

## HAEMOPARASITES OF DOMESTIC POULTRY AND WILD BIRDS IN SELANGOR, MALAYSIA

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**ABSTRACT.** Avian haemoparasites are known to exert negative pressures on their hosts causing considerable pathology and mortalities. The present study reports on the prevalence of haemoprotozoa and microfilaria in various species of wild birds and domestic poultry in Selangor, Malaysia, and contributes to the database on the occurrence of these pathogens among avifauna in the country. Giemsa-stained thin blood smears were screened from 728 birds representing five avian orders, namely Galliformes, Anseriformes, Phoenicopteriformes, Pelecaniformes and Gruiformes. The most common haemoparasite was *Plasmodium*, with a prevalence of 8.0%. The aquatic/wetland species of birds (Anseriformes and Gruiformes) were the most common hosts for this pathogen with high infection rates (31.8% – 50.0%). The prevalence of *Plasmodium* in domestic poultry was moderate (2.7%). *Leucocytozoon sabrazesi* and *L. caulleryi* were confined to the Galliformes with relatively low average infection rates of 0.7% and 0.5%, respectively. *Haemoproteus* was detected for the first time in domestic poultry and Red Jungle fowls in the country, with an

average prevalence of 0.8%. Trypanosomes and microfilaria were only present in the village chickens and Red Jungle fowls, with high microfilaraemia rates (19.0%) in the latter. The current compilation will contribute to our understanding on avian haemoparasite transmission in the country.

*Keywords:* haemoparasites, *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Trypanosoma*, microfilaria, wild birds, poultry, Malaysia.

### INTRODUCTION

Avian haemoparasites are known to be pathogenic to their hosts causing high mortalities, reproductive failure, retardation of growth, reduced productivity, and may exert negative effects on behavior and community structure (van Riper III *et al.*, 1986; Atkinson *et al.*, 1988; Sørçi and Møller, 1997; Merilae *et al.*, 1999; Merino *et al.*, 2000; Cardona *et al.*, 2002; Sol *et al.*, 2003; Marzal *et al.*, 2005; Dunn *et al.*, 2011). These pathogens are widespread and commonly include species from the genera *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Fallisia* and *Trypanosoma*. (Valkiūnas, 2005; Braga *et al.*, 2011).

Infections with the avian malaria parasite (*Plasmodium*) have been reported in birds from all avian orders with the exception of the Struthioniformes (ostriches), the Coliiformes (mousebirds), and the Trogoniformes (trogons and quetzals), but it is estimated that only half of all avian species have been examined for these parasites (Valkiūnas, 2005). The greatest diversity of *Plasmodium* has been recorded from the Galliformes, Columbiformes, and Passeriformes (Valkiūnas, 2005; Martinsen *et al.*, 2008). In Malaysia, *Plasmodium* has been detected previously in wild Red Jungle fowls (*Gallus gallus spadiceus*) and domestic poultry based on morphological identification of the organisms in stained blood films (Omar *et al.*, 1962; Moorhouse and Wharton, 1962; Bennett and Cheong, 1965; Bennett and Warren 1966a; 1966b; Bennett *et al.*, 1966; Omar, 1968; Fernando and Dissanaik, 1975; Amin-Babjee *et al.*, 1985; Sani *et al.*, 1986; Lee *et al.*, 1991; Amin-Babjee and Lee, 1994).

The genus *Haemoproteus* is equally widely distributed and has been reported in birds in all continents except Antarctica where there is no vector for the transmission of the parasite (Garnham, 1966; Bishop and Bennett, 1992; Valkiūnas, 2005). These parasites have been detected in various species of forest birds (Paperna *et al.*, 2005; 2008), pigeons (Lee *et al.*, 1991), Spotted doves (Amin-Babjee and Lee, 1992) and crows (Amin-Babjee *et al.*, 1993; Ludin *et al.*, 1994) in Malaysia, but not in domestic poultry. *Leucocytozoon* has been reported in many avian orders, but only a

few species are known to be pathogenic to their hosts. The most susceptible groups of avian hosts include poultry, waterfowl, pigeons, raptors and ostriches (Bennett *et al.*, 1993; Valkiūnas, 2005). These haemoprotozoa are transmitted by blackflies (*Simuliidae*) (Atkinson and Van-Riper, 1991; Valkiūnas, 2005), while *L. caulleryi* (a chicken-specific parasite) is known to be transmitted by biting midges belonging to the genus *Culicoides* (Morii, 1992; Valkiūnas, 2005). To date only two species of *Leucocytozoon* (*L. sabrazei* and *L. caulleryi*) have been detected in domestic poultry (Kuppusamy, 1936; Griffiths, 1964; Omar, 1968; Colley *et al.*, 1971) and Red Jungle fowls (*Gallus gallus spadiceus*) (Chin *et al.*, 1974; Amin-Babjee *et al.*, 1985) in Malaysia.

The present study provides data on the prevalence of haemoprotozoa and microfilaria in various species of wild birds and domestic poultry in Selangor, Malaysia, with an emphasis on commercial broilers and layers, village chickens, and Red Jungle fowls. It is envisaged that the data will assist in identifying the host range and contribute to a long term database on the occurrence of these pathogens among wild and domestic avifauna in the country.

## MATERIALS AND METHODS

Blood samples were obtained from commercial poultry (broilers and layers), village chickens, Red Jungle fowls (*Gallus gallus spadiceus*) and various species of captive and free ranging wild birds (Table 1)

**Table 1.** Haemoparasites in domestic poultry and wild birds from Selangor, Malaysia. Data represent number of individuals infected with prevalence (in parenthesis) calculated for sample sizes (N) of 10 or more individuals.

Species	N	Plasmodium sp.	Leucocytozoon sabrazesi	Leucocytozoon caulleryi	Haemoproteus sp.	Trypanosoma sp.	Microfilaria
<b>Galliformes</b>							
Gallus gallus domesticus	183	3 (2.2)	0	1 (0.5)	0	0	0
Gallus gallus domesticus	58	3 (5.2)	0	1 (1.7)	0	0	0
Gallus gallus domesticus	342	10 (2.9)	3 (0.9)	0	2 (0.6)	7 (2.0)	13 (3.8)
Gallus gallus spadiceus	58	5 (8.6)	2 (3.4)	2 (3.4)	1 (1.7)	3 (3.4)	11 (19.0)
<b>Anseriformes</b>							
Aix galericulata	10	4 (40.0)	0	0	0	0	0
Tadorna radjah	4	0	0	0	0	0	0
Anas platyrhynchos domestica	14	7 (50.0)	0	0	0	0	0
Branta canadensis	4	4	0	0	2	0	0
Alopochen aegyptiacus	20	7 (35.0)	0	0	1 (5.0)	0	0
Cygnus olor	1	1	0	0	0	0	0
Cygnus atratus	7	1	0	0	0	0	0
<b>Phoenicopteriformes</b>							
Phoenicopterus roseus	5	3	0	0	0	0	0
<b>Pelecaniformes</b>							
Pelecanus onocrotalus	2	2	0	0	0	0	0
Pelecanus rufescens	2	1	0	0	0	0	0
<b>Gruiformes</b>							
Amaurornis phoenicurus	22	7 (31.8)	0	0	0	0	0
<b>Total</b>	<b>728</b>	<b>58 (8.0)</b>	<b>5 (0.7)</b>	<b>4 (0.5)</b>	<b>6 (0.8)</b>	<b>10 (1.4)</b>	<b>24 (3.3)</b>

in Selangor, through active sampling over a period of two years (2011–2013) and from cases received by the Parasitology Laboratory, Faculty of Veterinary Medicine over a period of 10 years (2003–2013). A total of 728 blood samples were screened from birds representing five avian orders, namely, Galliformes, Anseriformes, Phoenicopteriformes, Pelecaniformes and Gruiformes. Blood samples were collected from the wing vein and placed in ethylenediaminetetraacetic acid (EDTA) or heparin coated blood collection tubes. Giemsa-stained thin blood smears were prepared from each sample and screened for haemoparasites microscopically using the x100 oil immersion objective. A minimum of 10,000 red blood cells (and corresponding microscopic fields) were screened for each slide to determine the presence of haemoprotozoa and microfilaria.

## RESULTS

The most commonly encountered haemoparasite in both the wild birds and domestic poultry was *Plasmodium*, which was detected in 8.0% of the blood samples examined (Table 1). The common hosts for this malaria parasite were the aquatic/wetland species of birds from the order Anseriformes (prevalence of 35.0%–50.0%) and the White-breasted Waterhen (*Amaurornis phoenicurus*) (prevalence of 31.8%). Among the Galliformes, the Red Jungle fowl and commercial layers exhibited the highest infection rates with

*Plasmodium*, where the parasite was detected in 8.6% and 5.2% of the birds, respectively. The average infection rate of *Plasmodium* in domestic poultry (layers, broilers and village chickens) was moderate (2.7%). Two species of *Leucocytozoon* (*L. sabrazezi* and *L. caulleryi*) were readily detected in the stained blood films due to their distinct morphologies. The prevalence of these parasites was relatively low (0.8% and 0.6%, respectively) and they were confined to the Galliformes. With the exception of the Red Jungle fowl, none of the other wild birds examined had apparent *Leucocytozoon* infection. *Haemoproteus* was present in the village chickens, Red Jungle fowls and domestic goose, with a relatively low collective prevalence of 0.8%. Moderate *Trypanosoma* (1.4%) and microfilaria (3.3%) infection rates were detected in the birds examined. The Red Jungle fowls exhibited the highest prevalence of *Trypanosoma* (3.4%) and microfilaria (19.9%), whereas the other wild avian species examined were free of these parasites in the stained blood films.

## DISCUSSION

Various species of haematozoa have been reported to infect both wild and domestic birds in Malaysia. The avian malaria parasite (*Plasmodium*) has been the focus of a number of investigations and it is well established that domestic poultry are common hosts for this parasite in the country (Omar *et al.*, 1962; Moorhouse and Wharton, 1962; Bennett and Cheong,

1965; Bennett and Warren 1966a, 1966b; Bennett, *et al.*, 1966; Omar, 1968; Colley *et al.*, 1971; Fernando and Dissanaik, 1975; Amin-Babjee *et al.*, 1985; Sani *et al.*, 1986; Lee *et al.*, 1991; Amin-Babjee and Lee, 1994). These haemosporidians are known to be highly pathogenic to domestic poultry with mortalities as high as 90% (Soulsby, 1982; Springer, 1996). *Plasmodium gallinaceum* in particular, is known to cause severe disease in susceptible poultry from Asia (Africa *et al.*, 1940; Crawford, 1945; Rao *et al.*, 1951; Omar and Lim, 1962; Omar *et al.*, 1962; Omar, 1968). This species was implicated in an outbreak among White Leghorn crosses in Selangor, producing high morbidity and mortality with the animals showing signs of paresis, paralysis, anaemia and greenish diarrhea (Omar *et al.*, 1962; Omar, 1968). Typical post mortem lesions observed in avian malaria among local poultry include hepatomegaly, splenomegaly, pericardial effusion, and pathological changes in the kidneys and heart (Omar and Lim, 1962).

In contrast to the present study, previous reports have shown a higher prevalence of *Plasmodium* among poultry in Selangor. Colley *et al.* (1971) examined 34 blood samples from domestic chickens in Gombak, Selangor and found that 47% of the birds were infected with *Plasmodium*. Subsequently, Amin-Babjee *et al.* (1985) observed a higher prevalence of *Plasmodium* (50%) in blood samples collected from 16 Red Jungle fowls in Selangor (areas in Banting, Dengkil and Kajang). A survey by Sani *et al.* (1986)

involving 201 village chickens from Sg. Besi, Serdang and Ulu Langat in Selangor revealed that 3.5% of the birds were infected with this haemosporidian parasite. The most recent published survey on poultry *Plasmodium* in Malaysia (Amin-Babjee and Lee, 1994) reported an infection rate of 8.3% for *P. juxtannucleare* among the fighting breed of domestic chickens. It is likely that the differences in the prevalence of *Plasmodium* infection are due to factors related to the sampling effort and location, including poultry breed, immune status of the birds, habitat type, season and the abundance of arthropod vectors responsible for transmitting the parasites. It is also highly likely that the shift in the poultry farming system from free-range to close-housing in recent years would contribute to the lower prevalence of these pathogens among domestic poultry. In addition, rapid urbanization in Selangor and drastic land use change may also impact the breeding of the vectors responsible for the transmission of these haemosporidians. Apart from domestic poultry and its wild ancestor the Red Jungle Fowl, *Plasmodium* has also been reported in a number of free-ranging wild birds in Malaysia. These include *P. vaughani* from crows (*Corvus macrorhynchos macrorhynchos*) (Ludin *et al.*, 1994), *P. formosanum* from White-breasted Waterhens (*Amaurornis phoenicurus*) (Yap *et al.*, 1986) and an unidentified *Plasmodium* sp. from the Mountain Fulvetta (*Alcippe peracensis peracensis*) (Paperna *et al.*, 2008). Bisseru and Lim (1971) detected both

*P. gallinaceum* and *P. juxtannucleare* in crows (*Corvus splendens protegatus*) from Klang, Selangor and suggested that these feral and introduced birds may be reservoirs of the parasite for domestic poultry in the country. This study revealed that the wild water birds and wetland birds had high *Plasmodium* infection rates. This may be attributed to their close proximity with the mosquito vectors responsible for the transmission of the parasite, along waterways and tall reed beds.

The genus *Haemoproteus* is reported for the first time among Red Jungle fowls and village chickens in Malaysia. The only other records of the presence of this haemosporidian in the country are from wild forest birds, pigeons, Spotted doves (*Streptopelia chinensis*) and crows (*Corvus splendens*) (Lee *et al.*, 1991; Amin-Babjee and Lee, 1992; Amin-Babjee *et al.*, 1993; Paperna *et al.*, 2005; 2008). The paucity of reports on the presence of this parasite in local poultry may be due to the difficulty in differentiating it from *Plasmodium* in stained thin blood films (Atkinson *et al.*, 2008). The pathogenesis of *Haemoproteus* infection in poultry is still poorly understood (Atkinson *et al.*, 2008) although infections have been associated with muscle pathology, hepatomegaly and splenomegaly (Atkinson and Forrester, 1987; Atkinson *et al.*, 1988; Cardona *et al.*, 2002). The present identification of *Haemoproteus* infection among local chickens, and its recent separation from the avian malaria disease cause by *Plasmodium* (Valkiūnas *et al.*, 2005), opens new ground

for comparative studies on the pathological effects of these haemosporidians among domestic poultry in the country.

Trypanosomes have been known to infect Red Jungle fowls (Dissanaïke and Fernando, 1974) and domestic poultry (Colley *et al.*, 1971) in Malaysia. The prevalence reported by Colley *et al.* (1971) from chickens in Gombak, Selangor is much higher (29.0%) compared to the present investigation (3.4% in Red Jungle fowls and 2.0% in village chickens). Interestingly, these kinetoplastids were not detected in subsequent examination of blood samples from Red Jungle fowls (Amin-Babjee *et al.*, 1985). While trypanosome infections are known to be fatal in various species of domestic livestock and humans, the pathological effects of avian trypanosomes have not been fully investigated. It is currently believed that these parasites have minimal pathological effects on their avian host (Saif *et al.*, 2008). A number of *Trypanosoma* species have been identified in poultry including *T. avium*, *T. gallinarum*, *T. numidae* and *T. calmetti*, however, the exact taxonomic status of avian trypanosomes have not been fully resolved (Saif *et al.*, 2008). Filarial nematodes of the genus *Pelecitus* (Dissanaïke and Fernando, 1974), *Cardiofilaria* (Mak *et al.*, 1984; Lee and Amin-Babjee, 1986; Kyi and Poon, 1987; Amin-Babjee *et al.*, 1993) and *Lemdana* (Amin-Babjee *et al.*, 1985; Lee *et al.*, 1989) have been reported in local domestic chickens, Red Jungle fowls, crows and Spotted doves. In this study, microfilaria

were detected in the blood of the village chickens and Red Jungle fowls, with high infection rate in the latter (19.0%). Amin-Babjee *et al.* (1985) reported a higher prevalence (50.0%) of this parasite in the blood of Red Jungle fowls from Selangor. Among local population of Spotted doves (Amin-Babjee and Lee, 1992) and crows (Amin-Babjee *et al.*, 1993), microfilaria were observed in the blood of 7.0% and 16.7% of the birds, respectively.

The current compilation of avian haemoparasite hosts range and the establishment of a local database will contribute to the understanding of the dynamic interactions between disease transmission and hosts susceptibility. In Malaysia, constant habitat alterations and changes in farming systems may lead to pathogen transmission between wild birds and domestic poultry. In addition the influx of migratory birds into the country during the winter months in the northern hemisphere may lead to introduction of novel haemoparasite genotypes that may affect the local avifauna. This is of concern because pathogen transmission and sharing of parasite lineages between resident and migratory birds have been known to occur in many parts of the world (Rappole *et al.*, 2000; Waldenström *et al.*, 2002; Hellgren *et al.*, 2007, Ishtiaq *et al.*, 2007). As such, the occurrence and incidence of avian haemoparasites among domestic poultry, resident wild birds and migratory avifauna requires constant monitoring in order to detect and prevent potential outbreaks that

may be detrimental to the local poultry industry.

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