

ECOLOGY OF *HAEMAPHYSALIS WELLINGTONI* IN RED JUNGLEFOWL

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Abstract

The ecology of the parasitic stages of *Haemaphysalis wellingtoni* was studied in 30 Red Junglefowls in a farm in Jenderam Hulu, Sepang, Selangor, Malaysia. The free ranging Red Junglefowls are trapped using leg traps and individual bird was carefully examined for tick infestations. The ticks were collected from the neck, comb, and outer ear canal, counted and preserved in 70% ethanol. The identification of the tick species and their stages at each site was done through examination using a stereomicroscope and keys to tick identification. Tissue biopsies with ticks attached were also obtained to determine the pathology of tick-feeding sites. The comb tissues were processed with the standard histological technique and stained with hematoxylin and eosin to observe the cellular changes initiated by the ticks. Only one species of the tick was identified, which was *Haemaphysalis wellingtoni*. The mean of tick numbers in the outer ear canal was the highest (1.77 ± 0.193), followed by the dorsal aspect of the neck (1.32 ± 0.329) and the comb (0.72 ± 0.190). In this study, there are significant differences in stage of the ticks at different infestation sites suggesting that they have preferential site for different stages of their life-cycle. Generally, the mean count for tick stages (adult, nymph and larval) were almost similar on the dorsal part of neck and the outer ear canal. The mean numbers of nymph and larval stages in the neck and outer ear canal were similar, except for the adult stage which was 3.63 on neck and 3.33 in the outer ear canal. However, adult stage was absent on the comb while the mean of nymph stage was 0.16. The larval stage was lower in number in the comb (0.87) compared to the neck and outer ear canal (both 2.2). The cellular changes observed at tick feeding sites consisted of eosinophil and very high lymphocyte infiltrations which indicated chronic inflammation. The congestion, hyperkeratosis and detachment of the keratin layer were also observed at tick feeding sites.

Keywords: *Haemaphysalis wellingtoni*, Red Junglefowl, eosinophils, lymphocyte, Inflammation

INTRODUCTION

The common ectoparasites infesting poultry includes ticks, mites and also lice. The ectoparasites such as ticks and mites can cause heavy morbidity due to their blood sucking habits and cause irritation to the birds causing economic loss (Phulan *et al.*, 1984). Heavy infestation of ticks cause severe blood loss and leading to anaemia and

eventually death (Bergstrom *et al.*, 1999). Ticks and other ectoparasites are able to transmit viral, bacterial and parasitic diseases such as Pasteurellosis, Avian encephalomyelitis, Aegyptiniallosis, fowl Cholera and Borreliosis (Permin and Hansen, 1998).

The ticks can be divided into two families: Ixodidae and Argasidae. Basically the Ixodidae has a chitinous covering, known as the scutum which extends over the whole dorsal surface of the male ticks. However, the scutum only covers a small area behind the head in the larvae, nymph and female ticks. The tick species such as *Haemaphysalis cinabarina*, *H. leporispalustris*, *H. chordeilis*, *Amblyomma haebraeum* and others feed on the poultry and wild ground birds (Johannes, 1996). The species of ticks infesting birds in Malaysia has been reported in Malaysian Parasites XLIX, (Audy *et al.*, 1960), and they consist of the genera *Amblyomma*, *Haemaphysalis*, and *Dermacentor*.

Haemaphysalis species are inornate ticks and have festoons but the eyes are absent. The sensory palps for this genus are short and broad with the second segment extending beyond the basis capituli. In Malaysia, the species *Haemaphysalis wellingtoni* has been identified, infesting the poultry. The genus *Haemaphysalis* is the second largest in the family of Ixodidae. The ixodidae family undergoes four stages: eggs, larvae, nymphs and finally adult stage. This family only has one nymphal stage. Most of the Ixodidae family requires three hosts to complete their life cycle (Oliver, 1989).

The saliva of the blood sucking arthropod has been identified as the sources of toxins or allergens. There is a vast literature on both the toxic and antigenic properties of this secretion (Ribeiro, 1987). The type of cells infiltrating tick feeding sites can be predominantly mononuclear cells or neutrophils. The type of cells infiltrating tick feeding sites can change after multiple infestations (Heinz, 2001). This study was conducted to cover the species of ticks infesting the Red Junglefowl, the predilection sites of infestation, the stages of ticks found at each site and the description of microscopic lesions produced by the host-ticks reaction.

MATERIALS AND METHOD

Animals

This study was conducted on a one-acre Red Junglefowl farm situated in Jenderam Hulu, Sepang, Selangor, Malaysia. These Red Junglefowls were kept under a free-range system and was mixed with village chickens and geese. They were fed once daily in the morning with commercial poultry feed.

Sampling

Eight to 10 birds were randomly trapped daily using the leg trap. Thirty Red Junglefowls were used in this study and these birds were more than 6-month old. In this study the Red Junglefowls were randomly sampled. The individual bird was examined for the ticks on the head region especially the comb, neck and the ears. The ticks found on individual birds at different sites were collected and kept in separate bottles containing 70% ethanol. The numbers collected at each site were recorded. Each individual bird had 3 bottles to collect ticks from the 3 sites.

Identification and Counting the Number of Ticks

The counting and determination of the species of tick were done using stereomicroscope. For each of the 3 sites, the stages of the ticks were also recorded. For identification of the tick species, the specimen was first mounted and then identified based on the mouth parts, presence of eyes, anal groove, presence of festoons and also the basis capituli. *Haemaphysalis* sp. can easily be identified based on the following: the second segment palpi is laterally projected, the eyes are absent, the anal groove is situated below the anus and the basis capituli is rectangular and also the presence of the festoons.

The larval stages are identified based on the presence of 3 pairs of small legs. The nymphal stage is small, have 4 pairs of legs and do not have the genital aperture at the ventral site of the body. In the adult stage, the male and female ticks are identified based on the presence of the scutum. In the male, the scutum covers almost the entire body, while in the female ticks the scutum covers part of the anterior of the body.

Tissue Biopsy and Histopathology

The tissue biopsy of the comb containing the attached ticks was taken from one bird. The birds were anaesthetised locally with 0.1 mL of 2% Xylocaine. The site of combs attached with ticks and an uninfested site was cut and the samples preserved in the 10% Formalin before staining with hematoxylin and eosin.

RESULT AND DISCUSSION

Identification of Tick Species

In previous studies, the most common ectoparasite found was the tick *Haemaphysalis wellingtoni* in 60% of the village chickens (Amin-Babjee *et al.*, 1997). In this study, the species of the ticks identified to affect the Malayan Red Junglefowls was confirmed to be *Haemaphysalis wellingtoni*. The genus of *Haemaphysalis* was identified based on some characteristic morphological parts of these ticks. These ticks had broad palpal segment and the second palpal segment laterally projected. The anal groove of these ticks was seen contouring posteriorly of the anus and there was presence of the festoon.

Predilection Sites of Tick Infestation

Based on the descriptive analysis of variance, ANOVA, $p < 0.05$, there was a significant difference in the number of ticks at the different sites of infestation. The results showed that the ears were the site which has the highest percentage (46%) of ticks, followed by the dorsal aspect of the neck (34%) and the comb (19%). The results also showed the outer ear canal was the site with the highest mean tick number 1.77, followed by dorsum of the neck (1.32) and the comb (0.72). The results indicated the ticks preferred sites which are less exposed to grooming, such as the outer ear canal and the dorsal aspect of the neck.

Stages of Ticks at Specific Sites

This study shows that the stages of ticks found in different sites varied significantly ($p < 0.05$). The different stages of tick's life cycle have their own predilection feeding site on the host. The mean numbers of nymphs and larval stages in the neck and outer ear canal were similar, except for the adult stage which was 3.63 on dorsum of the neck and

3.33 in the outer ear canal. However, there was no adult stage on the comb, but the mean numbers of the nymphal stages was 0.16 was lower than in the neck and outer ear canal (both 0.3). The larval stage is lower in number in the comb (0.87) than in the neck and outer ear canal (both 2.2). This behavior could reflect the feeding stage in the tick life-cycle. The lower mean number of nymphal stages in the host could also be the result of them dropping off after feeding.

Histopathology of Tick Feeding Site.

There were evidence of separation and detachment of keratin layer as the result of tick attachment. The thickening of the keratin layer, hyperkeratosis was observed at the feeding site with monocytes being the predominant cells and presence of several eosinophils. Lima e Silva *et al.*, (2004) described the microscopic features of tick-bite lesions in anteaters and armadillos to include dermal changes with infiltration by variable numbers of inflammatory cells, oedema, haemorrhage and vascular dilatation. In our study, we observed similar cellular eosinophilic and massive lymphocytic infiltrations. High numbers of red blood cells were seen occupying the sinus of capillary in this tissue. In conclusion, there was only one species of tick identified which was *Haemaphysalis wellingtoni*. Based on the results, the outer ear canal is the most common predilection site of these ticks followed by the neck and the comb. Adult ticks were predominantly found on the neck and in the ear canal while nymph and larval stages of the tick appeared to be present at all the three sites. Tick infestations induced pathological changes to the skin structure especially at bite sites, which showed heavy cellular reactions.

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