. The mean parasite abundance was significantly lowered ($P<0.05$) after introducing the predator TSB into the tanks. Combination of both control methods did not further improve the effectiveness in reducing *Argulus* population. Hence this study showed that utilizing this method of biotic control among goldfish is definitely a future control solution. This study also showed that water current seemed to reduce *Argulus* transmission and population. The predator effect of TSB was lessened by the fast flowing current. The major finding in this study indicated that TSB was a very effective biological control where with only two TSBs, 96% of *Argulus* was removed in less than 24 hours. The number of TSB used in the biotic control did not affect the predator efficiency. It is suggested that the biological control tested in this study can be used to reduce *Argulus* population among goldfish in aquaculture settings.

Keywords: *Argulus*, biotic and abiotic control, Thai silver barb (TSB), goldfish

Effect of Pendulous and Erect Pinna on Population Size and Frequency of *Malassezia globosa* and *Malassezia pachydermatis* in External Ear Canal of Healthy Dogs

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Abstract

Forty clinically healthy stray dogs were used to determine the effect of type of pinna on the population size and frequency of *Malassezia globosa* and *Malassezia pachydermatis* in external ear canal of healthy dogs. Two methods were used: cytological examination and fungal culture. The prevalence of *M. globosa* were 25 and 40% in pendulous and erect pinna, respectively. Both pinna recorded a very low mean count of 0.03 yeast/hpf. However, no growth of colonies was seen on modified Dixon's agar, therefore, *M. globosa* could not be confirmed. This study confirmed a high prevalence of *M. pachydermatis* in dogs with pendulous pinna (90%) which was higher than in dogs with erect pinna (65%). High population size of *M. pachydermatis* can be isolated from dogs with pendulous pinna (1074 cfu per swab) and dogs with erect pinna (466 cfu per swab) using fungal culture. There was a strong association of occurrence of otitis externa and type of pinna which predisposed dogs with pendulous pinna to be twenty-one times more at risk of developing otitis externa than dogs with erect pinna.

Keywords: pendulous, erect, population size, frequency, *Malassezia globosa, Malassezia pachydermatis*, external ear canal.

Experimental Infection of Hamsters with a Local Leptospiral Isolate from Rats

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Abstract

This study was conducted to investigate the clinical signs and post mortem lesions of the experimentally infected hamsters with leptospiral isolate. It also examined the stage of leptospiremia and the antibody titres in the infected hamster. A leptospiral isolate obtained from a rat was used as the infective inoculum. Twenty hamsters were infected with the leptospiral isolate and another 5 hamsters were used as negative controls. The concentration of the inoculum was 1 x 10^8 leptospire/mL. Blood was taken and post mortem lesions were observed from two infected hamsters and one negative control hamster every alternate day. The blood samples obtained were tested for leptopiral antibodies by MAT and PCR for leptospiral DNA. Clinical signs were observed everyday for any changes in the infected hamster. The hamsters were hypothermic on day three onwards and they were weak, depressed and anorexic on day 12 onwards. Infected hamsters had petechial hemorrhages in the lungs on day 5 and "butterfly" hemorrhages on day 8 onwards in the lungs. There was little petechial hemorrhages on the margin of the liver on day 12 onwards. For MAT and PCR, the results were all negative.

Keywords: hamster, leptospira, clinical signs, post mortem lesions, MAT

A Preliminary Study of Methicillin-Resistant *Staphylococcus aureus* and Antimicrobial Resistance Profile of Bacteria in selected Pig Farms in Peninsular Malaysia

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Abstract

Staphylococcus aureus is one of the most common and devastating human and animal pathogens. In this study, conventional technique of isolation and identification was used to isolate *Staphylococus aureus*, *Staphylococcus hyicus*, *Streptococcus* spp., and *E. coli*. Detection and confirmation of MRSA was done using Staphytect® Kit and Oxacillin Resistance Screening Agar Base (ORSAB). Two farms from each pig industry rearing state namely Selangor, Perak, Johor and Penang were selected. Nasal and rectal swab samples were taken from 64 piglets and nasal swab samples were taken from 16 farm workers. Nineteen (13.2%) MRSA were isolated. Fourteen (21.9%) isolates were from pigs while 5 (31.3%) isolates originated from humans. These alarming findings from the present study indicated that MRSA is an emerging pathogen as well as a zoonotic potential in pigs and humans in Malaysia. This study also determined the antimicrobial sensitivity profile of some isolates towards commonly used antimicrobials in pig farms. Results showed that lincomycin is no longer effective for treatment in the farms while spectinomycin, florfenicol and enrofloxacin are starting to be less effective in controlling pathogens. Both colistin and ceftiofur are still effective as the bacteria tested are sensitive towards them. The findings from this study warrant that suitable measures must be undertaken to prevent the spread of MRSA as well as to control the rise of antibiotic resistant bacteria in the farms.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), antimicrobial sensitivity test, pigs.

Effects of ß-Glucan on Growth Performance and Immunomodulation in Weaned Piglets

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Abstract

Seventy-four weaned piglets were used in a 6-week experiment to determine the effects of ß-Glucan on growth performance and immunomodulation of weaned piglets. Piglets were randomly chosen and divided into control and treatment groups. Body weight gain and feed consumption were recorded at weeks 2, 3 and 6. The immunomodulatory effects of ß-Glucan were determined by gross examination of lung lesions during post mortem. Weaned piglets from the treatment group had overall greater growth performance compared to the control group. This was also evident from the overall higher body weight and percentage of body weight gain as well as a lower feed conversion ratio. The fecal coliform count also implied that fecal coliform count in the treatment group was lower than the control group. Piglets treated with ß-Glucan were observed to have positive immunomodulatory effects on piglets. This was shown by an overall lower lung lesion score in the treatment group. The post mortem revealed 2 piglets with fibrinous pneumonia (APP) and 1 pig with severe atrophic rhinitis (Grade 5). In conclusion, treatment with ß-Glucan may lessen inflammatory response towards Gram negative bacteria via the inhibition of inflammatory cytokines and promote the production of anti-inflammatory cytokines. Further studies are needed to determine the efficacy of ß-Glucan in reducing total coliform count and its effect on immunomodulation.

Keywords: ß-glucan, growth performance, immunomodulation, piglets

Correlation of Radiographic and Echocardiographic Findings with Clinical Outcome in Canine Heart Patients

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Abstract

Thoracic radiography and echocardiography are two of the most important diagnostic tools for structural heart diseases. In canine heart disease cases, both severity grade and prognosis of the case are equally important for the clinicians and owners to decide on treatment options for the dog. Forty-two canine heart patients with complete history, clinical signs, follow up details, complete radiography records and echocardiography recordings were included in this study. The forty-two dogs were free of liver, kidney and lung diseases. Data was tabulated in SPSS 16.0 software and was analyzed using Kaplan-Meier Survival Analysis technique to determine the median survival duration of canine heart patients according to severity classification based on radiographic findings and severity classification based on echocardiographic findings. The medical effects of furosemide, benazepril and pimobendan were also studied by comparison of median survival duration between the group treated with the particular medication and the group not treated with the particular medication. Kaplan Meier Survival Analysis showed that the median survival durations in canine heart patients classified by severity of radiographic findings are 449±42 days in heart patients with severe radiographic findings, 530±175 days in heart patients with moderate radiographic findings and 549±173 days in heart patients with mild radiographic findings. Meanwhile, canine heart patients with severe echocardiographic findings have median survival duration of 449±3.3 days compared to 515±189 days in cases with mild echocardiographic findings. Kappa's test of agreement showed moderate degree of agreement between severity classification based on radiographic and echocardiographic findings with a kappa value of 0.60 ($p=0.003$). Median survival duration of canine heart patients undergone medical intervention was markedly longer than canine heart patients without medical intervention.

Keywords: canine, heart disease, radiography, echocardiography, median survival duration

Ethiopathogenesis of Caseous Lymphadenitis in a Mice Model

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Abstract

Corynebacterium pseudotuberculosis is a facultative, gram-positive intracellular small club-shaped rod which produces lesions similar to those of tuberculosis. It is known worldwide to cause caseous lymphadenitis (CLA) in sheep and goats. Caseous lymphadenitis is characterized by abscess formation in lymph nodes and/or visceral organs. Recent outbreak of CLA in Taman Pertanian University (TPU) farm, UPM reported that the CLA lesions were also found in the visceral organs. In the present investigation attempts were made to study the ethiopathogenesis of CLA in mice models which involved comparing the clinical signs, haemogram and biochemistry, and histopathological changes in visceral organs between the diseased and non diseased group. As an overall summary of this project, CLA in mice resulted in clinical signs such as huddling together, dejection, anorexia, pasty feces and accompanied by rapid and shallow respiration pattern. The haemogram and serum biochemistry profile showed significant ($p<0.05$) differences in the mean values between the diseased group and non-diseased group which include lymphocyte, plasma protein, monocyte, eosinophil, total bilirubin, total protein and potassium. Lastly, the most pronounced histopathological changes in the visceral organs were septicemia with severe congestion and increased vascularization together with the presence of capsulated abscess, micro-abscesses formation, infiltration of neutrophils and macrophages, tubercule granulomas, necrosis and early signs of degeneration in majority of the infected mice.

Keywords: *Corynebactrium pseudotuberculosis*, caseous lymphadenitis (CLA), ethiopathogenesis, septicaemia, tubercule granuloma.

Effect of Stocking Density on Haematological Indices and Welfare of Grower Rabbits *(Oryctolagus cuniculus)* in Tropical Climate

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Abstract

Ten New Zealand white cross rabbits of mixed sexes aged 10 weeks were used to evaluate the effect of stocking density (1 m^2/rabbit and 0.1 m^2/rabbit for 29 days) on haematological indices and welfare parameters in tropical climate. The rabbits were divided equally and allotted to the two stocking densities corresponding to an area of 0.5 m^2 for Group A and 5 m^2 for Group B. The rabbits were fed *ad libitum* with commercial rabbit grower pellets and fresh water was freely available throughout the study period. Each treatment group was also given carrot every Saturday and alfalfa hay every Monday evening as dietary enrichment. Group B rabbits had higher packed cell volume, haemoglobin concentration, mean corpuscular volume and mean corpuscular haemoglobin concentration and recorded a steeper rise of these indices over time when compared to Group A rabbits. Neutrophil/Lymphocyte ratio showed a correlation between social hierarchy and the availability of more space. Group A rabbits had higher weight gain and feed efficiency compared to that of Group B rabbits. However Group B rabbits recorded higher locomotor activity and Group A rabbits were observed to be utilizing the raised platform more. Both groups enjoyed the dietary enrichment exhibiting increased locomotor activity. The results of the study indicate that rabbits kept at 1 m^2/rabbit had better haematological indices and locomotion activities compared to rabbits kept at 0.1 m^2/rabbit in the tropical climate of Malaysia.

Keywords: rabbit (*Oryctolagus cuniculus*), stocking density, haematology, welfare, tropical climate

A Retrospective Study of Caseous Lymphadenitis Cases in University Veterinary Hospital, University Putra Malaysia and Selected Farms around Selangor

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Abstract

Caseous Lymphadenitis (CLA) is a disease that commonly affects sheep and goats. It is caused by *Corynebacterium pseudotuberculosis*. Effects of CLA on the economy are tremendous as it causes loss of production due to carcass condemnation and poor reproductive performance. Due to the chronic character of the CLA disease, retrospective study was chosen to determine the predisposing factors that can influence the occurrence of CLA and the common site of CLA abscess. Records from University Veterinary Hospital, UPM were reviewed and goats with swelling on the head, neck, shoulder, and thigh region were noted. Six predisposing factors were age, gender, breed, management system, source of goats, and purpose of the goats. The exposure odds ratio (EOR) was then calculated determine the strength of association between the predisposing factors with the occurrence of CLA. The results of this study indicated that pure bred goats were found to be 3 times more at risk of having CLA compared to mixed bred goats. The submandibular or parotid lymph node was found to be the most commonly affected lymph nodes (66.7%), followed by the prescapular lymph node (20.8%), and prefemoral lymph node (12.5%).

Keywords: caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, goats.

Carriage of *Campylobacter* spp. and *Salmonella* spp. by House Flies

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Abstract

Sixty samples consisting of three house flies in each, were collected around a large animal ward of University Veterinary Hospital (UVH) UPM, a cafeteria and a poultry farm. Twenty samples were collected from each site. The external surface and internal content of each sample were examined for *Campylobacter* spp. and *Salmonella* spp. All samples were cultured for *Campylobacter* spp. via direct plating on CCDA. Three samples (2.5%) were positive for *Campylobacter* spp. Pre-enrichment, enrichment and direct plating on XLT4 agar were done for isolation of *Salmonella* spp. from the house flies. Ten samples were positive for *Salmonella* spp. Isolation of *Salmonella* spp. from all three sites suggested that the areas were contaminated with *Salmonella* spp. It is possible that the flies could have picked *Salmonella* spp. from other places around the campus. This study showed that house flies could carry both *Campylobacter* spp. and *Salmonella* spp. internally and externally. The flies could readily contaminate the environment with their droppings or come in direct contact with food or feed. It is advisable that the population of house flies must be controlled by proper manure management, sanitation and clean lifestyle.

Keywords: house flies, carriage, *Campylobacter*, *Salmonella*.

Effect of Simultaneous Injection of Classical Swine Fever Virus Vaccine and *Mycoplasma hyopneumoniae* Vaccine on Immune Response of Swine

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Abstract

Objectives of this study were (1) to compare sero-conversion in pigs following simultaneous and separate vaccination against Classical Swine Fever (CSF) and *Mycoplasma hyopneumoniae* and (2) to determine safety of CSF and *M. hyopneumoniae* vaccines when given simultaneously. Twenty-four weaned pigs were divided into 3 groups of 8 heads. Groups were designated as non-simultaneous vaccinated group, simultaneous vaccinated group and negative control, respectively. Vaccines used in study were *M.hyopneumoniae* vaccine (SPRINTVAC®MH) and CSF vaccine (PESTIFFA®). IDEXX ELISA test kit (HerdChek M hyo) and LSIVET SUIS HC/PPC Blocking ELISA test kit were used to detect antibody titre on weekly basis. Sero-conversion rate of CSF antibody titre and M.hyo antibody titre were calculated. Result showed both simultaneous vaccination and non-simultaneous vaccination for CSF antibody titre reached 100% sero-conversion rate at 5 weeks post vaccination. Therefore, simultaneous vaccination was able to accomplish similar results as in non-simultaneous vaccination. Sero-conversion rate for CSF antibody titre in non-simultaneous group was slower before it reached 5 weeks post vaccination. 12.5% of animal from negative control group sero-converted at 5 weeks post vaccination due to false-positive result or field infections. *M. hyopneumoniae* antibody titre sero-conversion rate in both simultaneous vaccination and non-simultaneous vaccination reached 100% sero-conversion rate after 6 weeks post vaccination. Control group showed negative result for *M. hyopneumoniae* antibody titre throughout whole experiment. Vaccines used in trial did not cause any adverse effect after post vaccination when given simultaneously.

Keywords: classical swine fever, *Mycoplasma hyopneumoniae*, sero-conversion, ELISA, simultaneous injection

Efficacy of a Commercial Probiotic in Protecting Mice against *Salmonella* Infection

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Abstract

It is believed that probiotics are able to inhibit pathogenic bacteria from colonizing the gastrointestinal tract and thereby prevent infection and even mortality. On this basis, this project was undertaken to examine the efficacy of a commercial probiotic in preventing infection in the mouse model. This probiotic is made up of 8 bacterial species from 3 genera. Forty 6-week old white ICR mice were used in this project. The mice were divided into 7 groups consisting of positive and negative controls, 2 preventive groups and 3 treatment groups. The infective inocula were made up of a *Salmonella typhimurium* isolate. All mice in the positive control and treatment groups were severely affected when inoculated with the *Salmonella* isolate. Eleven (73%) of the 15 mice in the treatment groups died from the *Salmonella* infection. *Salmonella* was recovered from the internal organs of the mice in the positive control group and the treatment groups. No *Salmonella* was isolated from the internal organs of the mice in the negative control and the preventive groups. This showed that the probiotic was not able to prevent serious infection if given during or after infection. When the probiotic was given earlier as a prophylaxis, it was able to prevent serious infection. In this project, it is seen that none of the mice from the preventive groups succumbed to the *Salmonella* infection. It was clearly shown that probiotics were able to prevent adverse infection if given earlier as a prophylaxis.

Keywords: probiotic, infection, mice, mortality, *Salmonella*

Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Stray Cats around Colleges of Universiti Putra Malaysia and Selected Neighbourhoods of Seri Serdang, Selangor, Malaysia

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Abstract

The existence of Methicillin-resistant *Staphylococcus aureus* (MRSA) is due to the widespread utilization of antimicrobial agents. Besides humans, MRSA has not only been reported in livestock animals but also in pet animals such as the dogs and cats. This study was designed to estimate the prevalence of MRSA in stray cats around colleges of Universiti Putra Malaysia (UPM) and in selected neighbourhood of Sri Serdang. The samples for this study were taken from the nostrils of the stray cats using small sterile cotton swabs. The swabs were then inoculated onto blood agar (BA) and suspected colonies were gram stained. Gram positive cocci bacteria colonies were then inoculated onto mannitol salt agar (MSA) and yellowish colonies that grew were subjected to a series of biochemical tests such as the catalase, coagulase and Staphytect Plus latex agglutination test before they were inoculated onto the oxacillin resistance screening agar base (ORSAB). A total of seven (12.73%) MRSA from 55 samples were isolated from the stray cats. The results showed that the prevalence of MRSA in stray cats at colleges of Universiti Putra Malaysia and Seri Serdang are high (>10%). The stray cats may have contracted MRSA from contact with infected humans or via contaminated environments at the health care facilities such as the Student Medical Centre which is located in the college area of UPM and also from consuming contaminated raw meat from markets in Sri Serdang.

Keywords : Methicillin-resistant *Staphylococcus aureus*, stray cats, prevalence

Effects of Feeding Probiotic Metabolites on the Growth and Carcass Characteristics of Broiler Chicken

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Abstract

The objectives of the study were to determine the effects of probiotic metabolites on the growth rates, feed conversion ratios and carcass characteristics of broiler chicken in an attempt to determine the potential use of probiotic metabolites to replace antimicrobial growth promoters. Three hundred day-old Cobb chicks were reared for 42 days. The five treatment groups comprised of one negative control without antibiotics or probiotic metabolites added into the feed, one positive control with antibiotic added, and the other three comprised of treatment groups with probiotic metabolites added at different concentrations, namely 0.5, 1.0 and 1.5%, respectively. The liquid metabolites added were from four strains of *Lactobacillus plantarum* isolated locally from fermented soya bean *(tempeh)* and tapioca *(tapai ubi)*. The 60 chicks were assigned to each treatment comprising of six replicates. Each replicate comprising 10 chicks was randomly assigned to battery cages which are kept indoors. The feed and drinking water were provided *ad libitum*. The chickens were weighed individually every week and the feed weights were also recorded. Nine chickens from each treatment selected, using a stratified random method, were slaughtered at the end of the third week and at the end of the sixth week. Liver and gizzard were weighed while the duodenum, jejunum and ileum were measured. The 1.5% concentration of probiotic metabolites used was an effective dose to be supplemented for growth promoting purposes. The effect of probiotic metabolites on growth was good especially at the early stages. Probiotic metabolites significantly ($p<0.01$) reduced feed intake especially in the beginning without significant ($p>0.05$) changes to the weight gain. On the contrary, probiotic metabolites improved weight gain numerically especially at the first three weeks. The live weight gain was comparable to those of the antibiotic treatment group. The 1.5% MET also had a low feed conversion at both week 1-3 and week 1-6, contrary to the antibiotic group, which had high feed conversion ratios during week 1-3, although not significant ($p>0.05$). The probiotic metabolites appear to be potential replacements for antimicrobial growth promotants. The effect of probiotic metabolites on the carcass characteristic was less prominent compared to the growth performances. It was deduced from the results that probiotic metabolites do not have a direct effect on the gizzard. The probiotic metabolites have protective effects on the liver as the liver of the 1.5% probiotic metabolite group was significantly ($p<0.01$) smaller compared to the others at week 3. Meanwhile at week 6 there were no significant differences ($p>0.05$) in liver weights between treatment groups. There was no particular effect of the probiotic metabolites on the intestinal length. However, there were correlations ($t<0.05$) between the feed intake and intestinal length.

Keywords: broiler chicken, probiotic metabolites, antibiotic, growth performance, feed conversion effeciency, carcass characteristics, liver, gizzard, small intestine.

Molecular Approach in Avian Sexing using Cheek Cells

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Abstract

This study was carried out to determine the sex of birds through the DNA extracted from cheek cells using polymerase chain reaction (PCR) technique. Sterile swabs were used to collect cheek cells from 39 adults of various psittacine species as a source of DNA. A pair of Eclectus parrots (*Eclectus roratus*), a sexual dimorphism species was used as a control group. Various quantities of DNA were extracted from the buccal samples by the conventional DNA extraction method. A set of primers, P2 and P8, was designed for the study. The positive control samples were tested with the primers that will bind to CHD W and CHD Z genes, in the chromosome Z and W of the birds. The PCR products of P2 and P8 showed a single band (300 bps) for male and double bands (300 bps and 400 bps) for female birds. Based on the PCR results, of the 39 samples tested, there were 15 males (39%), 12 females (31%) and 12 unknown (12%) sex. This study shows that avian cheek cells contain DNA and can be used as a tool for sex or gender determination for avian species.

Keywords: avian, birds, DNA extraction, PCR

Evaluation of Bone Marrow-Seeded Porous Scaffolds at Post-Intramuscular Implantation in a Rat Model

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Abstract

Improvements to current therapeutic strategies are needed for the treatment of skeletal defects. Bone tissue engineering offers potential advantages to these strategies. One of the approaches in the development of bone graft substitutes is through use of scaffolds. The purpose of this study was to evaluate the ectopic bone formation of the bone marrow-seeded porous scaffold. Local cockle shell, *Anadara granosa* was used as material for scaffold. Eighteen rats were divided into 3 equal groups. Three different scaffolds were prepared for each group. Each rat was implanted with 2 scaffolds; the bone marrow-seeded scaffold was implanted into right gastrocnemius muscle whereas the non-seeded scaffold implanted at the contralateral side. Serum alkaline phosphatase and calcium concentrations were determined before and 3 weeks after implantation. Radiograph examination was done every week. At the end of week 3, all rats were sacrificed for histological evaluation. The radiographic examination and serum assays revealed that the scaffold can induce osteogenesis without exerting harmful effect to the body. Histological examination showed presence of new bone formation and blood vessels in bone marrow-seeded scaffold. Therefore, the bone marrow-seeded scaffold has characteristics of osteoconductive, osteoinductive, biodegradable and biocompatible properties.

Keywords: scaffold, ectopic bone formation, *Anadara granosa*, osteogenesis.

Blood and Biochemistry Profiles of Sambar Deer *(Cervus unicolor)* under Different Adaptation Periods

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Abstract

Hematological and biochemical analyses were carried out on 53, 2-year old Sambar deer (*Cervus unicolor*) of which 44 were from Taman Pertanian Universiti, Universiti Putra Malaysia and 9 deer obtained from Lenggong, Perak, Malaysia in July, 2009. Haematology and serum biochemical parameters were analysed and compared between groups of deer. The study revealed that deer from Lenggong had significantly ($p<0.05$) higher packed cell volume (PCV), erythrocyte (RBC) and lymphocyte counts, mean cell volume (MCV), hemoglobin, plasma and total protein concentrations than those from TPU. The biochemical data also revealed significant ($p<0.05$) differences in eight parameters. Lenggong deer had significantly ($p<0.05$) higher serum Na^+, K^+, alkaline phosphatase (ALP) and albumin concentrations but lower Cl^-, glucose, total bilirubin and alanine transaminase (ALT) concentrations. Among the parameters analysed only ALT, aspartate transminase (AST), creatine kinase (CK) and ALP were higher than reference values.

Keywords: Sambar deer, blood, biochemistry, adaptation periods

Effects of Feeding Time on Adipocyte Characteristics and Fat Metabolism in Rats

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Abstract

Effects of different feeding time (day vs night feeding) on the weight gain, adipocyte cellularity, plasma fatty acid profile and plasma leptin levels in rats were examined. Thirty male 8-week old Sprague Dawley rats were randomly allocated into day feeding group (DFG) as control, and night feeding group (NFG). They were fed 10% of their body weight with standard rat chow once a day. The DFG was fed at 0800h and NFG at 1900h. Both groups were exposed to 12:12 h light-dark cycle. Daily feed intake and weekly body weight were monitored. Five rats from each group were sacrificed at weeks 1, 3, 5 to collect blood for plasma fatty acids profiling and plasma leptin levels analysis. Abdominal fat were collected for adipocyte cellularity analysis where the number and diameter of fat cells were measured. Weight gain, increment of adipocyte numbers and plasma leptin levels was significantly ($P<0.05$) higher in NFG than DFG rats. There was no significant ($p>0.05$) difference in feed intake, adipocyte diameter and plasma fatty acids profile in both groups. Clearly, night feeding has an effect on fat metabolism and deposition where more adipose mass are accumulated which leads to more weight gain in rats. In summary, although nutrient absorption and mobilization was not affected by feeding time of the day, night feeding promoted the accumulation of more fat mass.

Keywords: rats, feeding time, body weight gain, adipocyte cellularity, leptin.

Baseline Values of Canine Tear Production Determined by Schirmer Tear and Phenol Red Thread Tests

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Abstract

Although research on canine tear production and dry eye has been reported in temperate countries in different breeds, there is no Malaysian data. On the average, at least two new cases of dry eye are diagnosed weekly at the University Veterinary Hospital (UVH) of the Faculty of Veterinary Medicine, Universiti Putra Malaysia. Currently in UVH the guidelines for the diagnosis of keratoconjunctivitis (KCS) and monitoring of response to treatment are based on recommendations for temperate countries. Thus the objectives of this study were to determine the Malaysian baseline values for canine tear levels using Schirmer tear test (STT) and phenol red thread test (PRTT), the relationship between and diurnal effect on STT and PRTT in dogs. The average baseline values for canine tear production in healthy local dogs in Malaysia with normal tear film breakup time (TBUT) were within the range of average baseline values reported in temperate countries. The average STT value for both eyes was approximately 22 mm/min and the PRTT was approximately 26 mm/15 s. There was poor relationship between STT and PRTT. This might be due to the presence of one or more confounding factors. The red colour change in PRTT was more intense when STT was performed first followed by PRTT and vice versa. The intensity of red colour change in PRTT was even more when performed in KCS dogs. This is because the intensity of the colour change in the phenol red thread is due to increasing alkalinity. Thus, KCS dogs had more alkaline tears than normal dogs. The lowest tear level was in the afternoon when diurnal study was conducted. Hence, KCS tests should be performed in the afternoon in order to obtain a more accurate measurement of tear level. Tear levels fluctuate when normal dogs were exposed in clinic with air-conditioned environment. Therefore, KCS tests should be performed immediately when dogs arrived at the clinic.

Keywords: dog, Schirmer tear test, tear film breakup time, keratoconjunctivitis sicca

Apoptosis Pathways Induced by Recombinant Adenovirus in Cancer Cells

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Abstract

Apoptosis, or programmed cell death, is an essential physiological process that plays a critical role in development and tissue homeostasis. Expression of VP2 gene derived from infectious bursal disease virus (IBDV) has been shown to be able to induce apoptosis in the cancer cells, which possibly renders it useful in cancer treatment regimes. However, the mechanism of the on-going apoptosis process is not clear. In this study, the apoptotic effect induced by the expression of VP2 gene in cancer cells was investigated. This was done by detecting the caspase activities which are the important components of the apoptosis process. Five types of cancer cells, MCF-7, HepG2, HeLa, MDA and CT 26, were cultured and inoculated with 1×10^6 $TCID_{50}$ of adenovirus recombinant with the VP2 gene. Activities of caspase 8, 9, 2 and 3, 7, 10 were then studied by adding specific substrate to the samples, resulting in the release of free dye, which can be measured by spectrophotometry. Active caspase 9 and caspase 3, 7 10 were detected in all of the cancer cells. The findings suggest that the the apoptotic event in cancer cells treated with the adenovirus recombinant with VP2 gene followed the intrinsic pathway.

Keywords: apoptosis, VP2, infectious bursal disease virus, intrinsic pathway, caspases, cancerous cells.

Molecular and Antigenicity Characterisation of *Vibrio sp*. Isolates from Asian Seabass *(Lates Calcarifer)*

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Abstract

Two species of *vibrio* (*Vibrio fluvialis* and *Vibrio mimicus*) isolated earlier from *Lates calcarifer* was sub-cultured into thiosulfate citrate bile sucrose (TCBS) agar. They were then grown into brain heart infusion broth (BHI) with the addition of 1% sodium chloride (NaCl). This culture was incubated with gentle shaking (50 rpm) for 24 h at 37ºC. Outer membrane protein (OMP) of both vibrio species was prepared from the culture in brain heart infusion broth. The bacteria were pelleted and subjected to sonication to break the bacteria cells from its membrane. The outer membrane of the vibrio species was then separated using sodium polyacrylamide gel electrophoresis (SDS-PAGE) in running buffer and subjected to 300 ma, 100 v for 1 h and 20 min. The gel containing polypeptides was then transferred into a nitrocellulose membrane for immunoblotting. Hyperimmune serum against the OMP of the vibrio *spp*. raised rabbits was used in this study. Immunodetection was done by subjecting the nitrocellulose membrane to the antiserum against the respective vibrio species. Horseradish peroxidase (HRP) was used as conjugate to facilitate binding of antigen antibody complex reaction at the nitrocellulose membrane. All photographed gels were scanned and analysed using gel analysis software gene tool. The results were compared to protein standard markers. This study shows that for *vibrio fluvialis*, the most antigenic OMPs were of molecular weights 50, 60a and 75 kda whereas the most antigenic proteins for the whole cells of this strain were of 33 and 75 kda. For *vibrio mimicus* the most antigenic OMPs were of molecular weight 40 and 80 kda while the most antigenic protein for the whole cells were of molecular weights 24 and 35 kda. These antigenic proteins can be good vaccine candidates against vibrio *spp* infections.

Keywords: outer membrane protein, vibrio spp, Asian seabass, sodium polyacrylamide gel electrophoresis (SDS-PAGE), nitrocellulose membrane, immunodetection

Reproductive Performance of Boer Goats Imported from Australia

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Abstract

Assessment of reproductive parameters of Boer goats imported from Australia were made in a Boer Breeding Farm and a mixed-breed farm in Sabah. Data between 2008 and 2009 were sampled randomly and used to calculate pregnancy rate, prolificacy rate and kid mortality rate. The performance was compared with that of a mixed-breed goat farm. The pregnancy rate of imported Boer goats and the mixed-breed goats was 48 and 80% respectively. The difference was significant ($P<0.05$). Pregnancy rate was influenced by the nutrition, buck-to-doe ratio, health status of the breeding goats and environmental associate stress. There was significant difference ($P<0.05$) in the overall mean prolificacy rate between the two farms with the prolificacy rate in Boer goat at 145% while the mixed-breed goats at 113%. Prolificacy rate was related to the nutrition, age and parity of the dams. However, there was no significant ($P > 0.05$) difference in kid mortality rate between the farms. Kid mortality was attributed to inadequate nutrition, low birth weight, infectious diseases, helminthiasis, traumatic injury, poor mothering ability and lack of efficient management.

Keywords: Boer goat, reproductive performance

Assemblages of Ectoparasites and Haemoparasites in the *Gallus gallus* Complex in Selangor, Malaysia

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Abstract

Parasites are known to be host-specific through adaptive radiation and tandem evolution with their hosts. However, selective pressures like rapid environmental changes and the adaptation of the hosts to novel habitats often influence the assemblages of parasites. This research investigates the ectoparasite and haemoparasite fauna on the *Gallus gallus* complex in Selangor, with reference to two groups, namely, the wild Red Jungle fowl which is believed to be the ancestor of all the domestic chickens, and the indigenous free-ranging village chickens. Fifteen adult Red Jungle fowls (9 females and 6 males) and 15 indigenous village chickens (8 females and 7 males) were examined to determine the ectoparasite and haemoprotozoan assemblage among the *Gallus gallus* complex in Selangor. Blood was collected via venipuncture for detection of intra- and inter-erythrocyte haemoparasite. Five feathers from various parts of the body, namely, the primary wing, tail, axilla, thigh, neck, back and breast were plucked from each bird for examination of ectoparasites. Four species of blood parasites were found including microfilaria, *Trypanosoma*, *Plasmodium* and *Leucocytozoon sabrazesi*. The Red Jungle fowl and village chickens did not share the same kind of blood parasites apart from microfilaria. Six species of ectoparasites were detected, namely, *Lipeurus caponis, Menopon gallinae*, *Gonoides dissimilis*, *Megninia cubitalis, Goniocotes* sp. and *Haemaphysalis* sp. The species composition of ectoparasites was found to be similar between the Red Jungle fowl and village chickens. However, the prevalence and intensity of infection was higher in the Red Jungle fowl. There appears to be subtle microhabitat segregation between the species of ectoparasites. The *Lipeurus caponis* are distributed throughout the body in both the Red Jungle fowl and village chickens, indicating that they are not selective. *Menopon gallinae* and *Gonoides dissimilis* selected the shorter feathers on the neck, thigh, axilla, back and breast, which is closer to host body. *Megninia cubitalis* preferred the wing and tail feathers. The Red Jungle fowl harbors more ectoparasite and haemoparasite at a higher infestation rate compared to village chickens. This may be due to the differences in habitat, behaviour and diet, and warrants further investigation.

Keywords: ectoparasite, haemoparasite, *Gallus gallus*, Red Jungle fowl, village chicken

Molecular Survey of *Ehrlichia canis* in Blood and Ticks Collected from Stray Dogs in Kuala Lumpur, Malaysia

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Abstract

Ehrlichia canis is the etiological agent of canine monocytic ehrlichiosis, a potentially fatal disease of dogs and is transmitted by the brown dog tick, *Rhipicephalus sanguineus*. Recent studies have detected other possible tick vectors. Thus far, only one study has been carried out to determine the prevalence of *E.canis* using molecular methods and there are as yet no study to that detects the organism in ticks in Malaysia. Polymerase chain reaction (PCR) is known to be a sensitive and specific method for the diagnosis of canine ehrlichiosis and currently is the diagnostic method of choice worldwide. Polymerase chain reaction was performed using a species-specific set of primers for the detection of *E.canis* in blood and ticks collected from 60 stray dogs from Dewan Bandaraya Kuala Lumpur. Out of a total of 122 ticks, 120 were identified as *Rhipicephalus sanguineus* and two were identified as *Haemaphysalis* sp. *E.canis* DNA could not be amplified from any of the 60 canine blood samples or the 60 tick samples.

Keywords: *Ehrlichia canis*, *Rhipicephalus sanguineus*, ticks, PCR

Detection of Resistance of Gastrointestinal Nematodes to Albendazole and Ivermectin in Goats

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Abstract

Gastrointestinal nematodes infection is one of the most important diseases of small ruminants in Malaysia, particularly goats. Control of gastrointestinal helminthiasis in small ruminants relies almost exclusively on the use of anthelmintic drugs but the effective control is limited by the development of anthelmintic resistance. This study evaluated the efficacy of albendazole and ivermectin that are currently used as preventive medicine in herd health programme of small ruminants as well as to detect the presence of anthelmintic resistance to both anthelmintics. Faecal examination was done by the McMaster technique to determine the number of eggs/g faeces. Efficacy of albendazole and ivermectin were calculated based on arithmetic means of pre-treatment and post-treatment eggs/g (e.p.g). While detection of anthelmintic resistance was done by faecal egg count reduction test (FECRT) in which arithmetic means of post-treatment e.p.g. for treated and control group were used. In this study, albendazole was moderately effective with percentage efficacy of 86% and ivermectin was ineffective with percentage efficacy of 16%. Anthelmintic resistance was detected to both drugs used in this study in which albendazole with 87% of reduction on faecal egg counts (FEC) associated with 61% lower 95% confidence limit and ivermectin with 13% reduction of FEC associated with -91% lower 95% confidence limit. In this study the resistance of gastrointestinal nematodes to albendazole and ivermectin in treated goats was detected. There was also evidence of reduction in FEC in both treated groups but not to a desirable level.

Keywords: helminthiasis, effective, resistance, efficacy, goats.

Frequency of Isolation and Antimicrobial Susceptibility Pattern of *Staphylococcus intermedius* From Dogs and Cats

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Abstract

A six-week study was carried out to isolate *S. intermedius* and determine their antimicrobial susceptibility from dogs and cats. The bacteria was isolated and identified from the skin and oral cavity swabs from 30 pet dogs and cats presented to University Veterinary Hospital and 30 dogs and cats from animal shelter. 38.3% of *S.intermedius* was isolated from total of 120 samples. The highest frequency (34.8%) of isolates was found in pet dogs. This was followed by pet cats, animal shelter dogs and animal shelter cats. The antimicrobial susceptibility pattern was detected by disc diffusion method. A high percentage of resistance was observed in ampicillin, tetracycline, and sulpho-compound at 80.4, 71.7, and 65.2% respectively. The resistance towards methicillin and ceftriazone were similar at 54.4%. Most of the isolates were susceptible to ciprofloxacin with 19.6% resistance rate. Almost 70% of these isolates showed multiple-antimicrobial resistance. It is concluded that *S. intermedius* is present in dogs and cats, distributed on the skin surface and in the oral cavity. Their antimicrobial resistance rates are of public health concerns. This potential human pathogen should be given consideration in bite wound and other infected lesions.

Keywords*: S. intermedius,* dogs, cats, antimicrobial resistance, zoonosis

Diurnal Activity Pattern and Behaviour of Captive Prevost's Squirrels (*Callosciurus prevostii*)

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Abstract

Wild populations of Prevost's squirrel (*Callosciurus prevostii*) are currently threatened due loss of feeding and nesting sites as a consequence of habitat destruction and hunting activities. It is therefore important that efforts to conserve this species are initiated, to prevent the extinction of *C. prevostii* in the near future. In view of that, this study was undertaken to establish the diurnal activity pattern and behaviour of captive *C. prevostii* (n = 10), to warrant a better understanding of their biology which may assist in conservation efforts of this species. Data obtained through direct visual surveillance of the subjects whilst they were in their captive enclosure was feasible at a distance of 1.5 m. Behavioural measure were performed *via* scan sampling of the subjects every 10 minutes between 0700 and 1900 hours, over a period of 28 days. Captive *C. prevostii* spent a significant period in the daytime either resting or climbing. These behaviours were consistently high during both the conditioning and post-conditioning period which were 7 and 21 days, respectively. Locomotor stereotypy, namely pacing and circling, was also evident during the two observation periods and could be attributed to the natural active predisposition of *C. prevostii*, which is arboreal in the wild.

Keywords: Prevost's squirrel, *Callosciurus prevostii,* diurnal activity, stereotypy behaviour

Antibacterial and Anaesthetic Effects of Thiopental-Propofol Mixtures in Dogs

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Abstract

Antibacterial properties of 1:1, 1:2, 1:3 and 1:4 thiopental-propofol mixtures were determined by inoculating a known quantum of *Staphylococcus aureus* and *Bacillus subtilis* into each anaesthetic mixture. The number of colony forming units (CFU) grown from the subculture at 0, 6, 24, 48 hours, 7 days and 14 days were determined. Results showed that mixtures at ratio of 1:2, 1:3 and 1:4, supported while 1:1 mixture inhibited the growth of *S. aureus*. However, all anaesthetic mixtures did not support the growth of *B. subtilis*. Administration of fresh and 7-day old 1:1 thiopental-propofol mixtures in 6 dogs to maintain 15 minutes of intubation time showed that the 2 regime did not result in adverse cardiopulmonary and blood parameters. Transient apnea following induction was observed. C-reactive protein (CRP) of fresh thiopental-propofol mixture tended to decrease post-anaesthesia. Comparatively in 7-day-old treatment group, CRP only started to decrease at 24 hour post anaesthesia. Compared to fresh mixture, slightly more 7-day old mixture was used to induce and maintain anaesthesia and resulting in slightly longer recovery. No inflammatory signs, depression, inappetance, nausea or vomiting was observed in any of the dogs within the 2-week experimental period.

Keywords: thiopental-propofol, antibacterial effect, anaesthetic effect

Garlic as a Prophylactic Agent in *Aeromonas hydrophila* Infection in Red Tilapia (*Oreochromis* Spp)

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Abstract

A study was carried out to compare the effects of garlic (*Allium sativum*), as a prophylactic agent, on survival rate of the red tilapia (*Oreochromis* spp). Mono-sex tilapia around the size of 3 cm were used in the experiment. Fish were assigned to five treatment groups, with two replicates each. Treatment groups were given different level of *A. sativum* (20, 30, 40, 50 g/kg body wt) in their diets. The control group diet was free from garlic. Fish were measured and weighed on the first day of the experiment to obtain the initial length and weight. Diet was administered *ad libitum* for 30 minutes, twice a day for 30 days. Excess feed was removed after 45 minutes after each feeding. On the 30th day fish were again measured and weighed to obtain the final length and weight before experimental infection with *Aeromonas hydrophila* by immersion route done and mortality rate monitored for 96 hours. The results show that there was lower mortality when garlic was added to the diet of the fish. There was also a significantly ($p<0.05$) higher standard body length and body weight gain in the treatment groups than the control. These findings suggest that fish fed with garlic showed better growth performance. In conclusion the study showed that garlic can be used as a prophylactic agent against *A. hydrophila* infection and the addition of garlic at 50g/kg body wt in the diet can improve the growth performance of the tilapia.

Keyword: garlic, tilapia, *Aeromonas hydrophila*, mortality rate, growth performance

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