

STUDIES ON HAEMATOZOA OF FAMILY CORVIDAE OF KERALA¹M.J. ELIZABETH²¹Accepted December 2006²Department of Zoology, Catholicate College, Pathanamthitta 689 643, Kerala, India.

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From Family Corvidae, 121 House Crows (*Corvus splendens* Madarasz) and 33 Jungle Crows (*Corvus macrorhynchos* Sykes) from four districts of Kerala state were screened for blood parasites. 78% (94/121) *C. splendens* and 39% (13/33) *C. macrorhynchos* were positive for *Haemoproteus* species. In total, 69.4% (107/154) of the corvids showed haematozoan prevalence. No other species of blood parasite was observed. The parasitic intensity in *C. splendens* was greater (5.2%) than that of *C. macrorhynchos* (3.2%). The haematozoan prevalence observed in the present study has been compared and contrasted with that of the corvids from India, Eastern and Southern Asia, Neotropics, and North America.

Key words: *Corvus splendens*, *Corvus macrorhynchos*, *Haemoproteus*, prevalence

INTRODUCTION

Numerous surveys throughout the world have demonstrated the presence of haematozoa in a wide variety of avian hosts from varying geographical and environmental regions. Avian haematozoa have been implicated in mass mortalities of birds (Herman 1968; Laird and Bennett 1970; Atkinson *et al.* 1986; Bennett *et al.* 1988, 1993; Earle *et al.* 1993; Reppas *et al.* 1995; Simpson *et al.* 1996; Merino *et al.* 2000). To date, about 450 species of haemoparasites have been described from over 4,500 species of birds (Herman *et al.* 1976; McClure *et al.* 1978; Bennett *et al.* 1981, 1982; Threlfall and Bennett 1989; Bishop and Bennett 1992; Evans and Otter 1998; Munoz *et al.* 1999; Mushi *et al.* 1999, 2000; Deviche *et al.* 2001a, b; Adriano and Cordeiro 2001). The better known genera of blood parasites are *Haemoproteus* (140 species from 1,700 bird species of 110 families), *Plasmodium* (54 species from 1,000 species of birds of 97 families), *Leucocytozoon* (96 species from 1,000 species of birds of 98 families) and *Trypanosoma* (90 species from 800 species of birds of 93 families; Threlfall and Bennett 1989).

In India, Nandi (1978) has reported the presence of 96 species of haematozoa of 8 genera, including 51 species of *Haemoproteus*, 11 species of *Plasmodium* and 12 species of *Trypanosoma*. However, Nandi and Bennett (1997) have reported the presence of a total of 111 valid species of haematozoa composed of 64 species of *Haemoproteus*, 28 *Leucocytozoon*, 8 *Plasmodium*, 5 *Trypanosoma* and 6 species of other parasites from the Indian subcontinent. Other works on avian haematozoa in India include those of Acton and Knowles (1914), Bhatia (1938), Chakravarty and

Kar (1945a, b), Ray and Bhatnagar (1953), David and Nair (1955), Narang and Bhatnagar (1969), Sarkar and Ray (1969), Greiner *et al.* (1977), McClure *et al.* (1978), Reddi *et al.* (1980), Pal and Dasgupta (1982), Nandi and Choudhry (1983), Nandi and Mandal (1984, 1985), Nandi *et al.* (1984), Nandi (1985) and Mandal *et al.* (1989). Studies on haematozoans of the birds of Kerala have been scant (Pillai *et al.* 1990; Varghese and Elizabeth 1992).

Of the two species of birds examined from Family Corvidae Common Crow *Corvus splendens* Madarasz is slightly larger than a pigeon, overall black with a dusky grey neck. Jungle Crow *Corvus macrorhynchos* Sykes is larger than House Crow and smaller than a kite in size. This is a glossy, jet-black crow with a heavy bill. These birds are gregarious, omnivorous and are considered to be a commensal of man and an useful scavenger (Ali 1984).

METHODOLOGY

121 *Corvus splendens* were collected from Wayanad, Kozhikode, Kottayam and Pathanamthitta districts and 33 *Corvus macrorhynchos* from Wayanad and Pathanamthitta districts of Kerala, India. A minimum of five blood smears each was prepared from the blood drawn from the brachial vein of the birds. Soon after collection, the smears were air-dried, fixed in 100% methanol, and subsequently stained with Wright's Reagent. Each slide was scanned under 45x and 100x objectives of a calibrated Labo Triumph Research microscope equipped with an ocular micrometer and Yashica F-2 photomicrographic attachment. Positive slides were mounted using DPX. The morphology of the different developmental stages (50 each) of the blood parasites were observed

(e.g. Macro and microgametocytes), and infected and uninfected erythrocytes were studied and measured in micrometers. The parasites were identified according to the descriptions of Greiner and Bennett (1975), Greiner *et al.* (1975), and Bennett and Peirce (1988, 1990). Definitions of prevalence and intensity (per 10,000 erythrocytes) followed are those of Margolis *et al.* (1982).

RESULTS

Of the total of 154 birds examined, 107 (69.5%) were positive for *Haemoproteus* species. This included the 78% (94/121) prevalence in *C. splendens* and 39% (13/33) prevalence in *C. macrorhynchos* (Table 1). No other blood parasites were seen.

In *C. splendens*, in increasing order, prevalence of parasite was 75% (51/68) in Pathanamthitta, 80% (20/25) in Kozhikode, 82% (18/22) in Wayanad, and 83% (5/6) in Kottayam. The intensity of the parasite was 5.2% in Pathanamthitta district. The prevalence was 67% (12/18) in Pathanamthitta proper, 71% (20/28) in Thiruvalla and 86% (19/22) in Kozhencherry.

The macrogametocytes were circumnuclear (14.5 x 3.0 µm) occupying more than 75% of the host erythrocyte. Deep blue, granular and slightly vacuolated cytoplasm showed dark brown granules averaging 17.6 in number. A slight lateral displacement of erythrocyte nucleus was seen. Normal erythrocyte of *Corvus splendens* was

13.1 x 6.3 µm in size and erythrocyte nucleus was 5.1 x 2.5 µm. Infected erythrocyte with macrogametocyte was 13.4 x 5.7 µm and infected erythrocyte nucleus was 5.6 x 2.0 µm. Slight hypertrophy of the erythrocyte and atrophy of its nucleus were noted (Table 2).

The microgametocytes of *C. splendens* were also circumnuclear (11.7 x 3.6 µm) occupying 3/4 of the host cell. Cytoplasm stained light blue with coarse granules. Darkly stained pigment granules were seen clustered at the poles, with an average of 25.4 (in number), which is more than that of the macrogametocyte. The uninfected erythrocyte was 13.1 x 6.3 µm and the erythrocyte nucleus 5.1 x 2.5 µm. The infected erythrocyte was 13.2 x 6.3 µm and the erythrocyte nucleus 5.5 x 1.8 µm (Table 2). A degree of atrophy was noted in the size of the host cell nucleus.

Of the 33 *C. macrorhynchos* examined, 13 (39%) were positive for *Haemoproteus* (Table 1). District-wise the prevalence was 33% (2/6) in Wayanad and 41% (11/27) in Pathanamthitta. Within the district of Pathanamthitta, the increasing order of prevalence was: 0% (0/2) in Adoor, 25% (1/4) in Kozhencherry, 40% (2/5) in Pathanamthitta town and 50% (4/8) each in Thatta and Thiruvalla. The overall parasitic intensity was 3.2%.

The circumnuclear macrogametocyte (16.7 x 2.0 µm) occupied almost 75% of the host cell. Lateral displacement of the host cell nucleus was seen. Cytoplasm stained deep blue with Giemsa stain; it was coarsely granular. Pigment granules were dark brown, with an average of 22.8, scattered

Table 1: Prevalence of haematozoa in corvids

Location (District / Place)	<i>Corvus macrorhynchos</i>			<i>Corvus splendens</i>		
	TE	TP	PR%	TE	TP	PR%
Wayanad						
Sulthanbathery	6	2	33	22	18	82
Pathanamthitta						
Adoor	2	0	0	0	0	0
Thatta	8	4	50	0	0	0
Pathanamthitta	5	2	40	18	12	67
Kozhencherry	4	1	25	22	19	86
Thiruvalla	8	4	50	28	20	71
Kozhikode						
Kozhikode	0	0	0	25	20	80
Kottayam						
Changanacherry	0	0	0	6	5	83
Total	33	13	—	121	94	—

TE = Total number of birds examined; TP = Total number positive for haematozoa; PR% = Percentage of prevalence of haemoparasites

throughout the cytoplasm. In the parasites, pigment granules were seen clumped in two or more regions. Normal RBC of the bird was $12.3 \times 6.0 \mu\text{m}$, RBC nucleus $5.4 \times 2.6 \mu\text{m}$. Erythrocyte infected with the macrogametocyte of the parasite was $13.2 \times 6.9 \mu\text{m}$ and nucleus was $5.4 \times 2.0 \mu\text{m}$ (Table 2). Hypertrophy in length and width of the erythrocyte and atrophy in width of the erythrocyte nucleus were seen.

Microgametocytes found in the blood smears were circumnuclear, with coarsely granular cytoplasm staining light blue with Giemsa and measured $16.7 \times 3.0 \mu\text{m}$. Pigment granules averaged 27 and were clustered at the poles. Erythrocyte infected with microgametocyte was $12.8 \times 6.8 \mu\text{m}$ and infected nucleus $5.0 \times 1.5 \mu\text{m}$ (Table 2). Slight hypertrophy of the host cell and atrophy of the nucleus was seen.

DISCUSSION

In *Corvus splendens*, only one type of *Haemoproteus* – a circumnuclear parasite which occupied more than 75% of the host erythrocyte – was observed. Table 2 compares the

general characteristics of the *Haemoproteus* sp. seen in the present study with those of *H. danilewski* and indicates that the former differs from the latter in being smaller, (macro $14.5 \times 3.0 \mu\text{m}$ as opposed to $19.4 \times 3.2 \mu\text{m}$; micro $11.7 \times 3.6 \mu\text{m}$ as against $17.4 \times 3.4 \mu\text{m}$), also the macro possesses fewer number of pigment granules 17.6 as against 23.4 (Bishop and Bennett 1990). Whether or not the difference in size and number of pigment granules seen in the *Haemoproteus* species and *H. danilewski* in the present study indicate the existence of a new species of the parasite in *Corvus splendens* of Kerala could not be ascertained until detailed studies are completed.

In *Corvus macrorhynchos* too a single type of *Haemoproteus* sp., which resembled *H. danilewski*, was observed. The size of the macrogametocyte infected RBC in the present study ($13.2 \times 6.9 \mu\text{m}$) was very close to that of *H. danilewski* infected RBC ($13.0 \times 7.3 \mu\text{m}$). Hypertrophy of the host cell was seen in the present study as in *H. danilewski* infection. The size of the microgametocyte infected erythrocyte ($12.8 \times 6.8 \mu\text{m}$) was similar to that of *H. danilewski* infection ($12.9 \times 7.5 \mu\text{m}$; Table 2). The

Table 2: Morphometric parameters of *Haemoproteus* spp. of *Corvus* spp.
(All measurements are in microns)

		Present Study <i>Corvus splendens</i>	Present Study <i>Corvus macrorhynchos</i>	<i>H. danilewski</i> *
Uninfected erythrocyte	Length	13.1 ± 0.72	12.3 ± 1.18	12.4 ± 1.1
	Width	6.3 ± 0.95	6.0 ± 1.0	6.8 ± 0.5
Uninfected erythrocyte nucleus	Length	5.1 ± 1.45	5.4 ± 0.77	4.9 ± 0.6
	Width	2.5 ± 0.72	2.6 ± 0.72	1.9 ± 0.3
Infected erythrocyte (Macrogametocyte)	Length	13.4 ± 0.47	13.2 ± 0.95	13.0 ± 0.8
	Width	5.7 ± 0.95	6.9 ± 0.77	7.3 ± 0.5
Infected erythrocyte nucleus	Length	5.6 ± 0.72	5.4 ± 0.77	4.7 ± 0.5
	Width	2.0 ± 0.72	2.0 ± 0.72	2.0 ± 0.2
Macrogametocyte	Length	14.5 ± 1.01	16.7 ± 1.11	19.4 ± 3.4
	Width	3.0 ± 0.71	2.0 ± 0.72	3.2 ± 0.5
No. of pigment granules		17.6 ± 0.84	22.8 ± 0.85	23.4 ± 4.2
Infected erythrocyte (Macrogametocyte)	Length	13.2 ± 0.77	12.8 ± 0.79	12.9 ± 0.8
	Width	6.3 ± 0.63	6.8 ± 0.79	7.5 ± 0.6
Infected erythrocyte nucleus	Length	5.5 ± 0.71	5.0 ± 0.72	4.6 ± 0.5
	Width	1.8 ± 0.68	1.5 ± 0.0	2.0 ± 0.3
Microgametocyte	Length	11.7 ± 1.18	16.7 ± 1.11	17.4 ± 2.3
	Width	3.6 ± 0.77	3.0 ± 0.0	3.4 ± 0.5
No. of pigment granules		25.4 ± 1.58	27.0 ± 1.33	27.6 ± 2.9

*Source : Bishop and Bennett, 1990

Table 3: Prevalence of haematozoa in the avian Family Corvidae

Country/Region	T E	T P	Percent positive for					Source
			H	P	L	T	M	
North America	1970	49.9	25.1	3.4	30.7	10.3	9.6	Greiner <i>et al.</i> , 1975
New Jersey and Maryland	323	21.1	13.9	2.5	4.3	0.3	3.1	Williams and Bennett, 1978
Neotropics	67	43.3	17.9	17.9	3.0	1.5	3.0	White <i>et al.</i> , 1977
Eastern & Southern Asia	152	26.3	21.7	0.7	4.6	-	2.6	McClure <i>et al.</i> , 1978
Indian Subcontinent ⁽¹⁾	616	64.2	4.8	31.3	0.9	0.5	29.7	Nandi and Bennett, 1997
Kerala, India	154	69.5	69.9	-	-	-	-	Present Study

TE = Total number of birds examined; TP = Percentage of total number of birds positive for haematozoa; H = *Haemoproteus*; P = *Plasmodium*; L = *Leucocytozoon*; T = *Trypanosoma*; M = *Microfilaria*

(1) = Includes India, Pakistan, Bangladesh, Bhutan, Nepal and Sri Lanka

measurements and number of pigment granules of macro- and micro-gametocytes were in accord with those of *H. danilewski*. Table 2 confirms that of the two species of birds the *Haemoproteus* sp. of *Corvus macrorhynchos* resembled *H. danilewski* more than *Corvus splendens*.

The other species identified is *Haemoproteus picae*, which has a halteridial shape with lower number of pigment granules, and the form found in the present study is not in accord with this.

Surprisingly, the overall prevalence of *Haemoproteus* spp. in the *C. macrorhynchos* was only 39% (13/33), which was exactly 50% less than that in *C. splendens* of the two districts from where *C. macrorhynchos* were examined, Wayanad showed a lower prevalence of 33% (2/6) while Pathanamthitta showed slightly higher percentage (40%; 11/27).

The 69.5% (107/154) prevalence of haematozoa recorded from corvids was substantially higher than the 12.5% in corvids of West Bengal (Nandi *et al.* 1984), 21.1% in New Jersey and Maryland (Williams and Bennett 1978), 22.2% in Bharatpur, Rajasthan (Mc Clure *et al.* 1978), 26.3% in Eastern and Southern Asia (Mc Clure *et al.* 1978), 33% in Andhra Pradesh (Nandi and Mandal 1984), 40% in Goa (Nandi and Mandal 1985), 43.3% in the Neotropics (White *et al.* 1978) and 49.9% in North America (Greiner *et al.* 1975; Table 3). The prevalence in the present study is closer to the 64.2% in Indian subcontinent (Nandi and Bennett 1997)

In the corvids of North America, Neotropics, and New Jersey and Maryland, the workers reported the presence of

Plasmodium, *Leucocytozoon*, *Trypanosoma* and *Microfilaria* in addition to *Haemoproteus* (Greiner *et al.* 1975; White *et al.* 1978; Williams and Bennett 1978). The results of the present study differ from those of the above workers in not finding *Plasmodium*, *Leucocytozoon*, and *Trypanosoma*, and are in accord in finding *Haemoproteus*. Studies in the corvids of Indian subcontinent (Nandi and Bennett 1997) showed the presence of *Plasmodium* (31.3%), *Leucocytozoon* (0.9%), and *Trypanosoma* (0.5%), which is at variance with the present study.

Nandi's "Index catalogue of haematozoa from India" (Nandi 1984) has recorded the presence of *Haemoproteus* (de Mello *et al.* (1917), Mc Clure *et al.* (1978) and *Microfilaria* (Sen *et al.* 1965) in *Corvus macrorhynchos*, *Haemoproteus* (Donovan 1904; Bhatia 1978; Mc Clure *et al.* 1978), *Plasmodium* (Donovan 1904; Nandi *et al.* 1984), *Trypanosoma* (Donovan 1904) and *Microfilaria* (Mc Clure *et al.* 1978) in *Corvus splendens*.

The finding of only *Haemoproteus* in *Corvus splendens* is not in accord with that of Mc Clure *et al.* (1978) from Bharatpur, India, where *Microfilaria* was also found, and also differs from that of all other workers listed above who have also found other parasites (Table 3). The results obtained in the present study from *Corvus macrorhynchos* differ from that of Sen *et al.* (1965) in not finding *Microfilaria*. Nevertheless, confirmation that the corvids of Kerala are hosts exclusively of *Haemoproteus* and no other species of haematozoa must await results of further more extensive survey.

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