



UNIVERSITI PUTRA MALAYSIA

**NATURAL AND EXPERIMENTAL COCCIDIAL INFECTION IN THE
MALAYAN RED JUNGLE FOWL (*GALLUS GALLUS SPADICEUS*)**

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**NATURAL AND EXPERIMENTAL COCCIDIAL INFECTION IN THE
MALAYAN RED JUNGLE FOWL (*GALLUS GALLUS SPADICEUS*)**

By

LEE CHU CHONG

**Thesis Submitted in Fulfilment of the Requirements for the
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LIST OF ABBREVIATIONS

E	-	Eosinophil
EM	-	Electron microscope
FELDA	-	Federal Land Development Authority
H	-	Heterophil
IEL	-	Intraepithelial lymphocytes
MAAF	-	Ministry of Agriculture, Fisheries and Food (London)
MC	-	Mast cell
MN	-	Mononuclear cell
NIAH	-	National Institute of Animal Health, Japan
opg	-	Oocysts per gram
PBS	-	Phosphate buffered saline
pi	-	Post-infection
S.G.	-	Specific gravity
TEM	-	Transmission electron microscope
UPM	-	Universiti Putra Malaysia
ysd	-	yolk sac diverticulum



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By

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Chairperson: Professor Dr. Aini Ideris, Ph.D.

Faculty: Veterinary Medicine and Animal Science

Wild Malayan red jungle fowl faecal samples were examined to determine the prevalence and species of *Eimeria* from their natural habitat. The birds possessed at least five coccidial species, namely *Eimeria mitis*, *E. acervulina* var. *diminuta*, *E. praecox* var. *ceylonensis*, *E. maxima* var. *indentata* and an unidentified *Eimeria* species. No attempt was made to name this unidentified species as information on other stages was lacking.

Intensively reared Malayan red jungle fowl chicks were used to study the pattern of coccidial infections. The chicks suffered from coccidiosis caused by *E. tenella*, *E. necatrix* and *E. maxima*. The mean prepatency period in these chicks was 9 days; their peak oocyst counts ranged from 136,364 to 591,200 oocysts per gram of faeces. *Eimeria tenella* was the most prominent pathogenic species encountered.

Karnovsky's fixative and McNamara's Giemsa stain were used for the first time for processing mucosal tissue samples for the study of cellular response to *E. tenella*. Two groups of laboratory hatched chicks were each infected orally with *E.*



tenella (NIAH, Japan strain) with either 15,000 or 30,000 oocysts whilst the third group remained as controls. Eosinophils increased significantly ($P < 0.05$) while mast cells declined significantly ($P < 0.05$) from day-5 to day-7 post infection.

The cell types recognised in the caecal mucosae were ultrastructurally quite similar to those reported from the domestic chicken. Most mast cell granules were surrounded by halos in various degranulating stages. The eosinophils had regular membrane bound dense granules. The heterophils possessed large, oval to spindle dense granules, small round to oval dense granules and small light, round to spindle granules. Mononuclear cells had proportionally large distinct double wall nuclei. The cytoplasm of lymphocyte contained prominent round mitochondriae whilst that of the plasma cell was packed with several strands of endoplasmic reticulum.

This study showed that jungle fowls could suffer from coccidiosis when domesticated. However, proper management together with the necessary supportive treatment with drugs could be effective against such infections.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

JANGKITAN KOKSIDIA SEMULAJADI DAN UJIKAJI DALAM AYAM HUTAN MERAH MALAYA (*GALLUS GALLUS SPADICEUS*)

Oleh

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Mac 1998

Pengerusi: Profesor Dr. Aini Ideris, Ph.D.

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Tinja daripada ayam hutan merah Malaya telah digunakan untuk menentukan prevalens dan spesies *Eimeria* daripada habitat semula jadinya. Didapati ada sekurang-kurangnya lima spesies *Eimeria*, iaitu *Eimeria mitis*, *E. acervulina* var. *diminuta*, *E. praecox* var. *ceylonensis*, *E. maxima* var. *indentata* dan satu spesies *Eimeria* lagi yang belum dikenalpasti. Spesies *Eimeria* ini belum dikenalpasti sebagai satu spesies baru oleh sebab maklumat mengenai peringkat lain perlu diperolehi terlebih dahulu.

Anak ayam hutan merah dipelihara secara intensif telah digunakan untuk mengkaji pola wabak koksidiosis. Ayam hutan ini mengidap koksidiosis daripada *E. tenella*, *E. necatrix* dan *E. maxima*. Min tempoh prajelas dalam ayam ini ialah 9 hari; bilangan kemuncak oosistanya berjulat daripada 136,364 hingga 591,200 oosista setiap gram tinja. *Eimeria tenella* didapati merupakan spesies patogenik yang terkemuka.

Pengawet Karnovsky dan perwama Giemsa McNamara telah digunakan buat kali pertama untuk memproses contoh tisu mukosa untuk kajian responsi sel

terhadap *E. tenella*. Dua kumpulan anak ayam hutan yang didapat dari pengeram telur makmal telah diinokulat secara oral dengan *E. tenella* (strain NIAH, Japan) sama ada dengan 15,000 atau 30,000 oosista setiap satu dan kumpulan ketiga digunakan sebagai kawalan. Eosinofil meningkat secara signifikan ($P < 0.05$) sementara sel masta menurun bilangannya secara signifikan ($P < 0.05$) pada lima hingga 7 hari selepas jangkitan.

Jenis-jenis sel yang dikenalpasti dalam mukosa sekum adalah agak serupa secara ultrastruktur dengan yang pernah dilaporkan pada ayam bela jinak. Kebanyakan granul sel masta didapati dikelilingi oleh halo pada pelbagai peringkat penyahgranulan. Eosinofil mempunyai biasanya granul tumpat terikat membran. Heterofil mempunyai granul tumpat, besar, bujur hingga spindel; granul tumpat, kecil bulat hingga bujur; granul kecil ringan, bulat hingga spindel granul. Sel mononukleor mempunyai secara berkadaran nukleus besar jelas berdinding dua lapis. Sitoplasma limfosit mengandungi mitokondrion bulat jelas. Sitoplasma sel plasma tersesak dengan beberapa utas retikulum endoplasma.

Kajian ini menunjukkan bahawa ayam hutan boleh dijangkiti koksidiosis bila dipelihara secara intensif. Walaubagaimanapun, pengurusan yang baik di samping rawatan, di mana perlu, boleh mengawal jangkitan ini dengan lebih berkesan.

CHAPTER I

INTRODUCTION

There are four known species of jungle fowls in the Indian subcontinent and in Southeast Asia, namely *Gallus gallus* (red jungle fowl), *Gallus lafayettii* (Ceylon jungle fowl), *Gallus sonneratii* (Indian grey jungle fowl) and *Gallus varius* (Javanese green jungle fowl) (Crawford, 1990).

The red jungle fowl (*Gallus gallus*) has the widest distribution and is found from North India through Burma, Thailand, Vietnam, Peninsular Malaysia and Indonesia. Peninsular Malaysia is included as a natural habitat of the red jungle fowl (Beebe, 1931). In the Philippines, Celebes and the lesser Sunda Islands, it is an introduced species (Delacour, 1977). The red jungle fowl is widely recognised as the ancestor of the domestic chicken (Davies *et al.*, 1963; Collias and Saichuae, 1967). It consists of five recognised subspecies, viz. *Gallus gallus gallus* (Cochin Chinese red jungle fowl), *Gallus gallus jabouillei* (Tonkinese red jungle fowl), *Gallus gallus bankiva* (Javanese red jungle fowl), *Gallus gallus murghi* (Indian red jungle fowl) and *Gallus gallus spadiceus* (Burmese red jungle fowl) (Nishida *et al.*, 1992). The red jungle fowl is not known to exist naturally in Borneo. *Gallus gallus spadiceus* is the only subspecies occurring in Peninsular Malaysia (Nishida *et al.*, 1992) and is known by various widely used local names, such as ‘Ayam Birga’, ‘Ayam Beroga’,



‘Ayam Denak’ and ‘Ayam Hutan’. This gallinaceous bird is the commonest game bird in the Malay Peninsula and is found to forage in oil palm, rubber, tea, coffee, cocoa and fruit plantations and in secondary forests near open or cultivated areas (Dr. S.M. Amin-Babjee, 1997, personal communication).

The Malayan red jungle fowl has been observed to be infested with parasites found in the domestic fowl (Amin-Babjee *et al.*, 1985). Over 40 species of parasites have been recorded from this jungle fowl (Lee and Amin-Babjee, 1993). The nematodes *Pelecitus galli* (Dissanaike and Fernando, 1974b), *Lemdana latifi* (Lee *et al.*, 1989a), *L. sonneretta* (Lee and Amin-Babjee, 1990), acanthocephala *Mediorhynchus gallinarum* (Lee *et al.*, 1985a), kidney trematode *Tanaisia vietnamensis* (Lee *et al.*, 1985b) and the caecal trematode *Postharmostomum gallinum* (Lee *et al.*, 1989b) are some endoparasites found and described. *Leucocytozoon sabrazesi* (Chin *et al.*, 1974), *Trypanosoma* sp. (Dissanaike and Fernando, 1974a), *Plasmodium gallinaceum* and *P. juxtannucleare* (Fernando and Dissanaike, 1975) are the blood protozoa observed. *Eimeria diminuta* which was renamed as *E. acervulina* var. *diminuta* and *E. indentata* which was reclassified to *E. maxima* var. *indentata* after cross-protection studies in the domestic chicken by Long (1974b), are the protozoa of coccidial species identified from the Malayan red jungle fowl. Some authors are of the opinion that the red jungle fowl being the ancestor of the domestic chicken might harbour many or all of *Eimeria* species present in the latter (Fernando and Remmler, 1973a; Long *et al.*, 1974). The ability of coccidial species to develop in different species of jungle fowls can possibly be related to their interbreeding (Williams, 1986).

Malayan jungle fowl chicks hatched from incubators are found to be susceptible to fowl pox, Newcastle disease, respiratory diseases, helminth, arthropod and protozoal infections/infestations (S.M. Amin-Babjee, 1997, personal communication). However, no information is available on these diseases in the wild jungle fowl. They are probably less exposed to the diseases in their natural environments because of the wide areas available to them. When newly caught jungle fowls are exposed to limited space concentrated with parasites they suffer severely due to being stressed and to their lack of immunity and exposure to these parasites. Jungle fowl chicks kept in intensive system in the Universiti Putra Malaysia (UPM) farms are found to be susceptible to many diseases especially coccidiosis (Lee *et al.*, 1996; Liao, 1996).

Coccidiosis is one of the three most important diseases in the domestic chicken throughout the world (Trees, 1987). It occurs concurrently with other diseases and exists in young growing and susceptible birds. Birds raised under warm and humid conditions causing wet litter and kept in large numbers, can suffer from coccidiosis (Whiteman and Bickford, 1989).

There was no detailed study on the *Eimeria* species in the Malayan red jungle fowl. This study was therefore conducted to obtain information on their prevalence and intensity in the wild red jungle fowls, oocyst outputs in domesticated groups and cellular response to *E. tenella* infection in domesticated red jungle fowls. An ultrastructure study of the cell types induced by one species, *E. tenella* was also conducted.

The study was divided into three main parts. Initially, observations were made on the prevalence of coccidia in the wild Malayan red jungle fowl from the natural habitat. Attempts were also made to determine the species of coccidia found. The second part of the study consisted the rearing of naïve Malayan red jungle fowl chicks under intensive management system. The final part of this study was conducted on the cellular response to *E. tenella* infection in this red jungle fowl.

Thus the objectives of this study were:-

1. to determine the prevalence and the species of *Eimeria* in newly caught wild Malayan red jungle fowls.
2. to determine the daily output of coccidial oocysts from domesticated Malayan red jungle fowl chicks from one day old to 120 days old and species of *Eimeria* from clinical cases.
3. to study effects of coccidiosis and the caecal mucosal cellular response to *E. tenella* infection in the domesticated Malayan red jungle fowl.

CHAPTER II

LITERATURE REVIEW

Coccidia

Classification and Taxonomy

Eimeria, *Isospora* and *Cryptosporidium* are genera of protozoa of considerable veterinary importance. The term coccidiosis usually refers to diseases caused by the genera *Eimeria* and *Isospora*. They belong to the family **Eimeriidae** which are mainly intracellular parasites of the intestinal epithelium (Soulsby, 1986). The family belongs to the subkingdom **Protozoa**, phylum **Apicomplexa**, class **Sporozoasida**, Subclass **Coccidiasina**, order **Eucoccidiorida** and the suborder **Eimeriorina** (Levine *et al.*, 1980; Shirley, 1992). **Apicomplexa** refers to the possession of an apical complex, a structure which assists penetration of the host cells and visible via the electron microscope (Urquhart *et al.*, 1987).

Life Cycle of *Eimeria* in Domestic Chickens

Coccidia of the genus *Eimeria* are generally quite site, organ and host specific and self limiting in nature. Individual species differ in the oocyst sporulation time (asexual sporogony phase), the number of schizogonous generations (asexual cycle),

the sexual phases, the required time for each developmental stage and the site parasitised in the gastrointestinal tract of the host (Pellerdy, 1974).

The non-infective, unsporulated oocysts each containing a zygote or sporont, are released in the faeces from infected birds. Under favourable environmental conditions four sporocysts, each containing two sporozoites, are formed in the oocysts after about 24 hours. A susceptible bird gets infected by ingesting the sporulated oocysts.

The sporocysts are released in the gastrointestinal tract of the bird mechanically, mainly in the gizzard. Trypsin from the pancreatic juice is found to be necessary for excystation (Ikeda, 1960). The chymotrypsin together with the bile salt and carbon dioxide activates the sporozoites (Reid, 1978). Bile requirement for excystation in *Eimeria* is dependent upon the species. *Eimeria acervulina*, *E. tenella* and *E. maxima* excyst more rapidly probably due to the bile activity present in the intestine (Speer *et al.*, 1970; Rose and Hesketh, 1983; Shiotani *et al.*, 1992). They move through the sporocyst micropyle into the oocyst thence migrate through the oocyst micropyle. The fusiform, transparent sporozoites contract, elongate, glide rapidly and penetrate the epithelial cells of the appropriate site of the intestine according to the species involved. Those of *E. necatrix* migrate through the lamina propria towards the muscularis mucosa and are engulfed by macrophages (van-Doornick and Becker, 1957). *Eimeria acervulina* sporozoites are transported to the crypts in macrophages (Doran, 1966). Lawn and Rose (1982), however, found *E. tenella* and Fernando *et al.* (1987) found *E. maxima*, *E. acervulina*, *E. brunetti* and *E. praecox* sporozoite transportation to occur in intraepithelial lymphocytes (IEL).

Eimeria necatrix sporozoites are shown ultrastructurally to be transported by a granulated intraepithelial mononuclear cell (Al-Attar and Fernando, 1987). Trout and Lillehoj (1993) found *E. acervulina* sporozoite transportation to occur in T-lymphocytes and in macrophages. Lillehoj and Chung (1992) are of the opinion that the nature of transporting cells for sporozoites need to be better characterised as IEL consists of heterogenous populations that include T-, B-lymphocytes, natural killer cells and macrophages.

The sporozoites round up in the epithelial cells and form trophozoites in 12 to 48 hours. The nuclei of the trophozoites divide by multiple fission to form schizonts or meronts which contain several merozoites. This schizogonic nuclear division is of the mitotic type (Pellerdy, 1974).

The mature schizonts rupture and release the merozoites. Most of these merozoites invade other epithelial cells to repeat the schizogonous stage. Some or all merozoites may go through the third schizogonous cycle, depending on the species, before forming either the male gametocytes (microgametocytes) or the female gametocytes (macrogametocytes).

Lin and Feng (1993) have observed three generations of schizonts in pathomorphological studies of domestic chickens with *E. tenella*. Mature first generation schizonts of *E. tenella*, measure $24 \times 17 \mu\text{m}$ and have up to 900 merozoites, hypertrophy host cells in caecal glands to several times and bulge into the lumen at the bottom of the crypts of caecal glands. Second generation schizonts of *E. tenella* grow up to $50 \mu\text{m}$ in diameter containing 200 to 350 merozoites proximal to the

epithelial cell nucleus. These schizonts migrate to the sub-epithelial tissues into the submucosa and muscular layers of the caeca. The third generation schizonts of *E. tenella*, measure $9 \times 8 \mu\text{m}$ and contain only 4 to 30 merozoites. There may be more than three generations of schizonts in *E. tenella* (Pellerdy, 1974).

The small first generation schizonts of *E. necatrix* are located proximally to the nucleus of the epithelial cell of the fundus of crypts of Lieberkuhn in the small intestine. Merozoites of *E. necatrix* re-enter cells adjacent to the original position to form colonies of second generation schizonts (localisation of infection), each $63 \times 49 \mu\text{m}$, in the sub-epithelial tissues and in the submucosa. These second generation schizonts are released and carried peristaltically to the caecum for the third generation schizogony with 3 or 4 schizonts to each cell or to the gametogony cycle (Davies *et al.*, 1963; Soulsby, 1986).

Tyzzar (1929) and Long (1959) found only one generation of schizonts in *E. maxima*, but Scholtyseck (1963) and Davies *et al.* (1963) found *E. maxima* to undergo two generations of schizogony. The *E. maxima* schizonts are small, $10 \times 8 \mu\text{m}$ and each contains 8 to 16 merozoites.

First generation schizonts of *E. brunetti* are found at or close to the basement membrane of epithelial cells of the upper small intestine. They measure $30 \times 20 \mu\text{m}$ and each contains about 200 merozoites. The larger second generation schizonts measure $27 \times 16 \mu\text{m}$ each, containing 50 to 60 merozoites, whereas the smaller second