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RE# MJVR - 0038-2014

## EFFICACY OF NEEM LEAF POWDER FOR TREATMENT OF COCCIDIOSIS IN YOUNG GOATS

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**ABSTRACT.** A study was carried out to analyse the efficacy of neem leaf powder, administered in a capsule against coccidiosis in young goats as well as to identify the species of coccidia found in a selected private farm in Sungai Siput, Perak. A total of seven *Eimeria* spp were found, in faecal samples from the experimental goats, namely; *E. arloingi*, *E. hirci*, *E. alijevi*, *E. christenseni*, *E. jolchijevi*, *E. ninakohlyakimovae* and *E. caprina* at a rate of 40%, 23%, 14%, 7%, 5% and 2% respectively. Two types of treatment for coccidiosis, herbal and synthetic, were given to 24 young goats for a period of 8 weeks to evaluate the effectiveness of a herbal product, that is the neem leaf powder capsule, a product of the Veterinary Research Institute. Results show that there is no significant differences ( $p > 0.01$ ) between neem capsule treatment and a synthetic drug (sulphur based) treatment in treating coccidiosis infection. This study shows that neem leaf powder can be an effective substitute for controlling coccidiosis in goats.

*Keywords:* *Eimeria* spp., *Azadirachta indica*, sporulation

### INTRODUCTION

The Malaysian livestock industry is comprised of two major sectors, a highly commercialised pig and poultry sector and a comparatively lagging ruminant sector. Currently, the ruminant industry is dominated by cattle for meat production which has recorded a steady growth largely due to the participation of government land development agencies in cattle, but has continued to lag in meeting local consumer demand. A total of 80% of the Malaysian goat population is kept on traditional, smallholder farms which are lacking in basic amenities such as piped water and has nutritional deficiencies due to lack of animal husbandry knowledge (Mukherjee *et al.*, 1991). For higher productivity, farmers need to have the latest knowledgeable on time and cost saving husbandry methods as well as the ability to treat minor conditions to save

their animals from mortality and morbidity. In the last three decades, there has been a sharp increase in demand for animal-based protein sources in Malaysia (Kaur, 2006). This increase has been attributed to rapid economic and population growth with the resultant effects of urbanisation income growth and changing consumer preferences fuelling a strong demand for animal proteins (Devendra, 2006). This increased demand opens doors of opportunity for entrepreneurs to venture into animal production. Goat meat could be a nutritious alternative to other red meat consumption and its suitability as an additional income source to small farmers should be explored further. Globally, there is an increasing need and demand in goat meat production for agricultural diversification and meeting the requirements for healthier meat by health-conscious consumers.

According to Chandrawathani *et al.* (2013), sustainable feeds and economical control of diseases using fewer drugs would be one of the ways of improving productivity in the livestock industry. This is because prolonged drug usage may lead to residues and a change for the worse in food safety. Moreover a rising concern in livestock industry is the residue and toxicity levels of synthetic anthelmintics and drugs being used. More smallholders are turning to herbal remedies for solutions since these anthelmintics are generally more expensive and are losing their strength against the resistances of parasitic worms (Priscilla *et al.*, 2014). A better education among producers is also an

important step towards higher food safety on farm level. Besides that, worm control by local farmers using naturally occurring plants with minimal cost as compared to drugs, could increase returns and provide mutton free of drug residues for human consumption. *Azadirachta indica* was found to be one of the herbs used to expel parasitic worms from the gastro-intestinal system in goats (Chandrawathani *et al.*, 2013). Preliminary studies conducted by the Veterinary Research Institute in Ipoh showed that feeding neem foliage is safe, eco-friendly, cheap and most importantly palatable to small ruminants.

However, apart from helminthiasis, coccidiosis among young goats is gradually causing a detrimental effect on the local goat industry's productivity. Coccidiosis is an opportunistic disease which is significant in affecting the productivity of goats. It can become an infection of serious economic importance in small ruminants pertaining to clinical diseases like diarrhoea and poor weight gain in particular for subclinical infections. In intensive breeding conditions characterised by high animal density for high productivity, these diseases result in reduced production. The causative agent is a protozoan that has the ability to multiply rapidly and they vary tremendously in virulence.

The objective of this study is to determine the effectiveness of a locally produced neem leaf powder capsule to palliate the coccidiosis problem in young goats and also to identify the species of

coccidia currently occurring in local farm animals used in this study site.

## MATERIALS AND METHODS

### Study Site, Animals and Management

This study was carried out between February and May 2014, in a private, traditional, smallholder farm at Sungai Siput, Perak. The climate was hot (30-34 °C) and humid (>80%) during the study, with frequent rain showers. This study comprised of 24 young goats, weighing between 10 to 12 kg, between the ages 4-6 months old based on the dentition. The breeds involved were mixed breed of Katjang, Jamnapari and Boer. The goats were randomly allocated into 3 groups with eight animals in each group, including one control group. The goats were kept in a semi-intensive management system where they were allowed to graze for 5-6 hours in the afternoon by the road side and on nearby uncultivated land. At other times, they were penned in wooden sheds with raised, slatted flooring. Oil palm fronds were provided in the shed and concentrates based on animal live body weight were given daily by the owner, as well as *ad lib* water. Salt blocks were also provided in this farm and basic health management was practiced during the study period. At the start of the study, all the selected animals were ear tagged and FAMACHA (FAMACHA© Information Guide) for each animal was recorded in the first and last week of the study period. No treatment

is required for FAMACHA scores of 1 and 2 (dark pink) indicating that the animal is healthy. A score of 3 to 5 (pale pink to off-white colour) shows progressive anaemia. A score of 5 indicates poor condition and requires treatment. The experimental animals were treated with neem leaf powder capsules and triple sulphur-based drugs respectively. The animals were observed weekly for eight weeks during which their health condition was observed and recorded, and rectal faecal samples collected to monitor coccidial oocyst counts.

### Parasitology Analysis

The McMaster method to enumerate oocyst per gram (OPG) of faeces, was the diagnostic test conducted to estimate the number of oocysts in each individual goat's faeces (Coles *et al.*, 2006). Besides that, strongyle egg count (EPG) was also recorded. Oocysts were identified after sporulation at room temperature (26-33 °C) in 2.5% potassium dichromate solution following the Manual of Veterinary Parasitological Laboratory Techniques (1986). Oocysts were concentrated by centrifugal floatation using saturated sodium chloride solution. Measurements of oocysts were done with compound microscope (Motic Pro BA310 Microscope) under a 40× objective. The species of the oocysts were identified based on morphology of oocysts (shape, presence or absence of micropyle and its colour) and sporulation time (Manual of

Veterinary Parasitological Laboratory Techniques, 1986).

### **Preparation of neem leaf powder capsules and administration in goats**

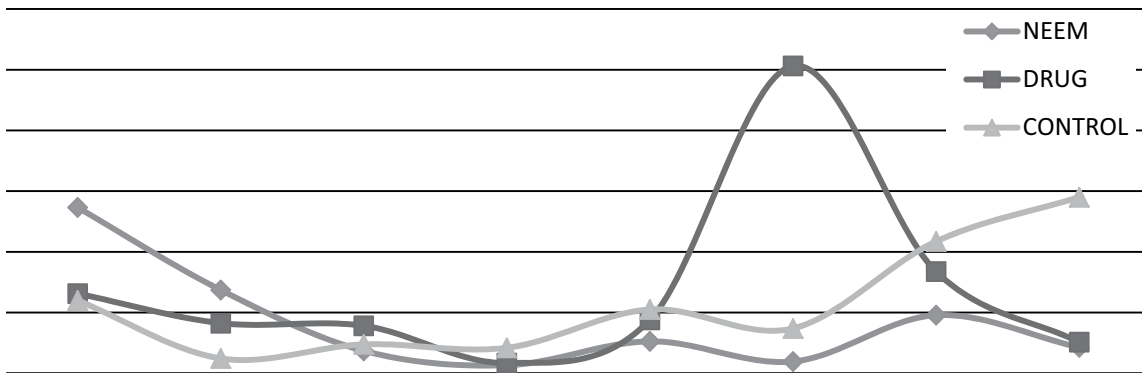
Fresh neem leaves were dried under the shade for 3 days and were finely ground to a powder form, in a grinder (Panasonic Blender MX337) and encapsulated in gelatine capsules of 0.3 grams of neem leave powder per capsule. It was stored in room temperature till used. In the first four weeks, each of the 8 goats in group 1 were fed orally at a dose rate of 0.1 gm per kg body weight, that is, 4 capsules (1.2 gm) per goat once a day. At the same time, each of the 8 goats in group 2 were given drug therapy (one treatment only) consisting of commercial sulphur-based coccidiostat at a rate of 0.5 mg/kg body weight, kaolin-pectin and electrolytes (5 ml per animal). The control goats of group 3 were not given any treatment but were observed for any signs of serious illness which if found, would be treated accordingly and removed from the experiment based on Animal Ethics Protocol.

### **RESULTS**

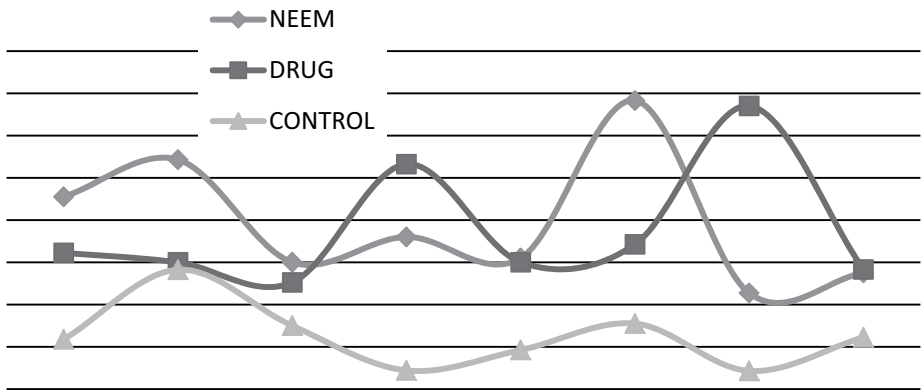
A total of seven *Eimeria* species were identified from the faecal samples of the goats. The most prevalent were *E. arloingi* found in 40% of the goats, followed by *E. hirci* (23%), *E. alijevi* (14%) and *E. christenseni*. Other species found were *E. jolchijevi*, *E. ninakohlyakimovae* and

*E. caprina*, present in 7%, 5%, and 2% of the goats respectively. High oocysts counts were mainly due to less pathogenic species such as *E. hirci*, *E. alijevi*, *E. jolchijevi*, *E. ninakohlyakimovae* and *E. caprina* whereas the pathogenic oocysts counts were still low such as *E. arloingi* and *E. christenseni*. Quantitative faecal examination performed weekly by McMaster technique to determine the number of oocysts per gram of faeces (OPG) as per standard procedure showed effectiveness in neem leaf powder capsule treated group. Its mean OPG count dropped by 84% compared to mean OPG count of drug treated group which decreased by 61% and mean OPG count of control group which increased by 59%.

Generally, the results show a trend towards a lower oocyst count after treatment with neem capsule and commercial drug. Figure 1 shows neem capsule treated group mean OPG count declined at a steady pace from week one (5462 OPG) up to week four (262 OPG). However from week five onwards its mean OPG count increased (1050 OPG) slightly due to the start of the rainy season beginning at week five. Mean OPG count of the drug-treated group showed a similar pattern. An obvious peak (10125 OPG) in mean oocysts was observed at week six of the study and this coincided with the rainy season which might have lowered the goats' immunity level further. Likewise, the mean strongyle egg count of the neem-treated group was between 2275 EPG and 1375 EPG whereas for the drug-treated group was 1612 EPG



**Figure 1.** The average OPG count over 8 week period for 3 groups of goats for various treatments



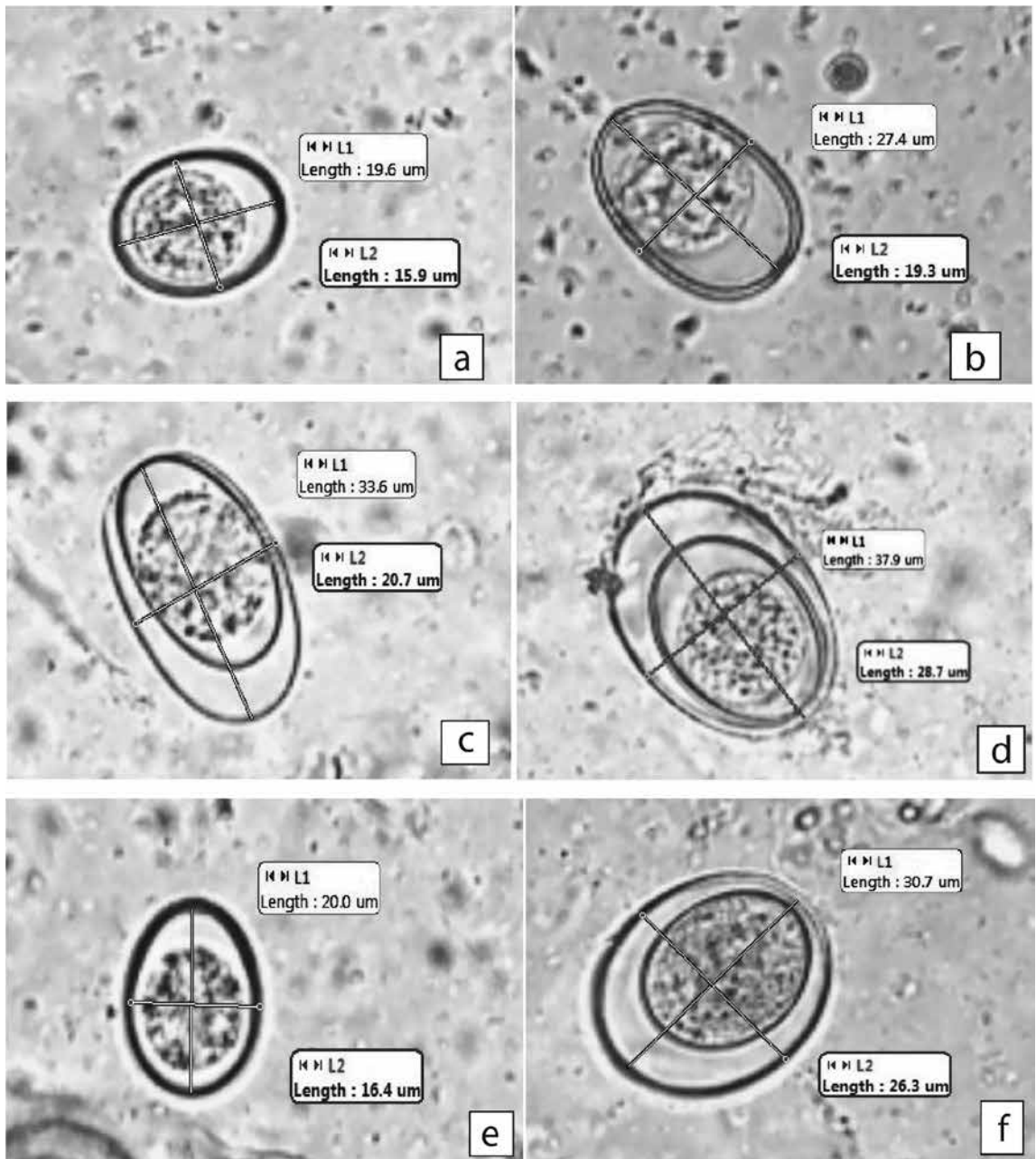
**Figure 2.** The average EPG count over 8 week period for 3 groups of goats for various treatments

and 1416 EPG. The mean egg count of the control group was between 587 and 612 as shown in Figure 2.

FAMACHA readings recorded at the start and end of the study remains almost constant for all the treatment groups throughout the project. It has been observed that the range of FAMACHA

scores for the farm on the first week of the study was 1 to 4, where a few animals were anaemic. However, at week eight there was an improvement in the anaemia status as seen by the FAMACHA score with a range of 1 to 3 in all groups. There was no significant difference between the treated animals and control animals.





**Figure 3.**

a) *E. alijevei*, b) *E. arloingi*, c) *E. caprina*, d) *E. christensenii*, e) *E. hirsi*, f) *E. jolchijevei*



## DISCUSSION

The findings of high prevalence of coccidial infections in young goats in this study is in agreement with observations made by an earlier study conducted in 10 smallholder farms in the state of Selangor (Peninsular Malaysia) where oocysts count were significantly higher in kids than in adults. In this study, seven species of *Eimeria* (Figure 2a-f) were identified in this traditional smallholder farm. *E. aspheronica* and *E. caprovina* which was described in Malaysia by Fatimah *et al.*, (1989) and Jalila *et al.*, (1998) respectively, was not found in this study.

Overall, it can be easily deduced that, both the herbal treatment and commercial drug managed to bring down the mean OPG count by more than 50%. However, the reduction differences between neem capsules and the drug was 23%, proving that neem capsules has a far better efficacy in keeping down coccidial infections in young goats. Furthermore, the neem capsule is a natural product with no drug residues making it safe for food animals. Since the selected young goats have no previous usage of synthetic drugs in them, there was no evidence of drug resistance. Even so, prolonged period of synthetic drug usage will evidently result in resistance (Basripuzi *et al.*, 2012). With the advent of drug resistance, there is a scarcity in the alternative availability of natural herbal remedies to curb coccidial infections and hence loss of opportunity to supply a better quality of local goat meat and improve on

productivity. Smallholder farmers may find it hard to purchase anthelmintic drugs due to its high cost and lack of supply in rural areas. Ethnoveterinary medicine mainly on herbal products is a good alternative (Chandrawathani *et al.*, 2013). In addition, the neem product has been found to reduce strongyle egg count and could be used to curb helminthiasis which is also rampant in goat herds.

Clinical signs like diarrhoea was observed. Studies correlating high oocyst counts with diarrhoea are rarely found, and in these samples, it is suggested to be due to the *Eimeria* species. One of the most common agents that precipitate coccidiosis is heavily contaminated environment. Most of the Malaysian smallholder farmers have a shed for small ruminants which presents opportunities for low level of oocysts contamination through its elevated slatted floor enabling easy clearing faeces on the floor (Jalila *et al.*, 1998). Even so, due to poor husbandry practice in smallholders, accumulation of faeces between the floor gaps and irregular removal of faeces from under the shed heightens transmission of coccidia.

Other factors contributing to the intensity of coccidial infection could be related to the number of kids in the herd, the feeding system, intensity of rainfall and poor nutritional status. The livestock industry in Malaysia needs to put more emphasis on improving hygiene in smallholder farms since it has a major impact on the infection level on farms. At the same time, introducing these

herbal anthelmintics like neem capsules to rural farmers to further ameliorate the productivity of local prime goat meat. This is in line with the National Key Economic Area (2014), NKEA, of agriculture sector whereby its focus is on transforming traditional small-scale production-based sector into a large-scale agribusiness industry that contributes to the Malaysian economic growth and sustainability. Economic transformation through NKEA is vital in providing more job opportunities, increasing the gross income of rural farmers and also to safeguard national food security.

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## BLOOD PROTOZOA FINDINGS IN PET DOGS SCREENED IN IPOH, MALAYSIA

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**ABSTRACT.** A total of 103 blood samples from pet dogs around Ipoh were screened for common blood protozoa. A total of 14 samples were found positive for *Ehrlichia canis* and one sample was found positive for microfilaria of *Dirofilaria immitis*. Both these diseases are transmitted by vectors; ticks (*Rhipicephalus sanguineus*) and mosquitoes respectively. In the hot and wet tropical environment where vectors are abundant, pet care, hygiene and regular screening will help veterinarians detect these infections early to facilitate treatment.

**Keywords:** vector borne blood parasites, pet dogs, *E. canis*

## INTRODUCTION

Humans and dogs share a long history of a close relationship. Dogs have known to become human companions in many instances such as hunting, working and pets. According to recent genetic analyses, they have been under human domestication for something in the order of

100,000 years—longer by several orders of magnitude than any other domestic species. The term domesticated dogs refer to *Canis lupus familiaris* that belong to Canidae family of the mammalian order Carnivora (Kjemtrup *et al.*, 2000). However, dogs are competent reservoirs as host to several zoonotic agents and their increased close relationship with humans in developing countries as pets and companions pose new concerns for public health (Otranto, Dantas-Torres and Breitschwerdt, 2009). As in previous years, the Veterinary Research Institute (VRI) has an initiative to monitor diseases of domesticated or pet dogs in Malaysia. According to Erwanas *et al.* (2014), the clinical signs of parasitic infection are variable and occasionally some infected animals are asymptomatic. As asymptomatic dogs can still transmit disease, it is very important to monitor the type of pathogen and the number of animals that carry it.

According to the VRI annual report, there are several types of blood parasites commonly found in Malaysian pet and

stray dogs, which are *Babesia gibsoni*, microfilaria (*Dirofilaria immitis*), *Ehrlichia canis*, *Babesia canis*, and *Hepatozoon canis*. Other than microscopic detection of parasites in peripheral blood smears, diagnosis can be by serological tests, flow cytometry and polymerase chain reaction (PCR) (Nalumba *et al.*, 2011). The other commonly found blood protozoan in Malaysia, as indicated in VRI annual report is microfilaria. *Dirofilaria immitis* is commonly found in the pulmonary arteries and right ventricle of dogs and other canids, and cause canine heartworm disease. In the past ten years, the geographic range of canine heartworm infection has been markedly increased (Simón *et al.*, 2009). Diagnosis of microfilaria are usually by microscopic examination and PCR. Next is *Ehrlichia canis*, which is a Gram-negative, highly pleomorphic bacterium, which is enveloped with a rippled thin outer membrane (Rikihisa *et al.*, 1985, 1997). *H. canis* infection is frequently diagnosed by microscopic detection of intracellular gamonts in stained blood smears, an indirect fluorescent antibody test (IFAT) for Anti-*Hepatozoon canis* antibodies, enzyme-linked immunosorbent assay (ELISA) and PCR (Baneth, 2011). Another blood parasite that is taken in consideration is Leishmania. Leishmania is a protozoan parasite belonging to the order of trypanosomatid that causes leishmaniasis. This disease is considered to be one of the “neglected tropical diseases” due to its increasing number of infection as well its relation with poverty, famine,

war and immunosuppression. PCR is the best technique to identify Leishmania because it enhances sensitivity and aids the identification of the infecting species and it obviates the need for parasite culture (Uezato 1998).

VRI has been consistent in studying the infections of blood parasites in dogs as shown in studies on incidences of parasites in pet dogs and cats that was conducted from the year 2009 until 2011. From routine analyses, results from the screening shows 1 positive case of *Babesia gibsoni* found each year. Besides that, 5 positive cases of microfilaria *Dirofilaria immitis* were recorded in 2009. One case of *Ehrlichia canis* and *Ancylostoma* sp. was recorded in 2010. Subsequently, *Ascaridia* (2 cases), *Toxocara* sp. (1 case) and *Babesia canis* (1 case) were identified in year 2011. Whereas, for cat samples only 3 parasite species were found which is mite (1 case) in 2009, 8 positive cases of *Toxoplasma gondii* in 2010 and 5 cases of *Ancylostoma* sp. Another study conducted by VRI, on the presence of ectoparasites and endoparasites found in pet and stray dogs showed that there were two positive cases of *Demodex canis*, one positive case of *Rhipicephalus microplus*, seven cases of *Rhipicephalus sanguineus*, four cases of *Ctenocephalides canis* and for endoparasites one positive case whereby an adult *Ancylostoma caninum* worm was found in one dog's gut contents, ova of *Ancylostoma* sp. in 8 faecal samples, *Toxocara canis* adult worm in one dog, *Gardia* sp. in one dog, and lastly *Ascaris* sp.

ova in 3 dogs (Erwanas *et al.*, 2014). Thus, the aim of this study was to determine the current situation of blood parasites in pet dogs, which have a close relationship with humans. Knowledge on the current disease scenario in pet dogs will enable a more structured control programme for these diseased dogs, thereby preventing transmission to humans.

## **MATERIALS AND METHOD**

### **Samples**

Blood samples from dogs were obtained from government veterinary clinic and one private practice which gave a total of 103 blood samples. The blood samples were taken when these dogs were brought to the clinics for routine examination, vaccination or treatment. The owners' approval was obtained prior to the sampling. The dog's information such as breed type, age and sex were obtained from the clinic registry. An informal interview was conducted with the pet's owner about the condition of their dog and also their feeding and management of their pet dog. The purpose of the interview was to get better information regarding their pet's living environment since habitat of the animal plays an important role in the pathogenesis of parasites. All blood samples were obtained from the cephalic vein using needles (23G) and 3 ml syringes before transfer into 3 ml EDTA tubes.

### **Parasitology techniques**

All blood samples were subjected to microscopic examination, by using two different types of smears, namely the thin blood smear using a drop of EDTA blood and thick smear using buffy coat. This is to differentiate between blood parasites that infect red blood cells and white blood cells. Both types of smears were stained with Giemsa solution. However, only the thin blood smear were fixed with methanol before staining. All sample slides were examined under 1000× magnification with a compound microscope.

#### **Thin Blood Smear**

A small drop of blood is placed at one end of the glass slide. Then, a spreader is placed on top of the drop of blood at 45° and waited till the blood extends along the edge of the spreader. The spreader is quickly pushed forward along the slide. This action pulls the blood over the slide, leaving a thin blood smear. The slide is left to air-dry overnight and stained with Giemsa solution. (MAFF, 1981)

#### **Thick Buffy Coat Smear**

For thick buffy coat smear, the blood sample was drawn into the capillary tube and sealed at one end. The tube is then centrifuged at 12000 rpm for 5 minutes to gain the buffy coat layer. The capillary tube is cut using a diamond cutter pen right above the buffy coat layer. The buffy

coat layer is smeared on to a clean glass slides to make a thick smear and left to air-dry overnight. The smear is stained with Giemsa solution. (MAFF, 1981)

RESULTS

Results obtained are shown in Table 1.

A total of 103 pet dogs were screened for blood protozoa whereby two isolations of parasites were found from 14 samples. Both isolations were identified as *Ehrlichia canis* (Figure 1) and microfilaria (*Dirofilaria immitis*) (Figure 2). No other parasites were identified. The *E. canis* is characterized by intracytoplasmic morulae inclusions in blood monocytes. Microfilaria of *D. immitis* is identified in blood smears as a multicellular organism about 300-500 µm in length. All animals tested appeared healthy, had good appetites and were active. All results were notified to the authorities for follow-up treatment.

DISCUSSION

Domestic pets are important carriers of zoonotic diseases. As such, regular screening of household pets is advocated. This study shows that vector borne

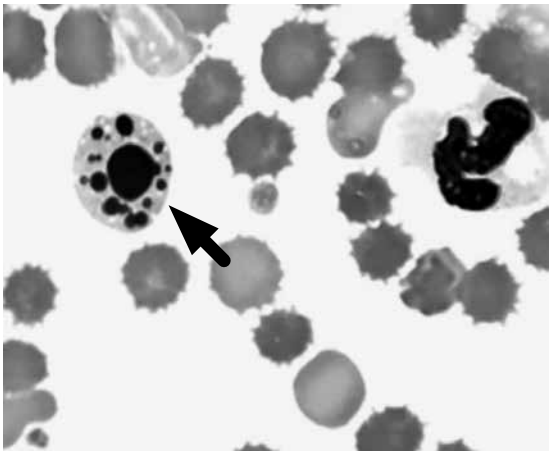


FIGURE 1. *Ehrlichia canis* in white blood cell.

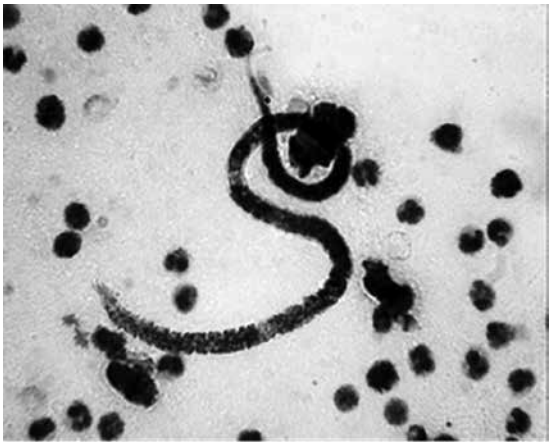


FIGURE 2. Microfilaria (*D.immitis*) in buffy coat smear.

TABLE 1. Blood protozoa identified in pet dogs in 2014

Parasites species	No. of dogs positive / total no. tested ( %)	
<i>Ehrlichia canis</i>	14/103	(13.6%)
Microfilaria ( <i>Dirofilaria immitis</i> )	1/103	(0.97%)
Total no. of isolations	15/103	(14.6%)



diseases are prevalent among domestic pet dogs. The presence of Ehrlichiosis also known as canine rickettsiosis, canine hemorrhagic fever, canine typhus, tracker dog disease, dog AIDS and tropical canine pancytopenia is a tick-borne disease of dogs usually caused by the organism *Ehrlichia canis* (Ettinger *et al.*, 1995). Traditional diagnostic techniques (hematology, cytology, serology and isolation) are valuable diagnostic tools for Canine Monocytic Ehrlichiosis (CME). However, a definitive diagnosis of *E. canis* infection requires molecular techniques. A common blood parasite found in Malaysia is *Hepatozoan canis* but was not observed in this study. Hepatozoon species are apicomplexan parasites that belong to the family Hepatozoidae and are phylogenetically closely related to the piroplasms and haemosporinids (Barta, 2001; Baneth *et al.*, 2003). However, one case of heartworm microfilaria was seen. Heartworm (*Dirofilaria immitis*) is a parasitic roundworm that is spread from host to host through the bites of mosquitoes. As these pets were kept in households, it is evident that good care in vector control such as giving dogs tick baths and keeping them in a clean environment free from mosquitoes will greatly reduce the infection rates. Canine babesiosis is another vector-borne disease caused by intra-erythrocytic protozoa that induces anaemia, fever, jaundice, splenomegaly, thrombocytopaenia and, occasionally, haemoglobinuria (Nalubamba *et al.*, 2011). According to

Nalubamba *et al.*, (2011) and Kjemtrup *et al.* (2000), although there are many strains in canine babesia species, two organisms *B. canis* and *B. gibsoni* are commonly the cause of the disease. Historically, babesia infection in dogs was identified based on morphological appearance in the erythrocyte. All large Babesia were designated *B. canis*, whereas all small Babesia were thought to be *B. gibsoni* (Boozer and Macintire, 2003). However, Babesiosis was not found in the dogs in this study. Regular screening is worthwhile as it will enable the veterinarian to intervene with reliable treatment to save the animal. Other blood protozoans such as *Babesia canis* or trypanosomes were not observed although it has been reported previously from diagnostic cases of the Veterinary Research Institute. This indicates that pet animal management is fairly good with fewer animals getting these infections. In view of the animal ethics and care protocols, it is important to disseminate awareness on proper management of pet animals so that household pets have a good quality of life with their owners.

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## PIGMENTARY KERATITIS IN DOGS – A STUDY ON INCIDENCE IN 83 CORNEAS

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**ABSTRACT. Objective:** To evaluate the incidence, etiology and progression of Pigmentary keratitis in dogs. **Materials and Methods:** A total of 83 corneas from 55 dogs of different breeds, sex and age were selected for the study. Signalment, anamnesis, nature of discharge and duration of illness was collected from all the animals. The progression of pigmentation was assessed by dividing the cornea in to four quadrants. Pigmentation grading, extent of pigmentation and mean pigment density were calculated by dividing the cornea in to 24 sectors. Schirmer tear test (STT), fluorescein dye test (FDT), tonometry, and slitlamp biomicroscopy and Corneal impression cytology were done. **Results:** Among the 55 animals, 51 dogs were Chinese Pug (92.7%) and the mean age was  $33.13 \pm 3.12$  months. Among 55 animals, 28 were females (50.9%) and left cornea was affected in 44 animals (53.01%). The

mean duration of the disease as noticed by the owner was  $07.21 \pm 0.65$  months. Most of the owners were totally unaware about the condition of the eye. Among 83 corneas, 40 (35%) showed pigmentation in all the sectors. 29 animals (53%) were affected with keratoconjunctivitis sicca (KCS) followed by 13 animals (24%) affected with entropion. The mean value of random blood sugar was  $107.84 \pm 0.99$  and the mean intraocular pressure in the animals under the study was  $40.64 \pm 2.38$ . The mean value of pigmentation grading, extent of pigmentation and mean pigment density was  $32.59 \pm 2.27$ ,  $15.67 \pm 0.83$  and  $1.37 \pm 0.07$  respectively. The mean value of Schirmer tear test was  $10.31 \pm 0.58$  and under high power microscopy, Leishman's stained corneal impression cytology revealed infiltration of neutrophils in all the slides. **Conclusion:** It was concluded that Chinese pugs under the age of 3 years

are mostly affected and females and left eye is mostly affected. All the animals with pigmentation is having KCS.

**Keywords:** dog, pigmentary keratitis, KCS, pigmentation grading, STT, glaucoma

## INTRODUCTION

The cornea, which comprises one-fifth of the fibrous tunic of the eye, in healthy conditions, is transparent in order to transmit light freely. The transparency of the cornea depends on number of features like its avascular nature, dehydrated state, unmyelinated nerves fibers, lack of pigmentation, non-keratinised epithelium, well organised lamellae in corneal stroma and the interval between collagen fibrils (1). The loss of transparency of the cornea is the first sign of corneal disease and when it is lost, due to any reason, the animal will become visually impaired or even blind.

The corneal pigmentation usually happens as a sequelae to chronic superficial keratitis or neovascularisation of cornea (panus) subsequent to chronic and repeated corneal irritation. The chronic phase of superficial keratitis is characterized by epithelial and stromal pigmentation associated with infiltrates of histiocytes, plasma cells and degranulated mast cells (2). When there is chronic inflammation of cornea, it will cause the underlying epithelium to become hyperkeratinized.

A high incidence of pigmentation of cornea in brachycephalic breeds like Chinese pugs is reported (3,4,5,6). Allgoewer and Hoecht (2010) reported

chronic superficial keratitis and subsequent pigmentation in German shepherd and its crosses and a high incidence of chronic superficial keratitis in German shepherds, Belgium shepherds, greyhounds and more rarely in Beaucerons, collies, poodles and Siberian huskies were also reported (3).

The exact reason for the development of pigmentary keratitis is still obscure. Hence, the study was undertaken to evaluate the incidence of pigmentary keratitis, its etiology and progression of pigmentation in dogs.

## MATERIALS AND METHODS

The dogs with ophthalmological complaints specific to cornea, presented to University Veterinary Hospital, Kerala, India for the period from January 2011 to June 2014, were screened for the evaluation for corneal pigmentation. Detailed examination of the selected 55 dogs presented with corneal pigmentation of different ages, breeds and sexes were conducted. Among 55 dogs, 28 were having bilateral pigmentation and 27 had unilateral affection. A total number of 83 corneas were evaluated for pigmentation. Signalment, anamnesis, nature of discharge and duration of illness were collected. The progression of pigmentation was assessed after dividing the cornea into four quadrants viz. ventro-medial, ventro-lateral, dorso-medial and dorso-lateral and the quadrant which affected with pigmentation was observed. Pigmentation grading, extent of pigmentation and mean pigment density

were calculated by dividing the cornea into 24 sectors (2). Schirmer tear test (STT), fluorescein dye test (FDT), tonometry and slitlamp biomicroscopy were done in all the corneas. Corneal impression was made for cytology and stained with Leishman's stain and examined under high power of microscope.

## RESULTS

A total number of 83 corneas with pigmentation were studied. Among the 55 dogs, 51 were Chinese Pug (93%), 2 were Lahsa Apso (4%) and 1 each Cocker Spaniel (2%) and Bull Mastiff (2%). The age of dogs, under the study, ranged from 2 months to 10 years with a mean value of  $33.13 \pm 3.12$  months. 29 (53%) animals were aged between 1 to 3 years followed by 17 (30.9%) animals below 1 year of age, 8 (15%) animals above 5 years of age and 2 (4%) animals were between 3 to 5 years of age. Among 55 animals, 28 were females (51%) and 27 (49%) were males. Left cornea was affected in 44 animals (53%) and right cornea was affected in 39 animals (47%). The mean duration of the disease as noticed by the owner was  $07.21 \pm 0.65$  months. Most of the owners were totally unaware about the condition of the eye and 5 (11%) owners reported that their pet is blind. 12 (27%) owners reported discoloration of the eye of their pet. 8 (18%) dogs had undergone treatment for corneal ulceration and under medication prior to presentation with the complaint of discoloration of the affected eye. Most of

the dogs were having purulent discharge from the eye at the time of presentation. Motile blood parasites were not observed in any of the dogs under the study.

Among 83 corneas, 40 (35%) showed pigmentation in all the sectors with varying pigmentation score and mean pigment density. Pigmentation of medio-ventral quadrant of cornea was the most affected quadrant in the study. Detailed ophthalmic examination of the corneas and adnexa of the animals affected with corneal pigmentation revealed that 29 animals (53%) were affected with keratoconjunctivitis sicca (KCS), 13 animals (24%) with entropion, 6 animals (11%) with periorbital dermatitis and subsequent entropion, excess nasal fold in 5 (9%) animals (Figure 1) and trichiasis in 2 (4%) animals. The mean value of random blood sugar (RBS) was  $107.84 \pm 0.99$  which was in normal range. 5 animals (8%) showed RBS values above normal range suggestive of hyperglycemia and diabetes (mean value  $220 \pm 0.76$ ). The mean



**Figure 1.** A pug with excess nasal fold

intraocular pressure of animals under the study was  $40.64 \pm 2.38$  and this was higher than the normal range and suggestive of glaucoma.

The mean value of pigmentation grading in 83 corneas was  $32.59 \pm 2.27$ . Among 83 corneas only 5 corneas (6%) showed pigmentation grade of 72 (fully pigmented). The values of pigmentation grading ranged from 8 to 72. The mean value of extent of pigmentation was  $15.67 \pm 0.83$ . Out of the 83 corneas, 26 corneas (31%) showed pigmentation extending in all the sectors. The values of extent of pigmentation ranged from 4 to 24. The mean value of mean pigment density was  $1.37 \pm 0.07$ . Out of the 83 corneas, 8 corneas (10%) showed mean pigment density of 3. The values of mean pigment density ranged from 0.3 to 3.

The mean value of Schirmer tears test (STT) was  $10.31 \pm 0.58$  which was below normal range suggestive of keratoconjunctivitis sicca (KCS). The values ranged from 2 to 24. Out of 83 corneas, 36 corneas (43.3%) showed STT value less than 10. Under high power of microscope, corneal impression cytology revealed infiltration of neutrophils in all samples. Degenerative changes and presence of necrotic debris were noticed in few samples and presence of squamous cells was noted in samples of 5 corneas.

## DISCUSSION

The popularity and increase in the number of Chinese pug may be the reason

for over-representation of this breed for ocular conditions affecting cornea (7). The presence of shallow orbits, excessive prominence of the globe, decreased corneal sensitivity, reduced tear film stability (8) the inherited corneal insufficiency, poor corneal reflex and lack of protective eye consciousness (9) were suggested as reasons for the high incidence of corneal lesions in pugs. Chronic pigmentary keratitis was considered to be an old age condition of eye in dogs and in the present study, the mean age of the animals affected with pigmentation was 33 months. Azoulay (2014) reported pigmentary keratitis in dogs with mean age of 7 years with a range from 3-14 years. Spontaneous Chronic Corneal Epithelial Defects (SCCED) and pigmentation was reported in middle aged to older dogs averaging 8 to 9 years of age. A high incidence of corneal pigmentation in females than males were reported (2,3,10) and this was contradicted by many (7,11). Bilateral affection of cornea was more and affection of left eye was predominant in the study and supported by many authors (3,7).

In the present study, it could be observed that pigmentation started mostly from the ventro-medial quadrant and progressed centrally. This might be due to increased irritation of the ventro-medial quadrant of cornea from concurrent conditions like entropion, trichiasis and periorbital dermatitis which were affecting ventro-medial quadrant of cornea (12). In many cases the pigmentation started at the periphery and progressed to the centre (13).

The dogs which were under treatment for corneal ulceration were presented with a central scar and peripheral pigmentation near the limbus which slowly progressed towards the centre. When a wound involving the corneal stroma heals there is transformation of keratocytes to fibroblast and collagen which is disorganized and resulted in scar formation. When neovascularization takes place for corneal healing the new blood vessel will carry melanin pigment from the limbal and perilimbal area and get deposited around the central scar and pigmentation results.

Keratoconjunctivitis sicca (KCS) affecting 29 animals was the most commonly occurring concurrent ophthalmic affection along with pigmentary keratitis and was supported by many (2,7,14). Entropion and entropion subsequent to periorbital dermatitis was the second highest concurrent affection or the reason for development of corneal pigmentation. This may be due to the fact that when there is entropion there can be inward deviation of eyelashes which constantly irritate the cornea, resulting in pigmentary keratitis (7). Trichiasis was another condition and this can also result in pigmentation by repeated irritation to the cornea and reported as major reasons for the development of epithelial and stromal pigmentation (15).

Diabetic dogs are predisposed to keratoconjunctivitis sicca because they have significantly reduced corneal sensitivity compared with control dogs (16). In the present study, 5 dogs were diabetic and the

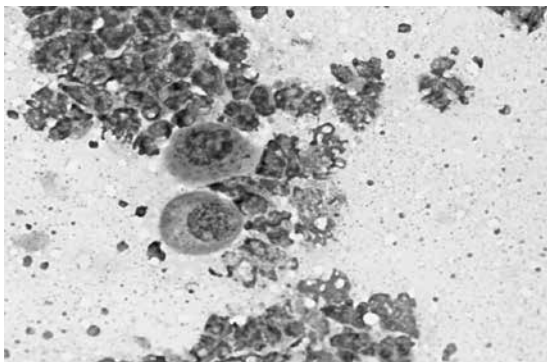
rest of the dogs had random blood sugar values in the upper range and decreased corneal touch sensitivity. Diabetic dogs have a significantly reduced corneal sensitivity in all regions and are prone for development of KCS (17,18). The mean value of STT in diabetic dogs were  $5.7 \pm 10$  which was suggestive of KCS and it was noted that when there was loss of corneal sensitivity, the constant irritation to the cornea by the excess nasal fold, entropion, periorbital dermatitis, trichiasis and KCS had resulted in hyperkeratinisation of the stratified squamous epithelium and subsequent corneal pigmentation (4).

The mean value of intraocular pressure (IOP) as measured with Tonopen-Vet on the day of presentation was  $40.64 \pm 2.38$  and suggestive of glaucoma (19,20). The increase in thickness of the central portions of cornea due to the hyperpigmentation may be a reason for increase in IOP. The deposition of melanin pigment in the iridocorneal angle and subsequent blockage of the filtering mechanism of aqueous humour could be attributed to a high IOP (5).

Leishman's staining was done for the corneal impression cytology and it revealed presence of epithelial cells and infiltration of neutrophils. Degenerative changes and presence of necrotic debris, noncornified corneal epithelial cells were also noted (17,1). Presence of a few squamous cells were noted in 5 corneas (Figure 2).

From the study, it was concluded that decreased corneal sensitivity subsequent to hyperglycemia and KCS and constant





**Figure 2.** Corneal impression cytology showing squamous cells

irritation of the cornea due to entropion or trichiasis can be the main reasons for the development of pigmentary keratitis in dogs. The increase in IOP which develops secondary to pigmentation and glaucoma is not the reason for the development of pigmentary keratitis.

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RE# MJVR – 0004-2015

## SHORT COMMUNICATION

**TAENIA TAENIAEFORMIS IN WILD RATS****PREMAALATHA B.<sup>1</sup>, CHANDRAWATHANI P.<sup>1\*</sup>, TAN P.S.<sup>2</sup>, THARSHINI J.<sup>3</sup>, JAMNAH O.<sup>1</sup>, RAMLAN M.<sup>1</sup> AND NOR IKHMAL S.<sup>4</sup>**<sup>1</sup> Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak<sup>2</sup> School of Marine Science and Environment, University Malaysia Terengganu, Terengganu<sup>3</sup> Department of Agro Technology and Bio-Industry, Nilai Polytechnic, Negeri Sembilan<sup>4</sup> School of Science, Monash University Malaysia Campus, Malaysia

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**SUMMARY.** *Taenia taeniaeformis* is a parasitic tapeworm that is commonly found in cats. The intermediate hosts for this parasite include mice, rats and other rodents. Cats can be infected by the taeniae by feeding on the rodents harbouring the intermediate stages. A total of 105 wild rats (*Rattus* sp.) in the vicinity of Kuala Lumpur were trapped and a post-mortem was carried out. Observation of whole liver samples showed the presence of cysts grossly on the surface. On cutting open the cysts, tapeworms were found and based on the morphological features of the parasites nine rats were confirmed to be positive for *Taenia taeniaeformis* tapeworms found in cysts in the liver. The cestode was identified based on helminthological keys by Soulsby, 1982.

*Taenia taeniaeformis* is known as a helminth which has been classified in the family of *Taeniidae*, the flatworm family. According to Mehlhorn and Aspöck (2008), the normal size of the eggs for this species is 35 µm, with the adult worm

having a length of 60 cm. This parasitic tapeworm is normally found in the small intestine of cats, specifically in the region of the duodenum (Combes, 2001). The scolex; which is the head, is the anterior region that is being used to attach to the mucosa of the small intestine.

The life cycle of this helminth begins with the eggs which are swallowed by the intermediate host, normally a rodent such as wild rats. These eggs are normally discharged by the tapeworms in the gut of infected cats or any definitive host and is excreted in faeces and contaminates potential food sources of rodents (Combes, 2001). Once these eggs have been consumed by the wild rats, the embryo in the eggs develop into the earliest larval stage, which is known as *Cysticercus fasciolaris*. At this stage, hooks exist in order to poke the muscle tissues of the wild rats and dig into the liver through the gut and bloodstream. Later, the larvae will emerge into the stage of strobilocerci. At this stage, fluid-filled cysts are produced and comprise of a

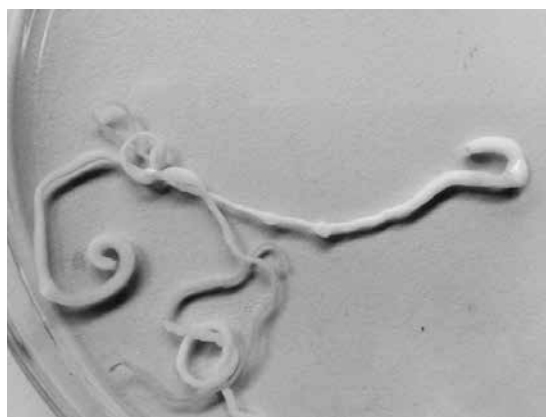
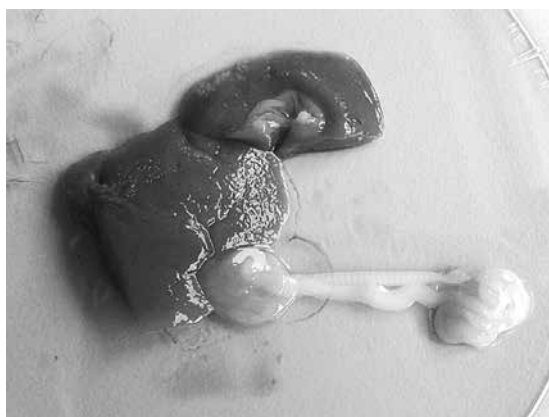
scolex which adheres to the mucosa and connects to its terminal bladder until the larvae matures (Rodríguez-Vivas *et al.*, 2011).

The adult of this parasitic tapeworm is a hermaphrodite. Hence, the eggs could possibly develop into mature worms with the strobila (the body) that contains numerous proglottids (the segments) with both male and female reproductive organs.

In fact, wild rats are responsible for the transmission of a number of diseases. Earlier studies by the Veterinary Research Institute, Ipoh has shown that from ten rats caught, seven are mite carriers and four of them are carriers of several bacterial species such as *Leptospira* sp., *Enterococcus* sp., *Escherichia coli* and *Mycoplasma arthritidis* (Premaalatha *et al.*, 2010). Besides that, a study by Mohd Zain *et al.*, 2012 has found that 346 *Rattus rattus* (black rat) and 104 *Rattus norvegicus* (brown rat) caught in Kuala Lumpur were positive with *Taenia taeniaeformis* (60%). In 2009,

Paramesvaran *et al.*, reported 24 out of 30 rodents from five wet markets (Chow Kit, Dato Keramat, Setapak, Jinjang and Kepong) in Kuala Lumpur were positive for cestode *Taenia taeniaeformis*. Thus, the animals that eat wild rats infected with *Taenia taeniaeformis* such as cats would act indirectly as definitive host of *Taenia taeniaeformis*. The cats would be infected by this parasitic tapeworm after ingestion and continue to defaecate infective proglottids containing eggs into the environment. Besides that, the infection of *Taenia taeniaeformis* is classified as asymptomatic since there is no specific sign observed in infected cats.

However, the infected cats may start to lose weight and vomit. This is because, the tapeworms feed on the nutrients from the cats leading to malnutrition in the infected cats. In addition, tapeworms can also be found around the anus of cats and in their faeces. Since a high percentage of rats are infected with taeniasis, it may be



**Figure 1.** Tapeworm *Taenia taeniaeformis* from the cyst in the liver of wild rat

useful to check faecal samples of domestic cats in the vicinity for tapeworm eggs. Faecal floatation technique is conducted in order to identify either the segmented proglottids or eggs in the faecal samples. The positive faecal floatation analysis would show either spherical eggs with a diameter of 31  $\mu\text{m}$  to 36  $\mu\text{m}$  or the shedding of segment in the faecal samples.

The infected cats could be treated by several prescribed drugs. Cestex, also known as epsiprantel, is a specific drug used to get rid of the *Taenia taeniaeformis* in infected cats. It immediately removes the tapeworms in the gastrointestinal tract as this drug persists within the tract and has minimal rate of being digested. Besides that, praziquantel is widely used in killing of wide range of tapeworms. Animals infected by *Taenia taeniaeformis*, even the wild rats, could be treated effectively by a single dose of praziquantel (Thomas and Gonnert, 1977).

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RE# MJVR - 0005-2015

## **AWARENESS OF OPERATORS ON THE REQUIREMENTS AND PROCEDURES FOR ANIMAL QUARANTINE AND THE DISTRIBUTION OF TEMPORARY ANIMAL QUARANTINE STATIONS IN PENINSULAR MALAYSIA**

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**Abstract.** Temporary quarantine stations (TQS) are transitory premises that have been approved to facilitate the quarantine of imported live animals in Malaysia. These stations must abide to the standard operating procedures (SOP) for animal quarantine as outlined by the veterinary authority in Malaysia. However, the level of awareness for the quarantine procedures among the TQS operators and managers has not been assessed. This study was conducted to describe the distribution of the TQS in 2012-2013 and the level of awareness among its operators on the quarantine procedures and the fundamental requirements for quarantine establishments. Eight TQS from 25 were selected and operators or managers were interviewed using a questionnaire and the facility was visited. The study found that majority (82.5%) of the TQS operators were aware of the quarantine procedures but the auditors from the veterinary authority revealed vice versa.

*Keywords:* temporary animal quarantine stations, awareness, quarantine procedures, Peninsular Malaysia

### **INTRODUCTION**

The animal quarantine station is the first point of entry for imported live animals and serves as an important barrier for the country against transboundary diseases that could be introduced via imported animal consignments. An animal quarantine station also potentially harbours various pathogens that could be transmitted from one consignment to another. Therefore, adherence to the quarantine procedures is important to minimise the risk of disease dissemination. Temporary quarantine stations (TQS) are transitory premises that have been approved to facilitate the quarantine of imported live animals. This premises supports the government quarantine station which has limited holding capacity of live animals especially

during the festive seasons. TQS was initially suggested and approved because of the limited capacity to quarantine live animals at the government quarantine stations. TQS is perceived to facilitate the quarantine process and prevent smuggling of animals into the country. These stations must abide to the quarantine procedures outlined by the veterinary authority.

Malaysia has been dependent on FMD endemic countries such as Thailand to import live cattle. Countries such as Australia that is FMD free have been the major exporter of live cattle to Malaysia but in recent years offered higher prices for cattle therefore making it less affordable for Malaysians. Importing live cattle from FMD-endemic countries increases the risk of introducing the disease to the local animal population if quarantine procedures are not abided to. Therefore this study was conducted to examine the temporary quarantine stations operators' awareness on cattle quarantine procedures and the fundamental requirements for quarantine establishments. This paper also describes the TQS distribution in the peninsula between 2012 and 2013.

## MATERIALS AND METHODS

A cross-sectional study was designed where 8 of 25 TQS that were accessible in Peninsular Malaysia and operating at the time of the study was selected and visited by the researcher. Two sets of questionnaires were developed to address the objectives of the study: a standard

questionnaire to assess the quarantine station managers/operators knowledge on essential quarantine procedures and a set of questionnaire to assess the perception of the veterinary officers involved in auditing on the level of quarantine awareness among the TQS operators. This questionnaire seeks their opinion on the compliance of the operators to the existing rules and regulations set by the Department of Veterinary Services Malaysia (DVS). The items in questionnaires used Likert scale measurement; 1 indicate highly disagree, 2 as disagree, 3 as neutral, 4 as agree and 5 as highly agree.

The locations of TQS that existed at the point of the study (2012-2013) were describe based on updated records from the Quarantine Services and Import and Export Section (SQIE), DVS and were plotted by using Google Map.

## Data analysis

The data were entered and managed in Excel and analysed using descriptive statistics.

## RESULTS

Figure 1 shows the distribution of the approved TQS. The concentration of TQS is higher in the northern part of peninsula, especially in Perlis (n=10) and Kelantan (n=6) states as these are the two most important entry points into Malaysia for consignments from Thailand and other countries from the north



**Figure 1** Distribution of approved temporary quarantine stations in Peninsular Malaysia (Portal Rasmi Jabatan Perkhidmatan Veterinar 2014)



**Table 1.** Availability of good biosecurity elements in selected TQS during on-site visit

	Biosecurity elements	TQS A	TQS B	TQS C	TQS D	TQS E	TQS F	TQS G	TQS H
a)	Signboard of 'Kuarantin Sementara Jabatan Perkhidmatan Veterinar'	✓	✓	✓	✓	✓	✓	✓	✓
b)	Vehicle dip facility	✓		✓	✓		✓		
c)	Waste management system	✓		✓					
d)	Location of the TQS is isolated from the housing community	✓		✓					
e)	Sanitary conditions inside the TQS premise	✓							
f)	Strong building structure	✓		✓					

During the visit, a list of important biosecurity elements was assessed as shown in Table 1. Firstly, the signboard indicating that it is a TQS '*Kuarantin Sementara Jabatan Perkhidmatan Veterinar*', is compulsory to be displayed at the TQS and must be visible throughout the quarantine process and removed when the managers are no longer actively importing cattle. This signboard helps DVS in differentiating the approved from the non-approved premises. Secondly, the vehicle dip facility at the main entrance of TQS must be available. Four out of 8 TQS (50%) did not have operational vehicle dipping facility. Thirdly, good waste management system is monitored regularly by DVS because based on the SQIE official record; most of the TQS are located near to residential areas. Only 2 out of 8 TQS (25%) have good waste management system as indicated by sewage treatment pond(s). Others appear not to have any type of acceptable sewage management system. It was observed that

the sewage from the premises drained into poorly managed and maintained ponds. As a result, the sewage spilled out to the surrounding areas and produced foul smell that attracted flies. Out of 8, only 2 TQS (25%) were located away from residential areas. Seven out of 8 TQS (87.5%) were observed to have poor sanitary conditions with heavy loads of faecal material on the floor. According to the managers, cleaning activity is only conducted before a new cattle consignment arrives for quarantine. No cleaning activity is conducted during and throughout the quarantine period.

Finally, only 3 out of 8 TQS were properly built with good physical structures using strong wood or iron frames. All the sampled TQS were previously holding yards of feed-lot premises. Hence, the owner or the managers rarely maintained the building to save costs. No auditing is required or performed by the veterinary authority for feed-lot premises.

### **Level of awareness and knowledge on the requirements and procedures for quarantine stations among the TQS managers**

Table 2 shows the results of the questionnaire used during the onsite visits to selected TQS. The purpose of the questionnaire was to assess the awareness of managers and operators on the biosecurity requirements of quarantine stations.

On average, 11.88% of the managers were ignorant on the elements of biosecurity and operational management required for a quarantine station. A small percentage (5.63%) were unsure but most (82.5%) appeared to be confident in their knowledge on the matter. A quarter (25%) of the respondents agreed that they did not have good knowledge on how to manage a TQS. More than 37% did not understand the requirements based on expand 2011. A quarter of respondents did not have any systematic documentation and records of consignments and activities at the quarantine station including blood results and FMD cases on the premises. More than 37% were not sure or were not aware that consignments exposed to FMD infection can only be released to designated areas approved by the DVS.

Table 3 shows the findings from the questionnaire given to the auditors to seek their opinion on the level of biosecurity and managerial knowledge among the TQS managers. The questionnaire was created to study the relationship between the TQS

and local FMD outbreaks and also to obtain information regarding the perception of the auditors on the TQS biosecurity management and level of awareness and understanding among the station managers/operators. The questionnaire consisted of 3 different parts; first was basic perception of the auditors to TQS. It covered the aspect of how far the auditors knew the managers' understanding to the APTVM SKH(S) regulations. The second part was to seek the opinions of the auditors on specific biosecurity and infrastructure requirements of the TQS based on their evaluation. The third part covered the observation of the auditors on the management of imported animals and the availability of the necessary records kept by the managers.

The first part of the questionnaire showed that 74% of the auditors disagreed or were unsure as to whether the managers really understood the APTVM SKH(S) 2011 rules and regulations. Only 26% agreed that the managers understood the APTVM SKH(S) 2011. The second part tested the opinion of the auditors on the suitability of the TQS infrastructure based on the biosecurity needs. More than 80% of the auditors disagreed or were unsure that the infrastructures of the TQS were suitable. On the aspect of managing imported animal, majority (80%) of auditors disagreed or were unsure that managers have good management records or if records were available. In addition, majority (60-80%) of auditors were not sure or disagreed that managers have

**Table 2:** Level of awareness on quarantine station requirements and procedures for quarantine stations among the TQS managers

List of the questions	Scale (%)		
	Disagree	Unsure	Agree
a. TQS is registered with DVS	0/8 (0%)	0/8 (0%)	8/8 (100%)
b. Have good knowledge on TQS management	2/8 (25%)	0/8 (0%)	6/8 (75%)
c. Will give full cooperation during DVS TQS annual auditing	0/8 (0%)	0/8 (0%)	8/8 (100%)
d. TQS establishment supports our cattle industry growth	0/8 (0%)	2/8 (25%)	6/8 (75%)
e. Procedures implemented in TQS is same with GQS expand	0/8 (0%)	1/8 (12.5%)	7/8 (87.5%)
f. Understands the procedures in <i>translate in English</i>	3/8 (37.5%)	0/8 (0%)	5/8 (62.5%)
f. Have systematic cattle importation records	2/8 (25%)	0/8 (0%)	6/8 (75%)
. Have cattle importation records for every imported cattle consignment: original copy of veterinary health certificate from the exporting countries, FMD vaccination record and original copy of DVS import permit	1/8 (12.5%)	0/8 (0%)	7/8 (87.5%)
g. Keep all blood sampling tests records	2/8 (25%)	0/8 (0%)	6/8 (75%)
. Any FMD occurrence in the premise is recorded	2/8 (25%)	1/8 (12.5%)	5/8 (62.5%)
. Every fatal case due to FMD is recorded	2/8 (25%)	1/8 (12.5%)	5/8 (62.5%)
h. All records mentioned in g) to k) are readily accessible when requested	2/8 (25%)	0/8 (0%)	6/8 (75%)
i. Operators -understands the clinical signs of FMD	0/8 (0%)	0/8 (0%)	8/8 (100%)
. veterinary officer will be informed as soon as possible if any FMD clinical signs are observed in the particular consignment	0/8 (0%)	0/8 (0%)	8/8 (100%)
j. The quarantine period will be prolonged whenever clinical signs of FMD are found in the consignment	0/8 (0%)	2/8 (25%)	6/8 (75%)
k. On the day of arrival, cattle with FMD clinical signs will be isolated	0/8 (0%)	0/8 (0%)	8/8 (100%)
. Consignment proven to be exposed to FMD infection will only be released to designated location approved by DVS	2/8 (25%)	1/8 (12.5%)	5/8 (62.5%)
. DVS is always informed of every cattle importation activity	0/8 (0%)	0/8 (0%)	8/8 (100%)
l. All imported cattle will be quarantined for 10 days according to the DVS import protocol	0/8 (0%)	0/8 (0%)	8/8 (100%)
m. Each imported cattle consignment is regularly by the State DVS officers	1/8 (12.5%)	1/8 (12.5%)	6/8 (75%)
Average percentage	11.875%	5.625%	82.5%

**Table 3.** Level of awareness on the quarantine station requirements and procedures among the TQS managers from the veterinary authority auditors' perspectives

Question list	Scale (%)		
	Disagree	Unsure	Agree
<b>a) Basic operational management observation and perception of TQS</b>			
i. All TQS are registered and licensed under DVS	0/6 (0%)	3/6 (50%)	3/6 (50%)
ii. TQS manager and staff have good understanding on how to run a cattle quarantine station	2/6 (33.3%)	4/6 (66.7%)	0/6 (0%)
iii. Managers of TQS give full cooperation during inspection	0/6 (0%)	3/6 (50%)	3/6 (50%)
iv. TQS practices the same procedure to examine imported animals from FMD as do the GQS	0/6 (0%)	4/6 (66.7%)	2/6 (33.3%)
v. TQS help the cattle industry in improving the cattle population numbers	0/6 (0%)	3/6 (50%)	3/6 (50%)
. TQS managers and staff obey the procedures required in <i>APTVM SKH(S)</i>	3/6 (50%)	3/6 (50%)	0/6 (0%)
vi. TQS managers are highly knowledgeable in cattle quarantine procedures	2/6 (33.3%)	4/6 (66.7%)	0/6 (0%)
<b>Average percentage</b>	<b>17%</b>	<b>57%</b>	<b>26%</b>
<b>b) Biosecurity and infrastructure evaluation</b>			
i. TQS have one entrance to its premise	2/6 (33.3%)	2/6 (33.3%)	2/6 (33.3%)
ii. Building design and layout is suitable for a quarantine station	0/6 (0%)	0/6 (0%)	6/6 (100%)
. Vehicle dip (properly built and disinfectant regularly added on) is available	2/6 (33.3%)	4/6 (67.7%)	0/6 (0%)
iii. Foot dips (properly built, with regular usage of disinfectant) is available	2/6 (33.3%)	4/6 (67.7%)	0/6 (0%)
. Feeding store room is clean, no disease vector like mice, cockroaches, and locked	3/6 (50%)	3/6 (50%)	0/6 (0%)
iv. Waste management (proper drainage, isolated waste pond) system is available	3/6 (50%)	3/6 (50%)	0/6 (0%)
v. Dead animals disposal management (proper place and not attracting flies, far from the quarantine buildings) is available	1/6 (16.7%)	4/6 (67.7%)	1/6 (16.7%)
. Quarantine station with a Generally acceptable in term of hygiene and cleanliness	1/6 (16.7%)	5/6 (83.3%)	0/6 (0%)
. The location of TQS is strategic and helps in preventing disease spread	2/6 (33.3%)	4/6 (67.7%)	0/6 (0%)
<b>Average percentage</b>	<b>30%</b>	<b>54%</b>	<b>16%</b>

Table 3 continues next page

**Table 3.** (continuation)

Question list	Scale (%)		
	Disagree	Unsure	Agree
<b>c) Management of imported animal</b>			
i. Scale of management and availability of records			
. Entry of imported animals	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Copy of exporting countries documentation record (health certificate, vaccination, condition upon departure	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Copy of import permit	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
ii. Daily expand record	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Treatment record (if any)	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Vaccination (in Malaysia) record	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Laboratory test record	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Morbidity and mortality record	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
. Movement permit record	2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)
iii. Overall health condition of the animals in the quarantine station is good on arrival	0/6 (0%)	4/6 (63.7%)	2/6 (33.3%)
iv. Managers of expand have a good knowledge on the FMD clinical signs	0/6 (0%)	4/6 (63.7%)	2/6 (33.3%)
v. The TQS has an attending veterinarian that manages the health of the imported animals	0/6 (0%)	5/6 (83.3%)	1/6 (16.7%)
vi. If an animal show signs of FMD, the managers would make sure that the quarantine period is prolonged	1/6 (16.7%)	3/6 (50%)	2/6 (33.3%)
vii. Animals with clinical signs of FMD are treated by a veterinarian	1/6 (16.7%)	3/6 (50%)	2/6 (33.3%)
. Animals with clinical signs for FMD upon arrival will be culled and disposed	1/6 (16.7%)	4/6 (63.7%)	1/6 (16.7%)
. Animals recovered from FMD will be released to its final destination in the peninsula	0/6 (0%)	5/6 (83.3%)	1/6 (16.7%)
. Cattle seropositive for FMD (field infection) will be treated and released to its final destination in the peninsula	0/6 (0%)	5/6 (83.3%)	1/6 (16.7%)
viii. Managers are committed attitude in helping the country to prevent infected animals from entering Malaysia	1/6 (16.7%)	5/6 (83.3%)	0/6 (0%)
<b>Average percentage</b>	<b>20%</b>	<b>60%</b>	<b>20%</b>

knowledge on the clinical signs of FMD, have veterinarians attending the animals and that animals with clinical signs of FMD should be culled and disposed. They were also not sure where the FMD positive (expand or clinical signs) cattle were distributed to.

## DISCUSSION

The purpose of many TQS in the two bordering states near entry points is to avoid cattle consignments transported far into the peninsula before the quarantine process starts. The local veterinary authorities have not provided any guidelines or limits to where and how many TQS each state should have. Therefore each state veterinary service could choose to approve the establishment of TQS based on their needs and requirements. There are also TQS located far from the borders into the peninsula as in Perak (n=2) and Johor (n=1), which raises concern over the dissemination of diseases when potentially infected animals travel into the country via various modes of transportation. Even though the animal consignments could be sealed throughout its journey to its destined quarantine facility, the probability of pathogens spreading via faeces and urine cannot be ruled out.

Lack of knowledge and awareness among traders about FMD and the failure to appreciate that illegal movement of cattle have enormously contributed to the outbreaks of FMD among local animals. By law, traders may be charged for illegal

cattle movements if caught by the *Anti-Smuggling Unit* (UPP). The introduction of TQS is perceived to have reduced the smuggling of cattle to a certain extent, although data has not been properly documented nor reported. Unfortunately these premises are often left to operate with little supervision by the state authorities and with minimum regulatory appraisals. DVS importation protocol imposes 10 clear days of quarantine for cattle consignments originating from Thailand. Every GQS operates based on the same operating procedure; arrival at the quarantine station at Day 1, blood sampling at Day 2 to detect FMD virus antibodies and to further determine whether a positive result is natural or requires vaccination. On Day 2 also, FMD vaccine is given as P2 to boost the P1 injection as requested and assumed to be administered at the pre-exportation station in Thailand. No second blood monitoring of the protection level boost by P2 injection (DVS 2011) is required to be performed at the station. The daily physical examination is continued until Day 11 when the cattle consignment is released if the animal is found to be healthy. The TQS are supposed to follow the same procedures as GQS, however since its establishment in 2009, biosecurity and operational monitoring remains a large issue because of the lack of manpower at the level of veterinary authority. As a result, the operator/managers are very relaxed in their interpretation of rules and regulations leading to poorly managed quarantine stations.

It appeared that only a small proportion (16%) of TQS managers agreed that they may not have good knowledge or were aware of the biosecurity needs and requirements to operate quarantine stations. This showed that some of the TQS managers did not know the existing regulations which they have to follow and implement at their own stations. Ironically, most (82.5%) of the TQS managers believed that they were aware of the regulations and fully implemented them at their stations. However, in this study, the researcher's visits to the TQS sites proved that the reality were contrary to what was recorded in the questionnaires. A large percentage of TQS were poorly managed and did not have the basic requirement necessary for animal quarantine facility operations. This observation is consistent with the opinion and perception of the veterinary auditors interviewed in this study. Most of the auditors (60-80%) were not convinced (unsure and disagree) that the manager/operator had the necessary knowledge on the basic requirements to operate an animal quarantine establishment. And most auditors were not convinced that the operators had adequate knowledge about FMD and its clinical signs.

Assessing the quarantine procedures effectiveness in developing countries is a difficult task due to unavailable or poorly recorded data (Wongsathapornchai *et al.* 2008). According to the OIE, animal quarantine is a crucial process to defend the country from potential harmful biological threats. The level of compliance of the

quarantine stations profoundly depends on the knowledge and understanding of the station managers/operators on the impact from the magnitude of diseases should imported infected animals be introduced to the local animal population. Therefore, their understanding and knowledge on basic regulatory and biosecurity requirements for a quarantine facility is necessary to ensure that quarantine procedures can efficiently minimise disease introduction and spread. According to Thailand DLD Animal Epidemic Act 1956 (amended 2003), any livestock being moved into Thailand expand Zone, must be quarantined at the place of origin for at least 21 days but the effectiveness of Thailand's quarantine procedure was difficult to estimate due to lack of available data (Wongsathapornchai *et al.* 2008).

It is uncertain if the quarantine measures as subscribed by the APTVM SKH(S) 2011 are practiced in TQS as no close monitoring have been performed on these premises since temporary stations were first allowed to operate. The veterinary authority faces lots of challenges such as scarcity of personnel and time to conduct assessment and monitoring of these facilities. As a result, many premises although approved to operate, may not necessarily meet the basic requirements for quarantine operations. In addition, this study could not gain enough cooperation from the operators because most of them lack awareness and basic knowledge on the essentials of biosecurity.



## CONCLUSION

Animal quarantine stations serve a vital mode of protection for the country from the introduction of potential animal diseases. The study found that the reported level of biosecurity knowledge and understanding among the operators of TQS contradicts the observation made in this study by authorised auditors. It is suggested that the TQS operators be educated on the basic quarantine facilities requirements before their premises is approved for operation. Regular assessment and monitoring of these facilities are needed to ensure compliance to the procedures. A standard operating procedure must be established by the authorities and followed

by quarantine managers to ensure a high level of compliance to related regulations. The veterinary authority need to look into creating awareness and training the operators so that they are educated on the essentials of good quarantine practices in order to fulfil quarantine measures.

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RE# MJVR – 0006-2015

## GROSS AND HISTOMORPHOLOGY OF THE OVARY OF BLACK BENGAL GOAT (*Capra hircus*)

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**ABSTRACT.** Ovary plays a vital role in the reproductive biology and biotechnology of female animals. In this study, both the right and left ovaries of the Black Bengal goat were collected from the slaughter houses of different Thanas in the Mymensingh district. For each of the specimens, gross parameters such as weight, length and width were recorded. Then they were processed and stained with H&E for histomorphometry. This study revealed that the right ovary ( $0.53 \pm 0.02$  g) was heavier than the left ( $0.52 \pm 0.02$  g). The length of the right ovary ( $1.26 \pm 0.04$  cm) was lower than the left ( $1.28 \pm 0.02$  cm) but the width of the right ( $0.94 \pm 0.02$  cm) was greater than the left ( $0.90 \pm 0.03$  cm). The diameter of ovarian follicles in the cortex was measured as primordial  $39.6 \pm 6.61$   $\mu$ m, primary single layer  $54.0 \pm 4.06$   $\mu$ m, primary multi-layer  $147.6 \pm 11.04$   $\mu$ m, secondary with C-shaped antrum  $449.5 \pm 75.71$   $\mu$ m and graafian  $1.3 \pm 0.20$  mm. In the graafian follicle, the thickness of the granulosa cell layer was  $79.2 \pm 11.04$   $\mu$ m, theca interna  $75.76 \pm 6.82$   $\mu$ m, theca externa

$130.07 \pm 12.53$   $\mu$ m and the oocyte diameter was  $109.8 \pm 5.75$   $\mu$ m. These results will be helpful to manipulate ovarian functions in small ruminants.

**Keywords:** Morphometry, ovarian follicles, cortex, medulla, oocyte.

## INTRODUCTION

Black Bengal goat is the national pride of Bangladesh. The most promising prospect of Black Bengal goat in Bangladesh is that this dwarf breed is a prolific breed, requiring only a small area to breed and with the advantage of their selective feeding habit with a broader feed range. It is very popular to consumers for its delicious and tender meat. Its skin is also highly valued in the world market due to some unique features of yielding fine leather that is light in weight and fine in texture. Considering the paramount importance and bright prospects of Black Bengal goat in Bangladesh, goat production level should be maintained properly by increasing fertility and conception rate.

The ovary is the key female reproductive organ of all the vertebrates. The reproductive physiology of goat is least understood compared to cattle, sheep and pig. A search of the literature for specific and detailed information on the ovary of goat is not rewarding. Description of goat is usually made as if it is identical with sheep (Smith, 1986). Some work on the morphology, physiology and pathology of reproductive organs of the goat (Epelu-Opio *et al.*, 1988; Moreira, *et al.*, 1991; Sattar and Khan, 1988; Torres and Badiangan, 1989) have been reported in many countries. But no comprehensive study has yet been undertaken on the ovary of Black Bengal goat in Bangladesh. Therefore, the study was designed to clarify the morphology and morphometry of the ovary of Black Bengal goat. The knowledge of the present study will contribute significantly in the reproductive biology and biotechnology of small ruminants.

## MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

### Collection and transportation of ovaries

Both the right and left ovaries of the female non-gravid adult Black Bengal goats (1-2 years of age) were collected

from the slaughter houses of different Thanas in Mymensingh district. The ovaries were then kept in a collection vial containing 0.9% physiological saline in a Thermos flask at 25 °C to 30 °C and transported to the laboratory within 4 to 5 hours of slaughter. The ovaries were then transferred to sterilized Petri dishes and rinsed thoroughly by physiological saline at 25 °C before further processing.

### Measurement of weight, length and width

After trimming individually, the right and left ovaries were weighed with the help of an electric balance. The length and width were measured with the help of measuring scale.

### Histomicrometry

The ovarian tissues were fixed by Bouin's fixative for 4 hours and then processed by routine paraffin embedding technique. The paraffin sections were cut by microtome (5-6  $\mu$ ) and stained with routine Hematoxylin and Eosin (H&E) for histomorphometrical analysis. The diameter of ovarian follicles and thickness of follicular layers along with oocyte diameter were measured by micrometry methods.

### Data analysis

All the data were recorded in a tabular form and analyzed by Student's *t*-test.

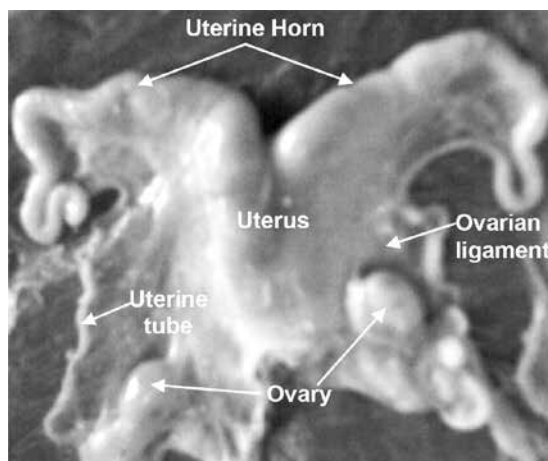
## RESULTS AND DISCUSSION

### Gross study of the ovary

The ovaries were found almond-shaped, pale colored structures situated in the edge of the mesovarium near the lateral margin of the pelvic inlet. This report corresponds to the report of Getty, 1975; May, 1970. Each ovary had an irregular surface by follicles of various sizes projecting from the surface. It also supports their observations. The weight, length and width of the left ovary were  $0.52 \pm 0.02$  g,  $1.28 \pm 0.02$  cm and  $0.90 \pm 0.03$  cm and of the right were  $0.53 \pm 0.02$  g,  $1.26 \pm 0.04$  cm and  $0.94 \pm 0.02$  cm, respectively. The length of the left and right ovaries were  $1.71 \pm 0.27$  cm and  $1.73 \pm 0.27$  cm, respectively in Nigerian goats (Adigwe and Fayemi, 2005); 1.5 cm reported for small ruminants (Sisson and Grossman, 1975); 2.2 cm reported in goats (Smith, 1986). The uterine extremity of the ovaries was connected with the extremity of the horn of uterus by a proper ligament of the ovary. There was no demarcation between the horn of the uterus and the very flexuous uterine tubes (Figure 1).

### Microscopic study of the ovary

The ovary consisted of two distinct zones, peripheral cortex and central medulla. This report is in agreement with Dellmann, 1971; Banks, 1986.



**Figure 1.** Photograph of the ovary and its associated structures of adult (2 years of age) Black Bengal goat

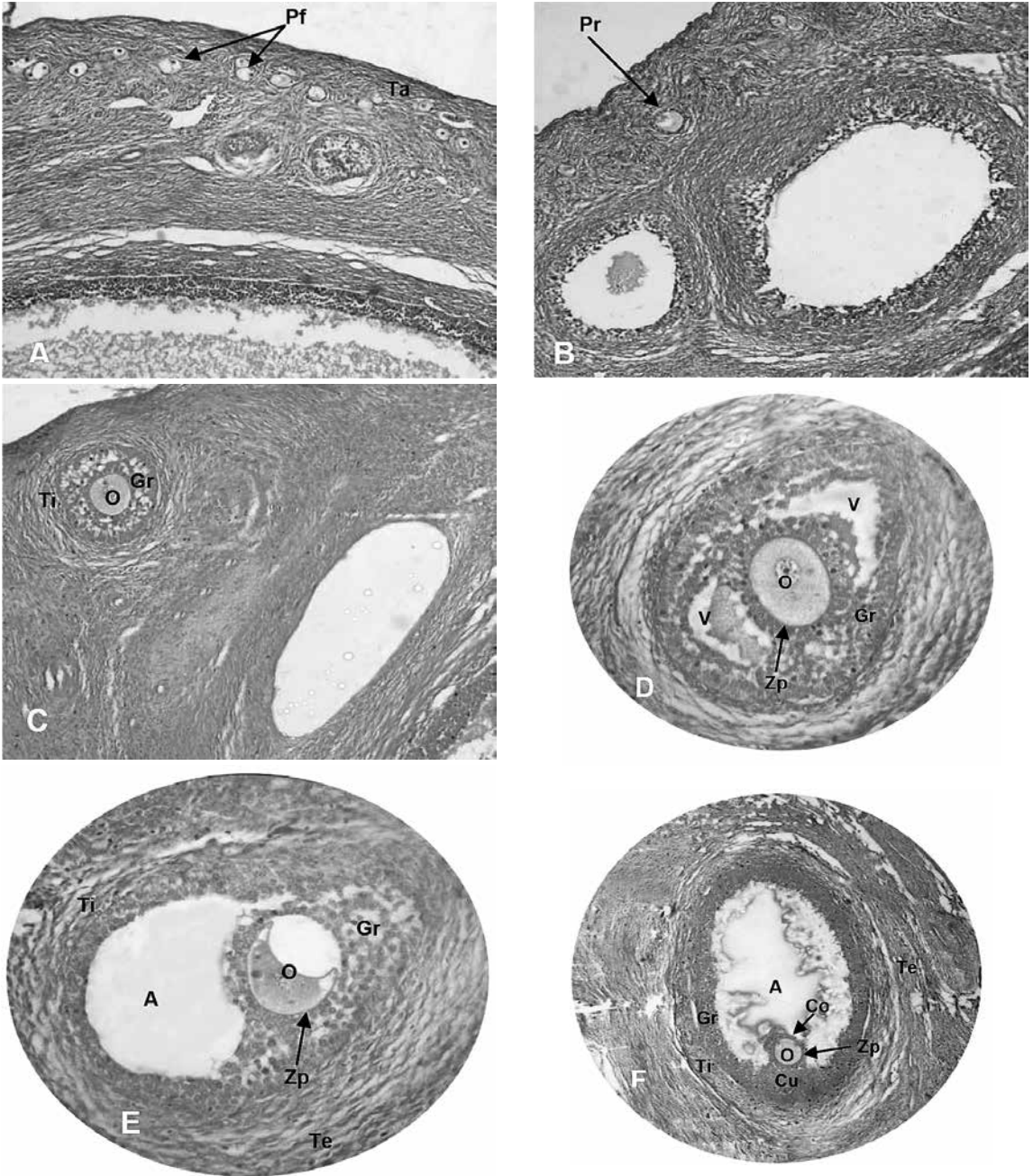
### A. Ovarian cortex

**Germinal epithelium:** The ovary was covered by a surface epithelium of simple cuboidal cells.

**Tunica albuginea:** Underlying the surface epithelium was a capsule of dense irregular connective tissue.

**Follicles:** Follicles of various stages of development were found in the cortex surrounded by the stroma. They were distinguished as:

*i. Primordial follicles:* They were located immediately beneath the tunica albuginea. Each was found to contain an oocyte with a large nucleus lined by simple squamous epithelium in contact with the smooth surface of the oocyte (Figure 2A). The diameter of primordial follicle was  $39.6 \pm 6.61$   $\mu$ m.



**Figure 2.** Photographs showing ovarian follicles in the cortex (H & E). A. Primordial follicle X10 B. Primary follicle (single layer) X10 C. Primary follicle (multilayer) X10 D. Secondary (vesicular) follicle X40 E. Secondary follicle with C-shaped antrum X40 F. Graafian follicle X40. Pf= Primordial follicle, Pr= Primary follicle, Ta= Tunica albuginea, Gr= Granulosa cells, Zp= Zona pellucida, Co= Corona radiata, Cu= Cumulus oophorus, O= Oocyte, V= Vesicle, A= Antrum, Ti= Theca interna, Te= Theca externa



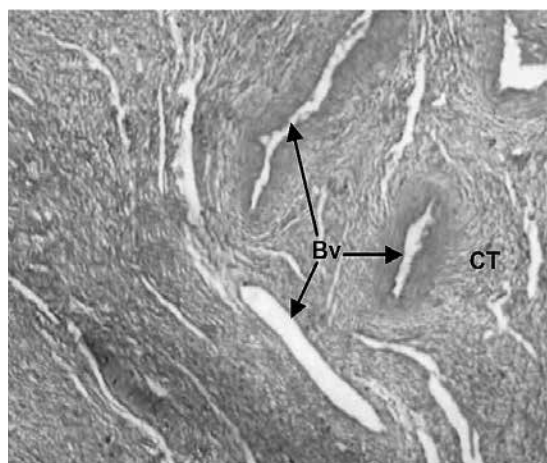
*ii. Growing follicles:* The growing follicles were found into the deep layers of the cortex. In primary follicles, the follicular cells were found to columnar with alteration of primary oocyte. The diameter of primary follicle, single and multi-layer were  $54.0 \pm 4.06 \mu\text{m}$  and  $147.6 \pm 11.04 \mu\text{m}$ , respectively (Figures 2B and 2C). The secondary follicles were identified by an increase in follicular cell population associated with the primary oocyte and development of zona pellucida between primary oocyte and follicular cells (Figure 2D). The stromal cells differentiated into theca interna and externa. The thecal cells were separated by a basement membrane. The theca interna consisted of large, epitheloid cells and an extensive vascular network. The theca externa was fibroelastic layer of cells. The diameter of secondary follicle with C-shaped antrum was found as  $449.5 \pm 75.71 \mu\text{m}$  (Figure 2E).

*iii. Graafian follicle:* This was accompanied by the continued growth of the follicle. The primary oocyte was still surrounded by a cluster of granulosa cells that was continuous with the peripherally displaced membrane granulosa. The mound of cells was cumulus oophorus. The granulosa cells immediately adjacent to the primary oocyte found columnar that were oriented radially known as corona radiata. The cells of the cumulus oophorus constituted a visceral layer of granulosa cells separated by follicular antrum from the parietal layer of granulosa cells. The parietal layer was separated from the theca interna by a basement membrane. This

preovulatory follicle was the graafian or mature follicle (Figure 2F) which extended from a protrusion at the surface to the depths of the cortex. In the graafian follicle, the thickness of granulosa cell layer was  $79.2 \pm 11.04 \mu\text{m}$ , theca interna  $75.76 \pm 6.82 \mu\text{m}$  and theca externa  $130.07 \pm 12.53 \mu\text{m}$  where as the oocyte diameter was  $109.8 \pm 5.75 \mu\text{m}$ . The diameter of ovarian follicles and thickness of graafian follicular layers (granulosa, theca interna and theca externa) along with oocyte diameter differ from the report of Mohammadpour, 2007 in Iranian native goats.

### **B. Ovarian Medulla**

The medulla consisted of dense irregular connective tissue with extensive network of vessels and nerves (Figure 3).



**Figure 3.** Photograph of medulla in the ovary of Black Bengal goat (H & E) X10. Bv= Blood vessels, CT= Connective tissue



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## FEASIBILITY OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY FOR PREDICTING AMINO ACIDS COMPOSITION IN EDIBLE BIRD'S NEST

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**Abstract.** A preliminary study of near-infrared reflectance spectroscopy (NIRS) for the feasibility of analytical monitoring of amino acids and total protein compositions in edible bird's nest (EBN) was conducted. The training (n=134) and validation sets to develop the equations were built with local unprocessed EBN samples sourced from different states of Malaysia. The regression method employed was modified partial least-squares (MPLS). The values of standard error for cross validation (SECV) and the coefficient of determination ( $r^2$ ) of the calibrations of these constituents for use to predict of amino acids in EBN were determined, but with a low predictive ability. To find an acceptable accuracy for each constituent is to increase the number of training samples. The findings, however, showed a potential alternative for the implementation of near-infrared reflectance spectroscopy technology in this field of analysis.

**Keywords:** edible bird's nest (EBN), near-infrared reflectance spectroscopy (NIRS), amino acids composition

## INTRODUCTION

Edible bird's nest (EBN) refers to the nest produced by several different swiftlet species. The nests are constructed from the saliva of swiftlets, which has been secreted from the pair of sublingual salivary gland of swiftlets during nesting and breeding season. EBN consists of high valued glycoprotein rich with amino acids, carbohydrate, calcium, sodium and potassium (Norhayati *et al.*, 2010). Near-infrared reflectance spectroscopy technique has extensive application for the analysis of constituents of agricultural crops, feeds, and food (Roberts *et al.*, 2004). At present, there is a dearth of published data on this subject matter; this preliminary study therefore was to explore the possibility of near-infrared reflectance spectroscopy analysis to predict the value of amino acids composition and total protein of the EBN sample matrix .

## MATERIALS AND METHODS

### EBN Samples

A total number of 134 commercial unprocessed EBN samples were received throughout the year for routine analysis of amino acids and other parameters by this laboratory. The sources of these samples were mostly from the southern zone of the Peninsular Malaysia. Each remaining sample was crushed into fine grains using hand mortar, kept in 50 mL plastic test tube each, capped and stored at 4 °C storage prior to near-infrared reflectance spectroscopy analysis.

### Reference Analysis

(i) Protein analysis was performed according to Kjeldahl method, using 6.25 as a conversion factor (AOAC method, 1995). Briefly, finely grounded EBN sample (0.1 g) was digested with 12 ml concentrated sulphuric acid in the presence of a catalyst (3.5 g potassium sulphate, 0.4 g copper sulphate) to convert sample nitrogen to ammonium sulphate. The acid solution was made alkaline with 40% sodium hydroxide solution. The ammonia was distilled and collected in an excess of 4% boric acid solution followed by titration with 0.2N sulphuric acid solution.

(ii) The 18 amino acids of the samples were analyzed based on Waters method of analysis (Waters AccQ-Tag Chemistry Package Instruction Manual) using Waters Aquity H-Class UPLC System

with photodiode/fluorescent detector. The amino acids determined include: histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine, aspartic, glutamic, serine, glycine, alanine, proline, tyrosine, cystine. Total amino acids were extracted by acid hydrolysis while tryptophan was extracted by alkaline hydrolysis.

### Near-Infrared Reflectance Spectroscopy Analysis

A FOSS NIRSystems model 5000 (Silver Spring, USA) was used to measure reflectance spectra of the same set of samples from 1100 to 2500 nm. A standard ring cup with 4.7 cm diameter was used for the measurement. The reflectance spectra were collected in duplicates. Calibrations were developed using WinISI II software version 1.5e. Near-infrared reflectance spectroscopy equations for the parameters under study were obtained using modified partial least squares (MPLS) regression. Outliers were removed for selection of calibration and validation sets. Predicted results were summarized as the standard error of cross validation (SECV).

## RESULTS AND DISCUSSION

In this study, several statistics of interest could be obtained. It is intended that the standard error of calibration (SEC) needs to be as small as possible. The second statistic of interest is the coefficient of determination ( $r^2$ ) between the spectra

**Table 1.** Cross validation of amino acid composition and total protein of EBN samples

Constituent	N	Mean	SEC	SECV	RSQ	1-VR
Total Protein	101	53.8277	0.4262	1.1715	0.8543	0.362
Proline	107	3.0185	0.2888	0.6177	0.8235	0.233
Aspartic acid	104	3.6459	0.3028	0.6001	0.8207	0.291
Glutamic acid	107	2.7825	0.2076	0.4052	0.8327	0.220
Serine	98	4.0711	0.2819	0.6486	0.8485	0.285
Glycine	90	1.2858	0.1347	0.4298	0.9109	0.365
Histidine	103	1.4046	0.1071	0.2447	0.8203	0.228
Arginine	105	4.5075	0.4965	1.15021	0.8578	0.474
Threonine	105	2.4639	0.2255	0.4968	0.8328	0.307
Alanine	103	1.1527	0.0904	0.2017	0.8109	0.203
Tyrosine	98	2.5676	0.2464	0.5119	0.7447	0.264
Valine	107	1.4756	0.1534	0.3184	0.8100	0.337
Methionine	120	0.3795	0.1176	0.2115	0.8433	0.568
Cystine	107	1.0283	0.2133	0.3939	0.8676	0.631
Isoleusine	106	0.6043	0.0633	0.1275	0.8229	0.394
Leusine	103	2.4445	0.2130	0.4678	0.8392	0.431
Phenylalanine	104	2.1802	0.1458	0.3904	0.8937	0.405
Tryptopan	114	0.6542	0.0791	0.1339	0.8403	0.547
Lysine	103	1.1455	0.1294	0.3011	0.8384	0.421

SEC = Standard error of calibration; SECV = Standard error of cross validation; RSQ = R square; 1-VR = 1-variance ratio

and the analytical values, whose values range from 0 to 1. A value of 1.0 indicates that all of the spectral differences between samples correspond perfectly with differences in analytical values. From this study,  $r^2$  values obtained for each constituent ranged between 0.74 to 0.91 (Table 1). The third statistic of interest is the proportion of variation explained by cross validation, “1-VR” (variance ratio) or the coefficient of determination of cross validation. This should be close to 1 and similar to the  $r^2$  of calibration. Therefore 1-VR is the coefficient of determination

( $r^2$ ) between the laboratory values and the predictions made during cross validation. The fourth statistic is the standard error of cross validation (SECV) that needs to be low values, similar to the standard error of calibration. Based on these statistics, the calibration equations obtained present a low predictive ability for the majority of constituents. According to their 1-VR values (<0.90), the equations for these analyses need to be further improved for quality assurance purposes.

One of the important parameters to be considered, beside homogeneity, is the

moisture content. The major components of food (water, protein, carbohydrates, and lipids) contain the overtones and combination of these molecules' fundamental vibrations particularly those involving hydrogen (Osborne, 1993). It is not sure whether a significant difference of moisture contents between each EBN's sample could give rise to a certain degree of inaccuracy between laboratory and near-infrared reflectance spectroscopy results. A different set of EBN samples (n=40) was tested for moisture content. The results obtained were between 8.73-18.63%, with an average of  $13.68 \% \pm 4.55$ . The tolerance for moisture content according to Malaysian standard (MS2334:2011) is <15%.

## CONCLUSION

In this feasibility study, the results demonstrate the capability of near-infrared reflectance spectroscopy technology to analyze important amino acids and total protein of unprocessed EBN samples. To increase the predictive ability more training samples need to be introduced in order to strengthen the quality of these equations. Currently, there is not much published data by other authors for the purpose of comparison, as well as the scarcity of EBN samples, the accuracy of the equations obtained in this study could not be confirmed.

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## **PATHOGENS ISOLATED FROM *Batagur affinis* (TUNTUNG SUNGAI) FROM CONSERVATION CENTRE FOR RIVER TERRAPINS IN 2014**

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**Abstract.** In 2014, a total of 16 river terrapins (*Batagur affinis*), locally called *tuntung sungai*, died due to various causes and a post mortem was carried out. Complete diagnostic evaluation of parasitological and bacteriological findings were recorded. Significant parasitological findings consisted of seven terrapins showing presence of Ascarid (*Sulcascaris sulcata*) and Strongyle (*Oesophagostomum* sp.) worms in the gut contents. Strongyle and strongyloides eggs were detected using McMaster's method on faeces of 12 terrapins. Bacterial cultures from organs indicated the presence of *E. Coli*, *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Aeromonas hydrophila* (4+) dan *E.Coli* (4+). This indicates that common parasites and bacteria are important in the conservation programmes for river terrapins and measures to curb the infection is highly recommended. Continuous data collection will enable its management and assessment in control programmes for a more effective conservation programmes.

**Keywords:** *Batagur affinis*, gastro intestinal helminthes, river terrapin

### **INTRODUCTION**

The *Batagur affinis* or southern river terrapin are commonly found in the rivers of Cambodia, Myanmar, Thailand and Malaysia. In Malaysia, it is called "tuntung sungai", and the rivers of Kedah, Perak and Terengganu are major nesting grounds though the population continues to dwindle despite conservation efforts undertaken by Malaysian Wildlife Department for over 20 years (3). One of the main reasons for the reduction in terrapin numbers is due to the threat of human activities such as overharvesting of adults and eggs coupled with habitat degradation. In efforts to maintain and increase the population of these terrapins, studies on breeding, management and environmental investigations into the nesting sites have been undertaken.

According to Ernst *et al.*, 2000; *B. affinis*, *B. baska*, and *B. borneoensis* are the only species living in tidal, brackish

areas of the estuaries of medium and large rivers. Rhodin *et al.*, 2010 reported that there are two subspecies of *Batagur affinis* namely *Batagur affinis affinis* and *Batagur affinis edwardmollis*. Terrapins are prone to bacterial and parasitic infections due to malnutrition and poor hygiene as they tend to become readily infected because of the polluted or contaminated aquatic environments. Common gastrointestinal parasites such as *Sulcascaris sulcata* have been recorded (5). An adult male measures about 9 cm long whereas a female is about 11 cm long and can be found in the stomach of terrapins. The terrapins are the definitive hosts while scallops and other mollusks are intermediate hosts, generally consumed by the terrapins. Eggs pass out in the faeces of infected terrapins and fall to the sea or river floor. The larva develops in the egg and undergoes 2 molts to the third larval (L3) stage. The L3 hatches from the eggs, beginning at 7 days after the egg is laid. The L3 are taken up by mollusks (scallops and possibly others) and go to the tissues. The L3 will molt to the L4 in the scallop in 3 to 4 months. When the infected mollusk is eaten by the terrapin, the L4 attaches to the stomach wall (at the esophago-gastric junction) and will molt to the adults in 7 days. Adults will become gravid in 5 to 6 months and produce eggs which will contaminate the aquatic environment. Apart from this, *Oesophagostomum* sp. and *Trichostrongylus* sp. also have been reported in turtles and terrapins. As for bacterial infections, *Pseudomonas* or *Aeromonas* related infections are common,

also due to the contaminated aquatic environment (6).

One of the crucial factors which can affect the productivity of terrapins are the diseases or infections which cause morbidity and mortality. In this regard, the Terrapin Conservation Centre in PKHL Bukit Pinang consistently monitors all mortality cases by sending dead terrapins for post mortem and disease investigations in order to elucidate the common infections in terrapins. This information is vital in formulating control programmes to prevent future infections and diseases as well as create awareness on the common infections in terrapins. Thus, this report is a collation of information from post mortem cases of *Batagur affinis* from the Conservation Centre for River Terrapins submitted for disease investigation and cause of death in 2014, conducted by the Regional Veterinary Laboratory, Bukit Tengah.

## MATERIALS & METHODS

### Management

The total population of the terrapins in Wildlife Reservation Centre Bukit Pinang is 494 and they are kept in an individual pond within same age group. There are total of 9 individual ponds in the Wildlife Reservation Centre. Newly hatched terrapins are grey in colour and each weighs around 100-120 g with a carapace length of 6-7 cm. Terrapins are fed with vegetation (*kangkung*), fish, banana and



pellets. The water supply for the Centre is from tap water. The terrapins are routinely given anthelmintics (fenbendazole).

Post mortem

Routinely, the management of the Conservation Centre will submit cases of mortality to the Regional Veterinary Laboratory, Bukit Tengah. In the year 2014, 16 terrapins were received and autopsies carried out. Samples were collected for bacteriological and

parasitological examination based on the gross pathological findings.

Laboratory diagnosis

Parasites found in the gut were put in alcohol and identified based on taxonomy (1, 5). Faecal samples were analysed using the McMaster method (2) to identify helminthic ova. Bacteria was cultured (7) from organ specimens.

**Table 1.** Results of Parasitological and bacteriological findings in 16 Terrapins from PKHL Bukit Pinang

No.	Helminth	Strongyle epg	Strongyloides epg	Bacteria
1	<i>Sulcascaris sulcata</i>	800	0	NSF
2	<i>Oesophagostomum</i>	0	0	NSF
3	<i>Oesophagostomum</i> (1 worm)	0	0	NSF
4	<i>Sulcascaris sulcata</i> (32 worms)	0	0	<i>Aeromonas hydrophila</i> & <i>E. coli</i>
5	NSF	1100	100	NSF
6	NSF	200	0	NSF
7	NSF	300	100	NSF
8	NSF	500	200	NSF
9	NSF	800	300	NSF
10	NSF	500	900	NSF
11	NSF	100	0	NSF
12	NSF	4300	400	NSF
13	NSF	900	500	NSF
14	<i>Sulcascaris sulcata</i> (12 worms)	2200	0	<i>E. Coli</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus chromogenes</i>
15	<i>Sulcascaris sulcata</i> (14 worms)	800	0	<i>E. Coli</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus chromogenes</i>
16	<i>Sulcascaris sulcata</i> (26 worms)	0	0	<i>E. Coli</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus chromogenes</i>

## RESULTS

Table 1 shows the pathogens isolated from 16 terrapins. The helminth worms found in the terrapins are *Sulcascaris sulcata*, recovered in 5 terrapins (1-32 adult worms); and *Oesophagostomum* sp. from one terrapin. The faecal examination revealed strongyle ova (0-4,300 epg) in 12 terrapins and strongyloides ova (0-900) in 7 terrapins.

Bacteriological cultures showed the presence of the following pathogens; *E. Coli*, *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Aeromonas hydrophila* (4+) dan *E.Coli* (4+) in four terrapins. These are common pathogens in the aquatic environment.

Figures 1 and 3 shows the presence of worms in the gut of the terrapins after post mortem. The heavy infection of worms impacting the stomach was observed. On cutting open part of the intestines, several worms were found to be coiled up thus blocking the passage of food.

## DISCUSSION AND CONCLUSION

River terrapins were once abundant in the major river systems of South and Southeast Asia, from the Mekong to the Ganges. However, a variety of human activities now threaten the survival of these large turtles. Five of the six species in the genus *Batagur* are ranked critically endangered by the IUCN Red List and face imminent extinction. *Batagur* eggs are widely harvested for domestic consumption



**Figure 1:** River Terrapins (*Batagur affinis*) before postmortem



**Figure 2:** Stomach of river terrapin filled with helminths



**Figure 3:** Digestive system filled with worms

every year, a process made easier because females congregate every year at about the same time to deposit eggs at known beaches and sandbars. Large adults are also harvested for food. Nesting females, the most important segment for sustaining the population, make up the majority of this catch as they are easy prey when they emerge to lay eggs. In order to help conserve Batagur populations, the nesting beaches are the focus in the conservation efforts. Monitoring nesting beach activity is generally the only opportunity to determine wild population numbers. The annual emergence of females to lay eggs, on well-known and often historic nesting beaches, is the most vulnerable stage of their annual life cycle, and it is here where protective conservation measures have proven the most effective. Apart from this, raising hatchlings in captivity until they are large enough to escape predation, is being used successfully in Batagur conservation programmes in India, Myanmar, and Cambodia. (8)

Reducing morbidity is another method of increasing the population of terrapins. As such, identifying infections and carrying out treatment as well as practicing good management at conservation sites will ensure terrapins are healthy with reduced common infections. Terrapins inhabit rivers and riverine areas in the tropics and subtropics filled with thick vegetation, sharing their habitat with many other reptiles and amphibians. In such a habitat, infections are easily transmitted, such as bacterial and parasitic

infections. As a result, it is common to find *Strongyles* and *Strongyloides* infections in terrapins, as a result of consuming plants and vegetation contaminated with these *strongyle* larvae. The faecal egg counts for *strongyles* too was more than 500 epg for 9 terrapins. *Strongyles* too can damage the intestinal wall causing poor digestive function and loss of weight apart from protein leak resulting in dehydration and weakness. Fenbendazole at 25 mg/kg once every second week for 8 weeks can be administered.

Generally, most species of river turtles have an omnivorous diet that is primarily made up of aquatic plants, grasses and leaves. Many river turtle species also hunt fish and mollusks in the water along with small reptiles and amphibians. Thus it was observed that large numbers of *Sulcascaris sulcata* were found in terrapins. Worms may aggregate and cause ulceration of the stomach with almost complete destruction of the upper layer of mucosa. This can lead to poor appetite and death.

Depending on the species, female terrapins lay between 5 and 100 soft, leathery eggs each time which it then buries in the sand. After a couple of months, the hatchlings head for the water. The average lifespan of the river turtle is about 30 years (Orenstein, 2001). Thus, it is important that the terrapins are healthy in order to produce eggs with the best chance of hatching. Terrapins also perform cloacal respiration apart from pharyngeal respiration, and aestivation and brumation (burying in sand) and these pose an added

risk to getting infections from the ground. Common infections include shell rot, salmonellosis and pneumonia caused by a variety of common bacteria. Antibiotic therapy can be instituted for group or individual terrapins.

In conclusion, the information gathered from the 16 terrapins that were autopsied gives an indication of the possible types of pathogens found in terrapins. This gives better awareness to veterinarians and conservation site managers on the care and management of terrapins so as to maximize the productivity to improve the population. A gradual and consistent recording of this information will definitely be useful for future generations, in helping to conserve wildlife holistically.

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## ORIGIN AND DISTRIBUTION OF BRACHIAL PLEXUS OF WHITE NEW ZEALAND RABBIT (*Oryctolagus cuniculus*)

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**Abstract.** The study was conducted to know the anatomy of the brachial plexus of White New Zealand Rabbit (*Oryctolagus cuniculus*). Ten healthy male and female White New Zealand rabbits were dissected in this study. It was found that the brachial plexus of the White New Zealand Rabbit was formed by ventral branches of C5, C6, C7, C8, T1 and T2 spinal nerves. The cranial trunk was formed by the caudal branch of C5, C6 and caudal trunk formed by rami ventralis of C7, C8, T1 and the cranial branch of ventral ramus of T2. Cranial pectoral nerve originated from the caudal trunk spreading into the pectoral muscles. The musculocutaneous nerve innervates into the brachial muscle and the axillary nerve into the subscapular muscle. The radial nerve was divided into two branches as ramus profundus and ramus superficial then divided into the digital dorsal common III and IV. The thoracodorsal nerve innervates the latissimusdorsi muscle. The median nerve was divided into digital dorsal common I, II, III and IV nerves. The ulnar nerve

formed the caudal cutaneous antebrachial then digital dorsal common IV and V nerves. Lateral thoracic and caudal pectoral nerves originated from the caudal trunk. The origin and distribution of brachial plexus resemble that of porcupines but differ from other mammals.

**Keywords:** White New Zealand rabbit, brachial plexus, forelimb

### INTRODUCTION

Livestock is one of the major economic resources of Bangladesh. Micro-livestock like rabbit may be considered an emerging sector for growth of the economy, in addition to other protein supplements from animal sources such as poultry. Use of laboratory animals like rabbits, rats and guinea pigs is increasing day by day for experimental purposes. Rabbits are raised for several purposes including meat and fur production, as laboratory animals, for show purposes and as pets (1). Rabbit meat is acknowledged as high quality lean meat, being high in protein

but low in fat and cholesterol (2, 3). The promising future demand of this animal warrants investigations into the different morphological systems of the rabbit for its use in clinical, surgical and research fields. Microsurgical techniques have largely improved the treatment of lesions in the peripheral nerve and brachial plexus. Other experimental work is limited. The rabbit model offers advantages, including rapid regeneration for a fairly short observation period (4). Special attention has been given to the dissection or neurological study of the different organs or regions of the body, because of variations among animal species. Some authors have studied the formation of nerve plexuses in domestic animals which showed that its organization varies. [The brachial plexus has been studied in mammals such as dogs (5, 6, 7, 8) and cats (5, 9, 10).] Rabbits have been used as an experimental model in diseases, such as peripheral nerve injury and brachial plexus injury. However, some aspects of their macro-anatomy need a more detailed description. The brachial plexus has been studied in mammals such as dogs (5, 6, 7, 8), cats (5, 9, 10), vervet monkeys (11), chacma baboons (12), mice (13, 14), rats (15, 16), porcupines (17) and rabbits (10, 18, 19). The White New Zealand Rabbits (*Oryctolagus cuniculus*) are from the order Lagomorpha. The aim of this present study was to make a detailed investigation of the origin and distribution of nerves arising from the brachial plexus in White New Zealand Rabbits to give support for experimental research for the

clinical, radiological and surgical practice of this animal.

## MATERIALS AND METHODS

### Statement of the experiment

The experiment is an *in situ* study of the origin and distribution of the brachial plexus of the White New Zealand Rabbit (*Oryctolagus cuniculus*), carried out in the laboratory of the Department of Anatomy and Histology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. The duration of study was about two months.

### Experimental animals

The study population was ten adult clinically healthy male and female White New Zealand Rabbits at 7 days old which were purchased from the local market in the Chittagong Metropolitan area. During purchasing, sexing of the rabbit was by observing the vent region. Before being used in the experiment, they were kept for 15 days to acclimatize them to the environment.

### Rearing and care

The rabbits were cared for at the Animal Care Room, Department of Anatomy and Histology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh, in proper hygienic conditions, with normal feed of green grass, leaf,



different types of grains and water *ad libitum*. The ventilation of the rearing house of Rabbit was sufficient as a standard one.

### **Selection and Identification of White New Zealand Rabbit**

White New Zealand Rabbit is becoming very popular as a pet animal in the country. Its availability in the local markets of Bangladesh makes it an important animal to investigate its anatomical significance. It is also widely used as a laboratory animal due to its short gestational length (30-32 days) and easy handling. White New Zealand rabbit is pure white in color with long erected ears.

### **Laboratory preparation**

#### ***Required reagents***

28 70% Alcohol and diazepam (Sedil®)

#### ***Required instruments and appliances***

Syringe with needle, tissue forceps, rat-toothed forceps, Allis forceps, scalpel with handle, cotton, gauze, gloves and thread

### **Anesthesia and killing of Rabbit**

The animals were killed according to the Ethics of Animal Research Committee of the institution. First, the rabbit was weighed using a weighing balance. Then, it was stretched out with the help of strings tied to the table at ventro-dorsal position.

In order to kill, an overdose of Sedil® (diazepam at 10 mg/kg body weight) was introduced into the external jugular vein.

### **Dissection of the fore limb of Rabbit**

The rabbit was kept in lateral recumbency after it was killed. The spinal nerves forming the brachial plexus with its branches was dissected out carefully. During dissection, the skin, fascia and adipose tissues covering the shoulder were removed carefully with the help of tissue forceps, rat-toothed forceps, Allis forceps and scalpel with a handle. The courses of the nerves emanating from the plexus were exposed. The muscles and tendons were dissected and reflected whenever necessary to trace the course of the nerves. The brachial plexus in both forelimbs were examined and photographed by using a 12 megapixel digital camera with 4× zoom (Sony®). See Figures 1, 2, 3, 4, 5. The organization of the main branches of the brachial plexuses of ten adult rabbits irrespective of sex was investigated. Observations were performed on the non-fixed material, immediately after killing the animals to draw the results.

### **Identification of the nerves**

Nerves of the brachial plexus were identified according to the innervation in muscles of the neck, arm, forearm and thorax.



## Nomenclature of the nerves

For the terminologies, the Nomina Anatomica Veterinaria (20) was used. The results obtained were compared, with the results from other studies of dogs (5-8), cats (9, 6, 10), Vervet monkeys (11), Chacma baboons (12), mice (13), rats (15, 16) and porcupines (17).

## RESULTS

The brachial plexus of the rabbit emerged between the dorsal scalenus and the ventral sclaneus muscle, just proximal to the first rib and medial aspect of the scapula. It constituted of the ventral branches of C5, C6, C7, C8, T1 and T2 spinal nerves. The ventral rami of C5 spinal nerve and T2 spinal nerve were divided into two branches. It also contributed to the caudal branch of ramus ventralis of C5 spinal nerve and the cranial branch of ramus ventralis of T2 spinal nerve. The cranial branch of ramus ventralis of C5 spinal nerve and the ramus ventralis of C6 spinal nerve formed the cranial trunk and cranial branch of T2 spinal nerve and rami ventralis of C7, C8 and T1 spinal nerves formed the caudal trunk which was the largest trunk. Two branches originating from the cranial trunk were bound to the cranial part of the caudal trunk (Figure 1).

## Origin of different nerves of the brachial plexus

### *Long thoracic nerve*

Before joining to the brachial plexus, the ventral branches of C6 and C7 spinal nerves, as each of C6 and C7 spinal nerves divided into two thin branches, passed beneath the scalenus dorsalis muscle and at the first rib, then turned to the caudal and dispersed into serratus ventralis thoracic muscle.

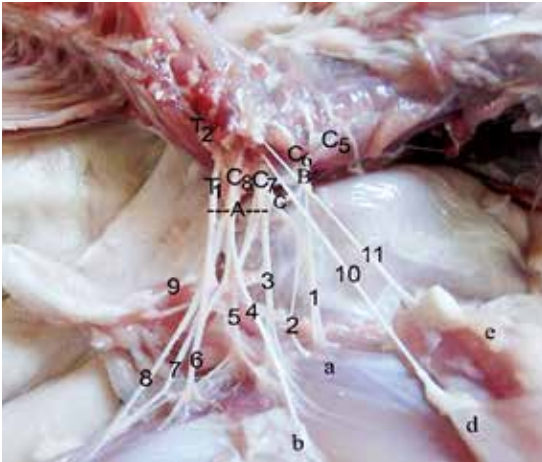
### *Nerves originated from cranial trunk*

Phrenic nerve, supra scapular nerve, the first branch of subscapular nerve and a branch bound to the caudal trunk were originated from the cranial trunk of the brachial plexus (Figure 1).

### *Nerves originated from caudal trunk*

Cranial pectoral nerve, axillary nerve, the second branch of subscapular nerve along with axillary nerve, thoracodorsal nerve, musculocutaneous nerve, radial nerve, ulnar nerve, median nerve, lateral thoracic nerve and caudal pectoral nerve were originated from caudal trunk of brachial plexus of rabbits (Figure 1).

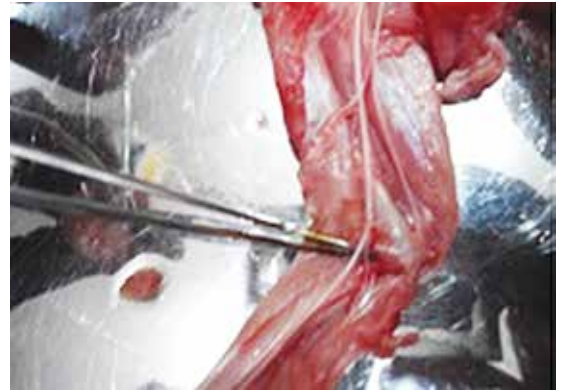
In rabbit, was found that the plexus brachialis was formed by two trunks, called cranial and caudal from which the nerves that spread through forelimbs originated.



**Figure 1.** Lateral view of the brachial plexus in the rabbit. C5 – Caudal branch of ramus ventralis of C5, C6 – Ramus ventralis of C6, C7 – Ramus ventralis of C7, C8 – Ramus ventralis of C8, T1 – Ramus ventralis of T1, T2 – Cranial branch of ramus ventralis of T2; 1–Suprascapular nerve, 2 – Subscapular nerve, 3 – Axillary nerve, 4 – Thoracodorsal nerve, 5 – Musculo cutaneous nerve, 6 – Radial nerve, 7 – Median nerve, 8 -Ulnar nerve, 9 – lateral thoracic nerve, 10 – Cranial pectoral nerve, 11- Caudal pectoral nerve; a – Subscapular muscle, b –Latissimus dorsi muscle, d-Pectoralis ascendens muscle, e- Pectoralis descendens muscle.



**Figure 2.** Branches of median nerve at the palmar part of metacarpus.



**Figure 3.** Separation of median nerve from musculocutaneous nerve at the level of distal end of humerus.



**Figure 4.** Digital dorsal and palmar nerve V (branches of the ulnar nerve) supplies the skin and subcutaneous tissues of the sole.



**Figure 5.** Caudal cutaneous antebrachial nerve (branch of the ulnar nerve) at the level of cubital joint.

## Distribution of nerves emerging from the brachial plexus

The supra scapular nerve originated from cranial part of cranial trunk, passed between subscapular muscle and supraspinatus muscle at collum scapula level, existed at lateral face of scapula and continued through supraspinatus muscle and infra spinatus muscle.

The subscapular nerve was two nerves. The first one originated from the cranial trunk which spread through the subscapular muscle. The second one originated from the caudal trunk with axillary nerve and dispersed to the caudal part of subscapular muscle and teres major muscle after leaving the axillary nerve.

The axillary nerve originated from the point that the branch coming from cranial trunk then joined the caudal trunk and after a short distance, became divided into two branches. First branch was given to the subscapularis and teres major muscles. This nerve at the level of collum scapula at the caudal of scapula coursed between teres major and supra scapular muscles to the lateral face of the scapula. After giving branches to teres minor and deltoid muscles, it passed to the lateral face of the forelimb through the space between caput lateralis of triceps brachii muscle and deltoid muscle and gave the cutaneous brachii lateralis cranialis which spread to the cranial of lateral of forelimb and then continued as the cranial cutaneous antebrachii nerve.

The thoracodorsal nerve arose from caudal trunk becoming two branches: the axillary nerve and radial nerve which spread through latissimus dorsi muscle.

The radial nerve originated from the medial part of caudal trunk together with median nerve and ulnar nerve. At midway down the humerus, it passed between capus medialis and capus lateralis of the triceps brachial muscle to the lateral face of the arm and gave rami musculares to tensor fascia antebrachii muscle and triceps brachii muscle. First, it gave lateral cutaneous antebrachii nerve between brachii muscle and caput lateralis of triceps brachii muscle and then divided into two branches called ramus profundus and ramus superficialis. Ramus profundus distributes through extensor muscles on antebrachium. Ramus superficialis separated into the digital dorsal common III and IV nerves on distal of antebrachium, descending on extensor carpi radialis muscle.

The median nerve was the longest nerve of the brachial plexus originating from the caudal trunk in common with the ulnar and musculocutaneous nerves as a common root. After leaving the caudal trunk, firstly as the ulnar nerve and then near to the distal of the humerus, it separated as the musculocutaneous nerve (Figure 2). The median nerve did not give any branch until the articulation cubiti level where it gave two branches called rami muscularis and interosseus antebrachial nerve on antebrachium. Then it ended as four branches; digital dorsal common I, II,

III and IV nerves, at the central level of the palmar part of the metacarpus (Figure 2).

Ulnar nerve originated from caudal trunk together with median and musculocutaneous nerves and then separated from them. It gave the caudal cutaneous antebrachii nerve at the caudal of antebrachium and rami muscularis at the level of cubital joint (Figure 5). The nerve divided in to digital dorsal and palmar nerve V (Figure 2) at caudomedial of antebrachium. Prior to spreading to the palmar and dorsal of the digiti V one branch originated from each of them spreaded to the skin and subcutaneous tissues of sole.

Cranial pectoral nerve had four branches, two spreading to pectoral descendens muscle, one to pectoral descendens muscle and pectoral transverses muscle, one together with lateral thoracic nerve and caudal pectoral nerve to cranial part of pectoral transversus muscle and pectoral ascendens muscle. Lateral thoracic nerve originated from caudal trunk and passed through pectoral ascendens muscle. After giving a branch to cranial part of this muscle, it spread into the cutaneous omobrochial muscle. Caudal pectoral nerve originated from caudal trunk with lateral thoracic nerve, and gave two branches which spread to lower part of cutaneous trunci muscle and caudal part of pectoral ascendens muscle. Musculocutaneous nerve initially coursed through the distal of the humerus together with the median nerve and then separated from each other. This nerve at level of articulation cubiti, became the

ramus muscularis distalis to the brachial muscle and centre of antebrachium, medial cutaneous antebrachii (going through skin fascia), and the other branch divided into the digital dorsal common I and II nerves at the level of the phalanx proxima.

## DISCUSSION

It has been reported that the formation of the brachial plexus varies in some species. The brachial plexus of rat is formed by contribution of ventral rami of C5, C6, C7, C8, T1 and T2 spinal nerves (15, 21). However, (4) reported that ventral branches of T2 spinal nerve are not involved. Another study was undertaken by (17) which revealed that the brachial plexus of porcupine is formed by ventral branches of C5, C6, C7, C8, T1 and T2 spinal nerves. (14) and (13) reported that the brachial plexus is formed by ventral branches of C5, C6, C7, C8 and T1 spinal nerves in mouse. (11) and (12) reported that, brachial plexus of Vervet monkey and Chacma baboon is formed by the ventral branches of C5, C6, C7, C8, T1 and T2 spinal nerves. In the cat, the contribution of the formation of brachial plexus is the ventral branches of C6, C7, C8 and T1 spinal nerves (6, 9). (7) reported that dog brachial plexus is formed by ventral branches of C6, C7, C8, T1 and T2 spinal nerves, while (5, 6) reported that T2 spinal nerve is involved occasionally. The brachial plexus of rabbit is formed by the contribution of ventral branches of C5, C6, C7, C8, T1 and T2 spinal nerves and its formation resembles that of rat

(15, 21), porcupine (17), Vervet monkey (11), Chacma baboon (12) and differs from that of rat (21), mouse (14, 13), cat (6, 9) and dog (6, 5, 7, 8). The ventral branches of C5 spinal nerve and T2 spinal nerve divided into caudal and cranial branches (17). The caudal branch of C5 spinal nerve and cranial branches of T2 spinal nerve contribute to form the brachial plexus in porcupine (17) to which the result of the present study is parallel. The brachial plexus of rabbit consisted of caudal and cranial trunks as that of porcupine (17) and in this respect it differs from those of rat (4, 12) which is formed from caudal, medial and cranial trunks. As in Vervet monkey (11), Chacma baboon (12) and cat (6) the ventral branches of C6 and C7 spinal nerves in porcupines gives a branch to form the long thoracic nerve before they contribute to the brachial plexus. This is different from the finding reported in dogs (7, 8) in which the nerve was originated from the ventral branches of C7 and C8 spinal nerves following the formation of plexus. Brachial plexus of rabbit form a network which resembled the rat (16), mouse (14, 13) and other mammals. Brachial plexus of rabbit consist of two trunks as cranial and caudal which were formed by ventral branches of C5, C6, C7, 13C8, T1 and T2 spinal nerves in rabbit, similar to porcupine (17) and differs from those of rat 14(16) and Chacma baboon (12) which are formed from caudal, medial and cranial trunks.

Emanating nerves from the brachial plexus in rabbit disseminates in different

muscles of the forearm is somewhat similar to porcupine (17) and in rat (4), but different from other mammals. In rabbit, nerves from the brachial plexus innervating the coraco-brachial muscle and brachial biceps muscle originated directly from the plexus, and transversely joined the musculocutaneous nerve after giving a branch to each muscle. After giving ramus musculer distalis to brachial muscle and cutaneous antebrachii medial nerve to the medial aspect of antebrachii, musculocutaneous nerve, continued and together with ramus superficialis of radial nerve and dorsal branch of ulnar nerve spread to the dorsal of fingers and palmar branch of ulnar nerve and the last parts of median nerve which supplied the palmar aspect of fingers. (6) Stated that, the musculo-cutaneous nerve in dog passes between the coraco-brachialis and the brachial artery and descends in the arm in front of the artery. At the shoulder joint it gives off branches to the biceps and coraco-brachialis, and in the distal third of the arm is connected with the median nerve by an oblique branch. It terminates near the elbow by dividing into a branch for the brachialis and a small cutaneous nerve which passes down over the medial face of the elbow and, inclining a little forward, descends over the deep fascia of the forearm to the carpus. Whereas (6) stated that, the musculo-cutaneous nerve in horse arises from the anterior part of the plexus and descends over the lateral face of the brachial artery, below which it is connected by a large but short branch



with the median nerve, thus forming a loop in which the artery lies. One or two branches to the pectoral muscles are given off from the nerve or the loop. In rabbit there is a smaller difference in this course with dog (6); where the musculocutaneous nerve initially coursed through distal of humerus together with median nerve and then separated from each other. This nerve at level of articulation cubiti, gave ramus muscularis distalis to brachial muscle and center of antebrachium, medial cutaneous antebrachii (going through skin fascia), other branch divided digital dorsal common I and II nerves at level of phalanx proximal. (6) Stated that, the radial nerve in dog descends behind the ulnar nerve, gives branches to the extensors of the elbow, dips in between the medial head of the triceps accessory head of the anconeus, winds around the arm, and divides between the brachialis and the lateral head of the triceps into two branches. The deep branch supplies the extensor and supinator muscles on the forearm. The superficial branch emerges upon the flexor surface of the elbow and divides into two branches which terminate by supplying two dorsal digital nerves to each digit, except the fifth, which receives its lateral dorsal nerve from the ulnar. The medial branch descends along the medial side of the cephalic vein to the carpus, where it divides into dorsal nerves for the first digit and the medial side of the second. The lateral branch is much larger. It descends on the middle of the front of the forearm and supplies the remaining dorsal digital nerves except that

to the lateral side of the fifth digit. In rabbit radial nerve originated from the medial part of caudal trunk together with median nerve and ulnar nerve. At midway down the humerus, it passed between carpus medialis and carpus lateralis of triceps brachial muscle to the lateral face of arm and gave rami musculares to tensor fascia antebrachii muscle and triceps brachii muscle. First, it gave lateral cutaneous antebrachii nerve between brachii muscle and caput lateralis of triceps brachii muscle and then divided into two branches called ramus profundus and ramus superficialis. Ramus profundus distributes through extensor muscles on antebrachium. Ramus superficialis separated digital dorsal common III and IV nerves on the distal of antebrachium, descending on extensor carpi radial muscle. But there is a similarity in the pathway of radial nerve in different muscles in ox and horse (6). The radial nerve in ox is continued below the elbow by a large cutaneous branch. Dorsal cutaneous antebrachial nerve which emerges at the lower border of the lateral head of the triceps and descends on the dorsal aspect of the limb. It communicates above the carpus with the lateral cutaneous branch of the median nerve and terminates in three dorsal digital nerves; two of these descend along the axial or interdigital side of the dorsal surface of the chief digits, and the third along the medial (abaxial) side of the medial chief digit. (6) Stated that, the radial nerve in horse arises from the posterior part of the plexus and is sometimes the largest branch. It descends

with the ulnar nerve over the medial face of the origin of the subscapular artery and the lower part of the teres major and dips into the interstice between that muscle and the long and medial heads of the triceps. (6) Stated that the ulnar nerve in dog is as large as or larger than the median, with which it is united for some distance. At the distal third of the arm it separates from the median and passes over the medial epicondyle of the humerus. At the proximal part of the forearm it gives off the dorsal branch, which supplies cutaneous twigs to the dorso-lateral surface of the distal part of the forearm and carpus and terminates as the lateral dorsal digital nerve of the fifth digit. Descending under cover of the flexor carpi ulnaris, the ulnar inclines medially under the tendon of insertion of that muscle and divide into superficial and deep branches. The superficial branch descends along the lateral border of the flexor tendons, gives off the lateral volar digital nerve of the fifth digit and a branch which descends in the space between the fourth and fifth metacarpal bones and unites with the deep branch. The deep branch descends in the carpal canal and divides under the deep flexor tendon into its terminal branches. The smaller of these supply the volar metacarpal muscles. The larger terminals are the three volar common digital nerves, which descend along the second, third, and fourth intermetacarpal spaces, subdivide, and concur with the volar metacarpal branches of the median nerve in forming the volar proper digital nerves. In horse,

the thickness, size of the nerve is different from that of dog. (6) stated that the ulnar nerve in horse arises with the median by a short common trunk. It descends behind the brachial artery and is accompanied a short distance by the radial nerve, from which it can be distinguished by its smaller size. (6) Stated that, the ulnar nerve in ox divides at a variable distance down the forearm into two branches. The dorsal or superficial branch emerges between the tendons of the ulnaris lateralis and flexor carpi ulnaris, and is continued as the lateral dorsal digital nerve on the lateral chief digit. The volar or deep branch descends along the superficial digital flexor, gives a branch to the suspensory ligament below the carpus, and unites with the lateral branch of the median nerve to form the lateral volar digital nerve. But there is much difference in the course and dissemination of the ulnar nerve in rabbit from other mammals. In rabbit, the ulnar nerve originated from the caudal trunk together with median and musculocutaneous nerves and then separated from them. It gave the caudal cutaneous antebrachii nerve at the caudal of antebrachium and rami muscularis at the level of the cubital joint. The nerve then divided into digital dorsal and palmar nerve V at the caudomedial of the antebrachium. Prior to spreading to the palmar and dorsal of the digital V, one branch originated from each of them which then spread to the skin and subcutaneous tissues of the sole.

(6) stated that there are similarities in the movement of median nerve and its



dissemination in different muscles and subcutis in dog, ox and horses; whereas in rabbit there is much difference in the courses of median nerve. (6) Stated that, the median nerve descends behind the brachial artery, passes over the medial epicondyle of the humerus, then under the pronator teres, and continues in the forearm under cover of the flexor carpi radialis. It gives branches below the elbow to the flexor and pronator muscles, and lowers down a volar branch to the skin on the medial and volar aspect of the carpus, and terminates between the superficial and deep flexor tendons by dividing into three volar metacarpal nerves. These descend in the first, second, and third intermetacarpal spaces and unite with the volar common digital nerves in forming volar proper digital nerves. (6) stated that, the median nerve is usually the largest branch of the brachial plexus. It descends over the insertion of the scalenus, crosses the medial face of the brachial artery, and reaches the anterior border of that vessel. It is easily recognized by its large size and the loop which it forms with the musculocutaneous nerve. (6) Stated that the median nerve in ox descends behind the brachial artery, passes over the medial epicondyle of the humerus, then under the pronator teres, and continues in the forearm under cover of the flexor carpi radialis. It gives branches below the elbow to the flexor and pronator muscles, and lowers down a volar branch to the skin on the medial and volar aspect of the carpus, and terminates between the superficial

and deep flexor tendons by dividing into three volar metacarpal nerves. These descend in the first, second, and third intermetacarpal spaces and unite with the volar common digital nerves in forming volar proper digital nerves. In rabbit, the median nerve was the longest nerve of the plexus brachialis originating from the caudal trunk in common with the ulnar and musculocutaneous nerves as a common root. After leaving the caudal trunk, firstly the ulnar nerve and then near to the distal of the humerus, the musculocutaneous nerve separated. Median nerve did not give any branch until articulation cubiti level and then it gave two branches called; rami muscularis and interosseus antebrachial nerve on antebrachium. Then it ended as four branches; digital dorsal common I, II, III and IV nerves, at the central level of the palmar part of metacarpus. This brief information about the White New Zealand Rabbit with regards to the topographical location of the brachial plexus including the course and distribution of nerves originating from it, may give suggestions for clinicians and practitioners for their application in practical field.

## CONCLUSION

Some suggested preventative measures are: aviary hygiene, cleanliness and preventive care.

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## ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM CHICKEN IN MALAYSIA

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**ABSTRACT.** Colibacillosis is an important disease affecting the poultry industry in many countries, caused by the Avian Pathogenic *E. coli* (APEC): it manifests as various clinical signs. It contributes significantly to economic loss for poultry farmers as a result of high mortality and morbidity in poultry. To overcome this, antibiotics have been widely used to eliminate *E. coli* infection in poultry farms in recent years. Treatment with antibiotics has been considered as a vital regimen to control *E. coli* infection at the farm level for many years. However, high frequency of antibiotic resistance of *E. coli* isolates from chicken has become the centre of attention due to public health importance. The aim of the present study is to determine the multidrug resistant profiles of *E. coli* strains isolated from chicken. *E. coli* isolates obtained from clinical cases were re-identified and classified by conventional methods. Multidrug resistant profiles against 13 different antibiotics of 125 *E. coli* isolates were determined by using disk diffusion method according to Clinical Laboratory Standard Institute (CLSI). Antibigram revealed that 81.6%

of the *E. coli* isolates showed multidrug resistant profiles to different antibiotics. Most of the *E. coli* isolates were highly resistant to erythromycin (52.8%), followed with tetracycline (52.0%), spectinomycin (39.2%), trimethoprim (38.4%) and flumequin (37.6%). Out of 125 isolates tested, 19.2% were resistant to more than eight antibiotics, with one isolates found to be multidrug resistant to most of antibiotics except polymyxin B. These findings also demonstrated that most of the isolates were susceptible to antibiotics commonly used for *E. coli* infections treatment in poultry with lowest resistant score against polymyxin B (92.8%) and colistin (92.0%). Moderate resistant profiles were observed towards amoxycilin (25.6%), apramycin (16%), kanamycin (8.8%) and streptomycin (8.0%). High percentage of multidrug resistance was found among the *E. coli* isolated from chicken as an indicator to more serious problems in animal health. Therefore, continuous surveillance of antibiotic resistance profiles in chicken and other food animals is crucial to ensure food chain safety.

**Keywords:** *E. coli*, antibiotic resistant, antibiotic, chicken

## INTRODUCTION

*E. coli* is one of the most important aetiological agents causing diseases in poultry which leads to significant economic losses related to high mortality, poor weight gain of infected chicken and poor carcass quality (Ewers *et al.*, 2004). The *E. coli* infection in poultry is usually considered as a secondary infection, which is triggered by various predisposing factors particularly environmental factors including poor ventilation, overcrowding and other biological predisposing factors such as viral or parasitic infections (Vandekerckhove *et al.*, 2004). Appropriate farm management approaches have been suggested to overcome *E. coli* problems in poultry farm but several studies reveal that management approaches are not applicable to prevent incidence of *E. coli* infection in the farms. Thus, antibiotics have been introduced into animal populations mainly for disease treatment for many years, just after the first antibiotic, tetracycline, were introduced to human health. Antibiotics also have been used to control disease outbreaks including *E. coli* infection, thus reducing morbidity and mortality rates due to the infections. Even though antibiotics are highly recommended for therapeutic purpose, certain class of antibiotics were also used for subtherapeutic reasons to prevent occurrence of disease outbreaks in farms. Emergence of multidrug resistant

*E. coli* in food animals including chicken arise due to the improper use of antibiotics, thereby reducing the clinical efficacy to antibiotics commonly used in human and veterinary medicine (Wang *et al.*, 2013). Most of the time, antibiotics have been administered to the healthy animals, instead of sick animals. This is because it is easier to treat whole flock of animals rather than individually, and sometimes usage of antibiotics did not follow the prescribed doses (Van Der Bogaard *et al.*, 2002). Unnecessary introduction of antibiotics to healthy animals induced those commensal bacteria strains to develop resistance against commonly used antibiotics. Usually, antibiotic resistant profiles were observed after a huge introduction of related antibiotics for therapeutic use in the human medicine and veterinary field. For example, resistance to tetracycline was observed after tetracycline was introduced in human medicine. However, later in the mid-1950s it was delivered in animal feed in order to enhance weight ratio in poultry. In Malaysia, multidrug resistant profiles of *E. coli* isolated from chicken against various antibiotics have been reported which involved chicken samples from poultry farms and diagnostic samples in veterinary laboratory which were limited to certain geographical locations (Geidam *et al.*, 2012; Khoo *et al.*, 2013). Concrete details on antibiotic resistant profiles of *E. coli* isolated from chicken in the country is useful for better implementation of antibiotics usage for effective control of disease caused by pathogenic *E. coli* in

poultry. Therefore, the aim of the present study is to determine the multidrug resistant profiles of *E. coli* strains isolated from chicken in Malaysia.

## MATERIAL AND METHODS

### Preparation of *E.coli* cultures

A total of 125 *E. coli* isolates were used in this study. The collection of *E. coli* stock cultures at the Veterinary Research Institute gene bank was used in this study with prior approval from the Department of Veterinary Services. All the isolates were selected from cases submitted for diagnostic purposes for *E. coli* infection, in broiler and village chicken with findings of profuse growth on agar medium. Visceral organ samples were inoculated onto 5% blood agar and MacConkey agar for selective isolation of Gram-negative bacteria. All the plates were incubated at 37°C for 18 to 24 hours. Well isolated presumptive *E. coli* colonies were subcultured on blood agar to obtain pure growth for further identification. Presumptive *E. coli* colonies were greyish in colour, small to medium sizes on blood agar plates, while they ferment lactose on MacConkey agar by producing bright pink colonies. The presumptive colonies were confirmed by conventional biochemical tests including Triple Sugar Iron (TSI), Indole, Methyl red, Simmons citrate, motility, urease and decarboxylase tests such as ornithine, lysine, arginine and malonate broth according to standard

protocols (Quinn *et al.*, 1994). The isolates were further classified into serogroups by using classical serological slide agglutination method according to Kauffmann-White scheme against commercially available specific antisera. The isolates were kept in maintenance medium until further tests were conducted.

### Antibiotic sensitivity tests

The Disk diffusion method was used to determine the susceptibility of *E. coli* isolates to several antibiotics of veterinary significance on Mueller Hinton agar. Thirteen different antibiotics which are typical for treatment of *E. coli* infections were selected based on the OIE List of Antimicrobial Agents of Veterinary Importance, 2014. The antibiotics with the following concentrations were used in this study: erythromycin (15 µg), tetracycline (30 µg), kanamycin (30 µg), colistin (10 µg), ceftiofur (30 µg), polymyxin B (300 µg), spectinomycin (25 µg), amoxycilin (30 µg), gentamicin (10 µg), flumequin (30 µg), trimethoprim (5 µg), streptomycin (10 µg) and apramycin (15 µg). Zones of inhibition were measured to the nearest millimeter using a ruler and reported either as sensitive (S), intermediate resistant (I) or resistant (R) on the basis set by the Clinical Standard Laboratory Institute (CLSI), 2012.

## RESULTS

### Biochemical profiles of *E. coli* isolated from chicken

In this experiment, several biochemical profiles showed reliable characteristics among *E. coli* isolates, with most of the isolates (>90%) showing similar expected *E. coli* reactions in biochemical profiles including citrate, lysine, urease, triple sugar iron (TSI) and methyl red. All 125 *E. coli* isolates tested (100%) were able to ferment lactose and were negative for malonate test, and these findings are similar to expected *E. coli* profiles. However, two isolates were negative for indole test, and one isolate was positive for citrate test; invariably these are different from expected *E. coli* reactions. The number of isolates with negative methyl red reaction was higher, with 16/125 (12.8%) isolates showing contradictory reactions with *E. coli* isolates. Generally, *E. coli* isolates showed Triple Sugar Iron (TSI) reaction to both acid butt and slant, with production of gas but no hydrogen sulphides. However, one isolate (0.83%) was found to show acid butt and slant reaction with no gas production observed. Most of the *E. coli* strains isolated from chicken usually showed variety in motility, arginine and ornithine reaction, with a small number of isolates showing differences in lysine reaction. Consequently, as reported in previous studies, contradictory reactions of biochemical properties by certain *E. coli* strains may occur (Bettelheim, 1994).

According to the results obtained, a total of 63/125 isolates (50.4%) were found to show contradictory reaction of *E. coli* in arginine, ornithine (60.8%) and motility (50.4%). Only 2/125 (1.6%) isolates produced hemolysis on blood agar, each one producing beta and alpha hemolysis reaction. The *E. coli* usually does not show hemolysis, and the colony is usually greyish white in colour, opaque, slightly moist or mucoid appearance with entire edges (Quinn *et al.*, 2004). Out of 125 *E. coli* isolates tested, only 24 isolates (19.2%) gave similar reactions compared with the reference strain *Escherichia coli* ATCC 25922 which was used as control in this experiment. Other isolates were found to show different profiles from reference strain mentioned above. Table 1 shows different biochemical profile results of *E. coli* isolates tested in this experiment.

### Identification of *E. coli* isolated from chicken serogroups

The highest number of *E. coli* isolates identified in this study as non-typeable *E. coli* (ECU), was 92 out of 125 (72%) isolates which could not be assigned to any serogroups tested. Several studies previously revealed different findings on prevalence of *E. coli* serogroups identified, with some studies reporting a low prevalence of *E. coli* non-typeable strain. However, findings from the present study correlate with other studies showing high prevalence of non-typeable strain as shown by Ewers *et al.*, (2007), whereby 50.4% of

Table 1. Biochemical profiles of *E.coli* isolated from chicken

Biochemical test	<i>E.coli</i> reactions	No. of isolates showed positive results	Percentage (%)
Indole	+	118/125	94.4%
Methyl red	+	109/125	87.2%
Triple Sugar Iron	A/A with gas	119/125	95.2%
Motility	V	63/125	50.4%
Citrate	-	119/125	95.2%
Urease	-	119/125	95.2%
Arginine	V	63/125	50.4%
Ornithine	V	76/125	60.8%
Lysine	+	113/125	90.4%
Malonate broth	--	125/125	100%
Hemolysis on Blood agar	V	2/125	0.016%

Table 2. *E. coli* isolates and serogroups identified from local village and broiler chicken from 2010- 2015

Serogroups	Number of isolates
<i>E.coli</i> Untypeable (ECU)	92
<i>E.coli</i> O1:K1	17
<i>E.coli</i> O2:K1	3
<i>E.coli</i> O78:K8O	13
Total	125

*E. coli* isolates tested were assigned to non-typeable serogroups. However, the results may vary depending on the study, due to the differences of antisera used in the study and geographical locations, meaning that different countries may show some variability in results. It was observed from various reports that the trend of *E. coli* non-typeable prevalence was found to increase steadily from the year 2010 in Malaysia (Maswati *et al.*, 2011). Most of the *E. coli*

isolated from year 2010 was not able to be assigned to any serogroup compared to the previous years, therefore the prevalence of non-typeable isolates showed an increase since 2010, with only a small percentage of the isolates being able to be identified according to their serogroups by available antisera. However, the most predominant *E. coli* serogroups identified in this study were O1 (14%), followed by O78:K8O (11%) and O2 (3%). Table 2 shows the



summary of serogroups identified from *E. coli* originating from chicken, isolated from diagnostic cases received in VRI from the year 2004 until 2015 used in this study.

A high percentage of multidrug resistant profiles, that is 81.6% against different types of antibiotics tested was observed among *E. coli* isolated from chicken in this study. Out of 125 *E. coli* isolates tested, 102 isolates exhibited multiple antibiotic resistant profiles, while the remaining 23 (18.4%) isolates were found to be resistant against less than four types of antibiotics. Most of the isolates were highly resistant to erythromycin (52.8%), tetracycline (52.0%), spectinomycin

(39.2%), trimethorpin (38.4%) and flumequin (37.6%). The majority of isolates were resistant to at least one antibiotic (99.2%), while only one isolate was found to be susceptible to all antibiotics tested. In this study, a total of 24 (19.2%) isolates were resistant to more than eight types of antibiotics, while only one isolate was found to be resistant to all antibiotics used in this study. High frequencies of isolates were susceptible to polymyxin B (92.8%) and colistin (92.0%). However, ten isolates (8.0%) showed moderate sensitivity towards colistin, with none of the isolates being resistant to this particular antibiotic. Most of the *E. coli* isolates also were highly susceptible to ceftiofur with the

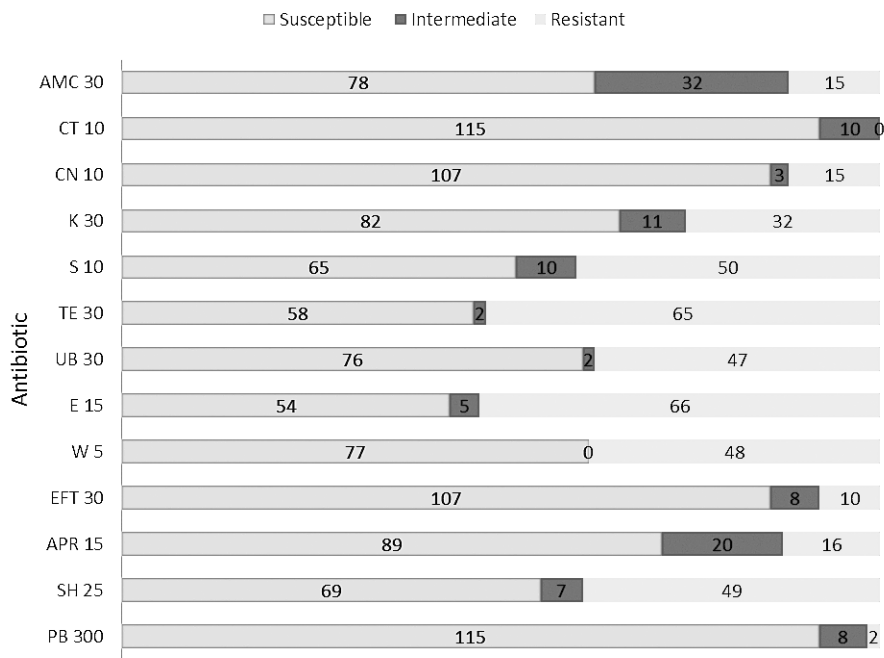


Figure 1: Antibiotic susceptibility of *E. coli* isolated from chicken.

lowest resistance score (8.0%), followed by gentamicin, apramycin and kanamycin. Moderate sensitivity profile was observed among isolates mostly towards amoxycillin and apramycin, with lesser extent towards kanamycin and streptomycin.

## DISCUSSION

Conventional biochemical tests have been used since the last decade for identification of *E. coli*. To date, these methods have been used intensively in many laboratories for biotyping and subtyping purposes, in order to get clear characteristics and phenotypic profiles of *E. coli* isolates. However, most of the studies revealed that *E. coli* itself has diverse characters, making it difficult to biotype the *E. coli* strains by using conventional methods alone. Recently, due to the diversity of the *E. coli* group itself, no particular biochemical characteristic was specific to the *E. coli* group that can be used for *E. coli* strain identification (Sojka, 1965). This finding is in agreement with the previous findings which revealed that *E. coli* strains isolated from chicken with colibacillosis poses a variable biochemical characters (Raji *et al.*, 2007). Another finding also showed that various *E. coli* biotypes with diverse biochemical profiles were identified in poultry isolates (Kika *et al.*, 2013). Even though certain biochemical tests have been used widely for *E. coli* identification in many laboratories, but still no definite results can be obtained by conventional methods alone. Certain *E. coli* strains produced slightly different

biochemical profiles compared to expected *E. coli* reaction, and have been reported previously in several studies (Bettelheim, 1994). However, the serotyping scheme available has been accepted internationally as a method for choice for epidemiological investigation purposes, but a large number of fully characterized antisera is largely restricted to most of laboratories. Some biochemical reactions which are particular for *E. coli* have been used to differentiate among the strains as reported previously, with extensive studies on the development of biotyping schemes for *E. coli* based on the ability of strains to ferment dulcitol, raffinose and sorbose and to decarboxylase ornithine (Bettelheim, 1994). Those studies concluded that when the strains from a variety of sources were tested, then good discrimination was achieved, however when the sources were limited, the discrimination was not so good, suggesting that the strains may be related to each other.

Lactose was one of the first substances used to determine the biochemical properties of *E. coli*. It is a reliable method to distinguish *E. coli* from other enterobacteriaceae. In this study, all the *E. coli* isolates tested were able to ferment lactose, which is in agreement with findings reported previously (Tonu *et al.*, 2011). However, certain *E. coli* isolates that did not give a positive reaction has been reported previously (Rodriguez-Siek *et al.*, 2005). Rodriguez-Siek *et al.* (2005) reported that 0.19% *E. coli* isolates haemolysed blood agar, in contrast with the

recent findings with 1.67% of the isolates able to haemolysed blood agar. In another finding, Rodriguez- Siek *et al.* (2005b) also reported that none of the APEC strains tested showed hemolytic activity on blood agar.

Certain properties including utilization of citrate, Voges-paskeur and methyl red are not constant but liable to fundamental changes e.g. after storage for a considerable time. Other earliest properties of *E. coli* was its ability to form indole in a medium containing peptone in the presence of tryptophan. However, there were strains which failed to produce indole (Bettelheim, 1994). Typical *E. coli* strains do not grow on an ammonium substrate containing sodium citrate or known to produce Simmons citrate negative reactions, nevertheless strains which are 'citrate positive' also occur in small number of isolates. Therefore, it is not surprising to observe the contradictory reactions in this finding. Fermentation of malonate on the other hand, gave expected results as 100% of the isolates tested gave negative reaction. Fermentation of malonate is very useful to differentiate *E. coli* from strains of other groups of enterobacteriaceae, such as *Aerobacter*. According to previous reports, almost 69.5% of *E. coli* strains produced positive reaction of lysine decarboxylase, 4% arginine decarboxylase and 52% ornithine decarboxylase. However, these findings do not correlate with recent findings whereby there was a higher percentage of *E. coli* isolates in this study positive for the above reactions

as compared to previous reports. Some of the *E. coli* strains are motile organisms, so variety in motility results were observed in this experiment. Khaton *et al.*, (2008) reported that all the *E. coli* strains isolated from poultry in Bangladesh were motile.

However, strains of *E. coli* may become altered in their biochemical characteristics following subculture, whereby both losses and gain in biochemical activity following subculture can occur or when the bacteria was kept in room temperature for a certain period as described previously, but strains stored at  $-70^{\circ}\text{C}$  or  $4^{\circ}\text{C}$  did not show any changes (Katouli *et al.*, 1990). Therefore, certain differences in the biochemical activity may relate to the storage condition of the isolates. As mentioned earlier, most of *E. coli* isolates used in this study were obtained from diagnostic cases and have been kept for various time periods from 1 to 5 years, in maintenance medium at room temperature. Besides, biochemical properties do not associate with their virulence properties, but are more correlated with the serogroups (Dho Moulin *et al.*, 1999). Based on the results obtained, it is concluded that no single feature is particularly characteristic of *E. coli*. Therefore, different tests are required for comparison of various reactions in detail for identification of *E. coli* in the laboratories.

Nowadays, usage of antibiotics is not restricted for therapeutic purposes only, but also widely used as feed additive and growth promoters in poultry farms.

Series of antibiotics such as amoxycilin, penicilim, trimethorpim, aminoglycosides, ciprofloxacin and semi synthetic antibiotics were suggested for treatment against *E. coli* infections, while antibiotics including tetracycline, trimethoprim, penicillin and bacitracin have been used as growth promoter and subtherapeutic purposes in livestock and poultry farms as well.

Based on this finding, the high percentage of multidrug resistance (81.6%) in *E. coli* isolated from chicken was in agreement with other studies in other Asian countries. In Thailand, higher prevalence (100%) of multidrug resistance in broilers was reported (Mooljunttee et al., 2010). While in China, Dou et al. (2015) found that multidrug resistance in avian *E. coli* isolates was 80.3%, with high resistance to erythromycin and tetracycline. In Malaysia, previous studies reported that high multidrug resistant profiles towards different antibiotics were observed with the highest percentage reported (100%) in *E. coli* isolated from chicken in different geographical locations (Geidam et al., 2013; Ong et al., 2014). This finding also revealed that high frequencies of resistance against erythromycin (92.8%) and tetracycline (92.0%) were observed among isolates tested. Several studies also indicated high resistance rate of avian *E. coli* against erythromycin and tetracycline (Kazemnia et al., 2014; Wang et al., 2013; Zhang et al., 2012; Dou et al., 2015), and this situation is usually related to their subtherapeutic usage in *Mycoplasma* infections in poultry farms.

Erythromycin has been used in poultry farms as prevention and reduction of respiratory distress caused by *Mycoplasma synoviae* and *Mycoplasma gallicepticum* (Talebiyan et al., 2014). Several factors related with high resistantance of *E. coli* towards tetracycline including other resistant mechanisms through presence of tetracycline alleles which is found in commensal *E. coli* as well. Miles et al. (2006) also reported that tetracycline resistance in avian *E. coli* isolates was mediated by presence of *tetB* and *tetD* gene, which usually acquired through horizontal transfer among *E. coli* isolates. Long term and frequent used of tetracycline for decades also leads to extremely severe tetracycline resistance (Dai et al., 2008). Meanwhile, cross contamination among antibiotics of the same class can also contribute to the high resistance rates to other antibiotics such as usage of tetracycline may cause high frequencies of resistance towards deoxycycline (Sharada et al., 2008). However, from the present study it was proposed that high resistance rate against erythromycin and tetracycline is not associated with the overuse or improper use of those antibiotics in poultry farms, as both antibiotics were not commonly used in poultry farms in the country. As described by Smith et al. (2007), usage patterns of antibiotics may not correlate with incidence of antibiotic resistance prevalence among avian *E. coli*.

The percentage of *E. coli* isolates resistant to quinolones (77.6%) observed in this study, was slightly lower compared

to studies in other countries (Moniri *et al.*, 2005; Rahimi, 2013; Allocati *et al.*, 2013). Resistance rate against quinolones related concomitantly related with consumption of quinolones in the farms (Moniri *et al.*, 2005), however other resistant mechanisms including mutation of certain resistant genes also found to related with resistant against quinolones among *E.coli* isolates (Szmolka *et al.*, 2013).

Based on this study, most of the *E. coli* isolates found in chicken were highly susceptible to colistin, polymyxin B (92.0%) and ceftiofur. The results indicated a good choice for treatment of *E. coli* infections in the farms as both were clinically available polymyxin class antibiotics that has been effectively used for treatment of Gram-negative bacterial infections including *E.coli*. This finding is in agreement with several reports demonstrating that resistance to polymyxin class of antibiotics is very uncommon among *E. coli* isolates from different geographical regions (Gales *et al.*, 2011). Low resistance score of *E. coli* isolates (8.0%) towards certiofur in this study contradict with previous studies which reported that more than 80% of *E. coli* strains isolated from chicken were resistant to ceftiofur.

Meanwhile, the isolates which exhibited a high percentage of moderate susceptibility profiles towards amoxycilin (25.6%) and apramycin (16.0%) should be taken into consideration, as the profiles might develop into resistant profiles to that particular antibiotic. The results of

this study also indicates that resistance to both antibiotics may increase over time. Therefore, regular surveillance on antibiotic resistance is important in order to monitor any emergence of resistant profiles of *E. coli* in chicken.

In conclusion, this study demonstrated that there were high multiple resistance of *E. coli* strains isolated from chicken. Polymyxin B and colistin is recommended for the treatment of *E. coli* infection in the farms, as it was shown to be effective for disease control. However, establishment of guidelines for prudent use of antibiotics in farms with effective enforcement is required, so that the occurrence of antibiotic resistance of *E. coli* in chicken can be managed in the future, thereby limiting the flow of these elements of resistance into the human food chain.

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## Short communication

**A CASE REPORT OF SCALY LEG MITE IN GREEN PEAFOWL (*Pavo muticus*)****SITI AMINAH Y.\*<sup>1</sup>, DONNY Y.<sup>1</sup>, ZUBAIDAH K.<sup>2</sup>, ZAITUL HAZLIN M.J.<sup>3</sup>, SIVANANTHAN E.<sup>1</sup> AND MISLIAH M.B.<sup>1</sup>**

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**SUMMARY.** This is a case report of a captive female green peafowl (*Pavo muticus*) that was presented with severe scaly legs with raised encrusted scales on both legs. Diagnosis of scaly leg mite was made based on history, clinical signs, and results of parasitological examination from deep skin scrapping from the area of lesions and response to treatment. Treatment consisted of Ivermectin solution, administered orally at a dose rate of 0.2 mg/kg. The gross lesions were completely resolved 28 days post treatment. It was concluded that based on the treatment given, knemidocoptiasis or scaly leg can be successfully controlled with good prognosis in captive birds. Care should be taken as the mite is transmitted from bird to bird through prolonged close or direct contact.

**Keywords:** scaly, encrusted, deep skin scrapping

The peafowl are birds in the genera *Pavo* of the Phasianidae family, and are known for the male's piercing call and, among the Asiatic species, his extravagant eye-spotted tail covert feathers which he displays as part of a courtship ritual (Davies *et al.*, 2012). Peafowls are forest birds that nest on the ground but roost in trees. In captivity, they need to be housed in large aviaries.

Scaly Leg Mite infection is caused by a burrowing mite (*Knemidocoptes mutans*) which causes scaly, raised encrusted scales on the legs of birds and other avian species. Scaly leg can cause intense irritation to the bird by burrowing under the scales, causing them to become raised and thickened. The scales often look like they are protruding outwards and parts of the scales will come off, making the legs look unsightly (Mercks, 2010). Birds need to be treated to kill the mites and then scales left to come away naturally through a moult. According to Bowman,

1995, *Knemidocoptes* species mites spend their entire three-week life cycle on their bird hosts. The females are viviparous and the larvae have three pairs of legs. After two nymphal stages, the mites mature into adults that have four pairs of legs. The mites burrow into the feather follicles and stratum corneum, primarily on the face, feet, and comb, where they feed on keratin. Most commonly, the unfeathered regions (beak, eyelids, legs, and vent) are affected. As the mites burrow, they form tunnels and may get further infected with secondary bacteria causing pain and eventually death. The mites are transmitted from bird to bird through prolonged close or direct contact. Although the mites are primarily transmitted from parent to unfeathered nestlings, knemidocoptiasis appears to be more opportunistic than infectious and seldom found in wild birds but more commonly in captive birds especially if there is overcrowding in the aviaries.

In this case, a female green peafowl (*Pavo muticus*) with the microchip identification number 900032000020507 weighing about 5 kg was presented with severe raised encrusted scales on the both legs. The bird was housed in an aviary with other peacocks and peahens, usually in pairs, in Pusat Pendidikan Biodiversiti Bukit Marak, Terengganu (Biodiversity Educentre). At this centre, conservation activities are carried out for peafowl which involves, breeding, rescue and treatment. There were a total of 13 birds in this centre; seven male and five female adults, and one juvenile. Another 10 birds were

placed here also belonging to another organization (UPEN) for conservation purposes. All birds were fed routinely with cracked grains, fruits and a variety of local vegetables and provided water *ad lib*. The keeper noted the skin lesions on the peafowl which showed clinical signs of difficulty in perching and walking. However, the bird was otherwise normal with good appetite, active and alert. On presentation, physical examination revealed raised encrusted scales on the both legs as shown in Figure 1. The skin of both feet was markedly thickened and covered with thick friable crusts (hyperkeratosis). The differential diagnoses when presented were parasitic infestation, that is, scaly leg mite infection (*Knemidocoptes jamaicensis*), fungal infestation, contact dermatitis and hyperkeratosis due to allergy.

A deep skin scrapping was done on the leg lesions and the sample was sent for laboratory analyses (Christopher *et al.*, 1992). The microscopic examination of the scraping did not reveal any mites. This could be due to the sampling method or preservation as the mites can move away very rapidly when disturbed. Moreover, a drop of glycerol on the scrapping may have helped to maintain the mites to facilitate microscopic examination.

However, based on the clinical signs and pathognomonic lesions, treatment was instituted with Ivermectin at a dose rate of 0.2 mg/kg, given orally three times every fortnight over a period of six weeks. The Ivermectin had an original concentration of 10 mg/ml solution but was diluted with



**Figure 1.** Condition of lesion when presented, indicating crusty lesions on both legs of peafowl.



**Figure 2.** Crusts on the legs of peafowl fall off after the second treatment about two weeks after presentation



**Figure 3.** Leg lesions completely healed after the third treatment about five weeks after presentation

sterile water to obtain a 1 mg/ml solution. With the dose rate of 0.2 mg/kg, the 5 kg bird was given 1 ml of the diluted Ivermectin orally, 3 times 2 weeks apart. Observations were made on the progress of healing of the lesions as shown in Figures 2 and 3. Two weeks after the onset of treatment, the scales were sloughing off and after the third treatment, the bird was fully healed with both legs devoid of crusty scales, with new skin growth. The bird was observed to be healthy, active and alert.

The prognosis in this case is good. The bird responded to treatment well since the gross lesions were reduced and completely healed after the third treatment. One important consideration in diagnosing parasitic infections of the skin, is the effectiveness in sampling technique which is crucial in identifying the accurate causative agent. In most cases, scrapings should be taken from the edge of the lesion, from obviously pruritic locations, and from where there are thick, crusty flakes. Take a skin scraping by holding a scalpel blade or other sharp instrument at a right angle to the skin and scraping off the outer surface of the skin. For those mite species that burrow into the skin, the scraping must be deep enough to cause a small amount of blood to ooze from the scraping site. A drop of mineral oil or glycerol may be placed on the blade to help hold the skin scrapings during the procedure. Skin scrapings should be placed in sealed containers (e.g. clean, empty salve tins; stoppered glass/plastic test tubes; small, sealable plastic bags) and promptly taken

or sent to a laboratory for more thorough examination (Klayman & Schillhorn van Veen, 1981). In this case, skin scraping is done at the site (aviary) and limitations due to handling and restraining the patient effectively as well as biosafety and security need to be resolved in order to obtain a good sample for diagnostic work.

Treatment of choice for birds with scaly leg mite lesions is Ivermectin given orally; which was very effective in this case. Some suggested preventative measures are: aviary hygiene, cleanliness and preventive care such as prophylactic acaracidal treatment (Merck, 2010). Knemicoptic mite infection is not zoonotic. It is important for avian species as it causes unsightly, uncomfortable, and potentially life-threatening lesions. It is economically important for zoos or captive bird management, especially of rare, exotic or valuable bird species.

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