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Prevalence of malaria parasites in indigenous chickens and ducks in selected districts of Bangladesh

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Abstract

Avian haematozoans are important pathogens of birds causing asymptomatic to fatal infections. The present study was undertaken to investigate the prevalence and associated risk factors of malaria parasite infections in domestic chickens and ducks in three localities namely Mymensingh, Tangail and Netrokona districts in Bangladesh. In total, 474 blood smears (266 chickens and 208 ducks) were screened for haemoprotozoan infection during the period from March, 2016 to February, 2017. Blood samples were collected from wing vein by needle puncture. The parasites were identified from Giemsa stained thin blood smears based on morphological features using standard keys. Malaria protozoa was found in 60 birds (12.7%), of which 35 were chickens (13.2%) and 25 were ducks (12.0%). Two species of blood protozoa were identified namely *Leucocytozoon* spp. (10.5%) and *Plasmodium* spp. (2.1%). The prevalence of haemoprotozoa was found high among the adults (13.9%) while it was 11.2% among young birds. Female (15.2%) was 1.46 times more susceptible than male (10.9%).The prevalence rate was highest in Netrokona (18.9%) followed by Tangail (12.2%) and Mymensingh (8.7%). In this study, the prevalence rate was highest in rainy season (18.3%), moderate in summer (10%) and less in winter season (9.3%). Present study revealed that these haemorpotozoa are capable of infecting indigenous domestic chicken and duck. Further studies should focus on characterization of the malaria parasites of indigenous chickens and ducks in Bangladesh.

Introduction

Avian haemosporidians (*Haemoproteus, Leucocytozoon* and *Plasmodium*) are common vector-borne globally distributed blood parasites, which occur in most species of birds. They are transmitted by blood-sucking dipteran insects (Krizanauskiene *et al.*,2006) which occur worldwide, irrespective of climatic barriers (Wiersch *et al.*, 2007).

Leucocytozoon, *Haemoproteus* and *Plasmodium* spp. also have been reported to affect the domestic poultry in Bangladesh (Dey *et al.*, 2008a,b; 2010). Amongst them, *Leucocytozoon* is considered to be host-specific at the family level (Peirce *et al.*,2005) and is found in birds worldwide. The prevalence of *Leucocytozoon* is 16% in domestic poultry in Iran. The parasites were found in chickens (5.2%), geese (4.3%), ducks (3.6%), and turkeys (2.9%) (Dezfoulian *et al.*,2013).A survey in South Carolina of USA reported that 13.6% of backyard chickens were infected with *Leucocytozoon caulleryi* (Noblet *et al.*, 1976).

At the population level, haemoparasites can affect their hosts by reducing fitness parameters such as body condition, reproductive success and survival (Stjernman *et al.*, 2004). Mortality in bird due the disease may be up to 90 % (Jordan and Pattison, 1998). Haemoparasites cause anaemia by invading the host's erythrocytes which are consequently destroyed by the bird's autoimmune system, leading to death (Soulsby, 1982; Permin and Hansen, 1998). *Leucocytozoon* typically causes anemia and enlargement of liver and spleen (Valkiunas, 2005; Dey *et al.*, 2010).

Few comprehensive studies related to prevalence of haemosporidians have done in domestic and wild/migratory bird in Bangladesh (Islam *et al.*, 2013, Dey *et al.*,2008a,b; 2010). Elahi *et al.* (2014) reported 15.1% prevalence of *Plasmodium* spp. and 0.6% of *Leucocytozoon* spp. in wetlands birds of Bangladesh. According to Nath *et al.* (2014), prevalence of *Leucocytozoon* was 12% in chickens in Bangladesh. In hilly areas of Bangladesh, prevalence of haemoprotozoa in domestic bird was 60.6% in summer season, 36.7% in rainy and 23% in winter seasons (Nath and Bhuiyan, 2017). There is limited information on the epidemiology of these important haemoprotozoan parasites in Bangladesh. Therefore, more information on the prevalence of haemoprotozoa in brids will help to understand the epidemiology of the diseases.

Considering these points, this study was undertaken to investigate the prevalence of malaria parasites along with seasonal dynamics and other associated risk factors (age, sex, and geographical area) related to malaria infection in indigenous chickens and ducks in Bangladesh.

Materials and Methods

Study area

Blood samples of chickens and ducks were collected from different villages in Tangail, Netrokona and

Mymensingh districts. The collected samples were examined in the Department of Parasitology, Bangladesh Agricultural University, Mymensigh.

Study Period

The study was conducted in three different seasons such as summer (March-June), rainy (July–October) and winter (November–February) from March, 2016 to February, 2017.

Selection of birds

In total, 474 birds (266 chickens and208 ducks) were selected randomly irrespective of age, sex and health condition directly from households of different villages in Tangail, Netrokona and Mymensingh districts of Bangladesh.

Antemortem examination

During collection of samples, age and sex of both chickens and ducks were recorded as described by the owners. According to sex, birds were divided into male and female. They were further grouped as young (less than 6 months) and adult (more than 6 months).

Collection and identification of samples

About 5 ml blood was collected from wing vein and placed into a test tube containing sodium citrate. Samples were then transferred to the Parasitology laboratory, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. Then a thin smear was made, which was air dried, fixed with methanol and stained with Giemsa's stain as described by Kaufmann (1996). The slides were then examined under microscope athigh magnification (40X and 100Xwith immersion oil) for the detection of malaria protozoa (Zajac and Conboy, 2012).

Statistical analysis

To compare the prevalence of blood parasites in relation to sex, age, area and season data were analyzed by χ^2-test and z-test using Statistical Package for Social Science (SPSS version 22.0, SPSS Inc., Illinois, USA).

Results

The overall prevalence of haemoprotozoa

Results revealed that 12.7% (60/474) birds were infected with a single or more types of haemoprotozoa in the study area. About 13.2% (35/266) chickens and 12.0% (25/208) ducks were infected. The identified protozoa in the current study were *Leucocytozoon* spp. (Fig. 1) and *Plasmodium* spp. (Fig. 2) and prevalence were 10.5% and 2.1%, respectively (Table 1).

Table 1. Overall prevalence of malaria parasites in indigenous chickens and ducks

Host	Prevalence (%)	
	***Leucocytozoon* spp.**	***Plasmodium* spp.**
Chicken (266)	30 (11.3)	5 (1.9)
Duck (208)	20 (9.6)	5 (2.4)
Total(474)	50 (10.5)	10 (2.1)
Overall (474)	**60 (12.7%)**	

Age-wise prevalence of haemoprotozoa

Results showed the age-wise prevalence of malaria parasite in adult and young chickens (10.4% and 12.8%) and ducks (6.7% and 13.6%). The prevalence was higher in chickens (23.2) as compared to ducks (20.2).Additionally, adults were 1.28 times more susceptible than young birds (Table 2).

Sex-wise prevalence of haemoprotozoa

In this study, higher infection rate was observed in female (15.2%) birds as compared to male (10.9%). Although, female ducks (16.7%) showed higher prevalence than female chickens (14.4%), however the differences were not statistically significant (p= 0.21). Moreover, the female birds were 1.46 times more prone to haemoprotozoa infection than male (Table 3).

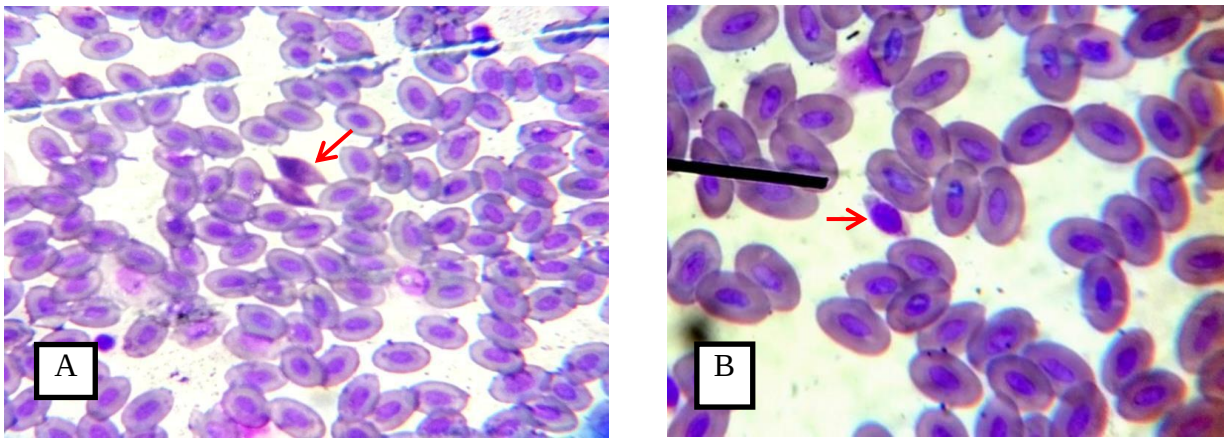

Fig. 1. Developmental stage of *Leucocytozoon* spp. (A) *Leucocytozoon sabrazesi*(B) *Leucocytozoon simondi*

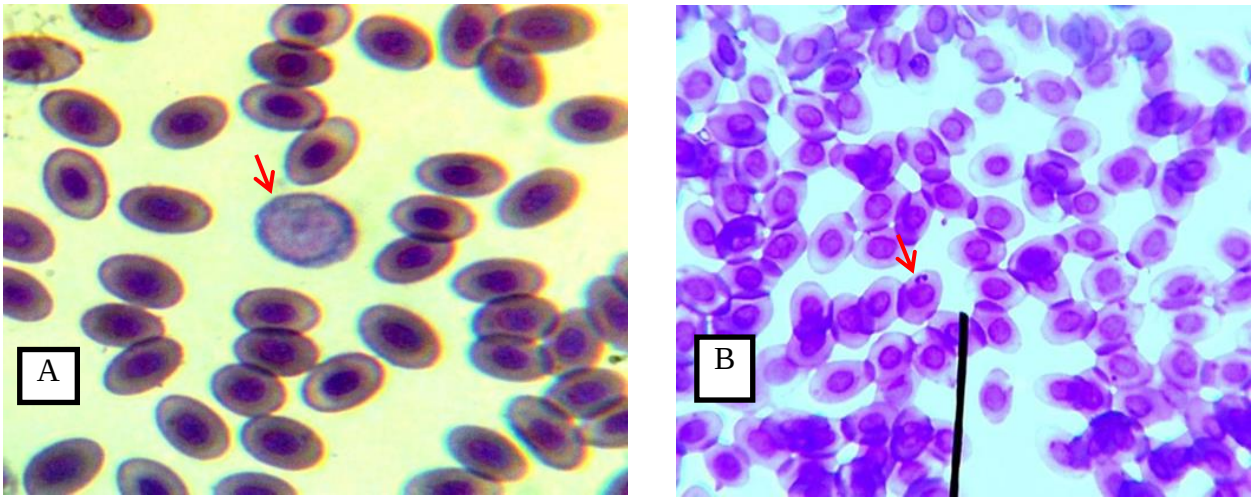

Fig. 2. Developmental stage of *Leucocytozoon* and *Plasmodium* spp. (A) *Leucocytozoon caulleryi* (B) *Plasmodium* spp.

District-wise prevalence of haemoprotozoa

Amongst three study areas, highest prevalence was recorded in Netrokona (18.9%) followed by Tangail (12.2%) and Mymensingh district (8.7%) in both chickens and ducks with significant variations (p= 0.02) (Table 4).

Season-wise prevalence of haemoprotozoa

The present study revealed that 13 (9.3%), 17 (10%) and 30 (18.3%) birds were infected with blood protozoa during winter, summer and rainy season, respectively. Amongst seasons, rainy season showed highest prevalence in both ducks (18.9%) and chickens (18.0%). The difference of infection rates of blood protozoa due to seasons were significant (p= 0.03) (Table 5).

Table 2. Age related prevalence of malaria parasites in indigenous chickens and ducks

Birds	Age group	Prevalence (%)		OR	χ^2 - value	p- value
		***Leucocytozoon* spp.**	***Plasmodium* spp.**			
Chicken	Young (102)	13 (12.8)	3 (2.9)			
	Adult(164)	17 (10.4)	2 (1.2)			
Duck	Young (120)	8 (6.7)	1 (0.8)			
	Adult (88)	12 (13.6)	4 (4.6)			
Total	Young (222)	25 (11.2)		1.28	0.79	0.40^{NS}
	Adult (252)	35 (13.9)				

NS = Not significant, OR= Odds Ratio

Table 3. Prevalence of malaria parasites related to sex in indigenous chickens and ducks

Birds	Sex	Prevalence (%)		OR	χ^2 - value	p- value
		***Leucocytozoon* spp.**	***Plasmodium* spp.**			
Chicken	Male (169)	18 (10.7)	3 (1.8)			
	Female (97)	12 (12.4)	2 (2.1)			
Duck	Male (112)	8 (7.1)	1 (1.0)			
	Female (96)	12 (12.5)	4 (4.2)			
Total	Male (276)	30 (10.9)		1.46	1.91	0.21^{NS}
	Female (198)	30 (15.2)				

NS = Not significant, OR= Odds Ratio

Table 4. District-wise prevalence of malaria parasites in indigenous chickens and ducks

Birds	Area	Prevalence (%)		OR	χ^2 - value	p- value
		***Leucocytozoon* spp.**	***Plasmodium* spp.**			
Chicken	Mymensingh (102)	5(4.9%)	3(2.94%)			
	Netrokona (83)	15 (18.07%)	2 (2.41%)			
	Tangail(81)	10 (12.35%)	-			
Duck	Mymensingh (93)	7 (7.53%)	2(2.15%)			
	Netrokona (49)	7 (14.29%)	1(2.04%)			
	Tangail (66)	6(9.09%)	2(3.03%)			
Total	Mymensingh (195)	17(8.7)[a]		1.00		
	Netrokona(132)	25(18.9)[b]		2.45	7.47	0.02**
	Tangail(147)	18(12.2)[a]		1.45		

**= P<0.05, significant at 95% level of confidence interval, OR= Odds Ratio
[a,b] Each superscript letter denotes a subset of area categories whose row proportions differ significantly from each other at the 0.05 level.

Table 5. Seasonal Prevalence of malaria parasites in indigenous chickens and ducks

Birds	Area	Prevalence (%)		OR	χ^2 - value	p- value
		***Leucocytozoon* spp.**	***Plasmodium* spp.**			
Chicken	Winter (85)	10(11.8%)	1(1.2)			
	Summer(70)	3 (4.3%)	1 (1.4)			
	Rainy (111)	17 (15.3%)	3 (2.7)			
Duck	Winter (55)	2 (3.6%)	-			
	Summer (100)	11 (11.0)	2 (2.0)			
	Rainy (53)	7 (13.2)	3(5.3)			
Total	Winter (140)	13(9.3)[a]		1.00		
	Summer (170)	17 (10.0)[a]		1.08		
	Rainy (164)	30(18.3)[b]		2.19	7.47	0.03**

** =P<0.05, significant at 95% level of confidence interval, OR= Odds Ratio
[a,b] Each superscript letter denotes a subset of Season categories whose row proportions differ significantly from each other at the 0.05 level.

Discussion

Various species of haematozoa have been reported to infect both wild and domestic birds in Bangladesh. The avian haematozoans have been the focus of a number of investigations and it is well established that domestic poultry are common hosts and black flies and mosquitoes are vectors for those parasites (Bennett *et al*., 1966). The present study indicates that 12.7% birds were infected with malaria protozoa which were similar to the findings of Bennett *et al*. (1991) in Northeastern Mexican birds (12.8%). However, higher prevalence rate(50.7%) was reported by Dey *et al.* (2008a) in Mymensingh district of Bangladesh and Silva-Iturriza *et al.*(2012) in the central Philippine islands (42.0%). The more or less similar prevalence (29.5%) was recorded by Elahi *et al.* (2014) in wetland birds in Bangladesh, Sehgal *et al.* (2005) in Equatorial Guinea and Ivory Coast (28.6%), and Momin *et al.* (2014) in poultry in Tangail district, Bangladesh (22.7%). These variations might be due to the differences in geographic niches, climatic conditions, breed of birds, management factors, availability of vectors and the method of study. Moreover, in this study, samples from indigenous chickens and ducks were collected from local house and market with relatively poor management. Infact, *Pseudolynchia canariensis*, *Simulium* spp and *Culicoides* spp. are abundant in Bangladesh. They act as potential vector of malaria parasite of poultry for both chickens and ducks. Probably, these factors play a vital role in the prevalence of blood protozoa in indigenous poultry in Bangladesh.

The prevalence of avian malaria species also varied across different countries. In this study, the prevalence of *Leucocytozoon* spp and *Plasmodium* spp. was 10.5% and 2.1%, respectively. Dey *et al.* (2008a) and Dey *et al.* (2008b) reported 33.3% and 60.0% prevalence of *Plasmodium* spp. and *Leucocytozoon* spp. in chickens and ducks, respectively, in Mymensingh district of Bangladesh. Similarly, Momin *et al.* (2014) recorded 34.6% and 58.3% prevalence rate for *Leucocytozoon* spp. in chickens and ducks of Tangail district in Bangladesh. Benenett *et al.* (1975) reported 30% haemoprotozoan infection in ducks in Nova Scotia and Prince Edward Island while Thul *et al.* (1980) found *Leucocytozoon simondi* infection in 20.0% ducks in Atlantic Flyway. For *Plasmodium* spp. Ishtiaq *et al*.(2007) recorded 28.0% prevalence in wild birds of India, but in Myanmar the prevalence rate was 60.0%. In addition, Dranzoa *et al.* (1999) and Okanga *et al.* (2013) recorded 29.4% and 27.0% infection rate with *Plasmodium* parasites in rock pigeons and weavers in

Uganda and South Africa, respectively. The lower prevalence is reported by Elahi *et al.*(2014), Borji *et al.*(2011), Permin *et al.*(2002) and Forrester *et al.*(2001) in wetlands of Bangladesh (0.6%), Eastern Iran (2%), Zimbabwe (3%) and Florida (9.2%) in wetland birds, pigeons, chickens and ring-necked ducks, respectively.

According to the present study, it was observed that the prevalence of malaria parasite was higher in female (15.2%) than in male (10.9%). Senlik *et al.*(2005) in USA and Gupta *et al.* (2011) in India reported higher infection in female birds (62.8% and 55.0%, respectively). But the result is contrary with the previous report of O'Dell and Robbins (1994) who reported higher prevalence in male ducks (19.7%) than in female (18.0%) in USA. The exact cause of higher prevalence in female birds cannot be explained but in general higher level of prolactin and progesterone suppress the immune system of the individual and make the female individual more susceptible to infection.

In this study, the prevalence of malaria parasite was higher in adult (13.9%) than in young (11.2%). Similar results were recorded by Islam *et al.*(2013) in Bangladesh (33.3% and 3.5%), Msoffe *et al.*(2010) in Tanzania (63% and 11%) and El-Magd *et al.*(1988) in Egypt (60.7% and 20.0%) in adult and young birds, respectively. On the contrary, in some studies, higher prevalence was reported in young birds than in adults (Van Oers *et al*. 2010; Hudson and Dobson, 1997). Higher prevalence in adults may be owing to long time exposure to the vectors. Moreover, another cause might be owing to absence of active transmission of malaria parasite from adult to young (Thull and O'Brien, 1990).

In case of district-wise prevalence, significantly higher rate was found in Netrokona (18.9%) than in Tangail (12.2%) and Mymensingh (8.7%). This variation was due to the abundance of vector (Culicoides, *Simulium* spp) in Netrokona which acts as vector of the malaria parasites. Besides, lack of quarantine procedure for newly acquired poultry, failure to prevent the contact with wild birds and host susceptibility were also may be the reason behind this variation. Elahi *et al.* (2014) found 29.5% infection with *Plasmodium* spp. and *Leucocytozoon* spp. in a wide range of wetland birds in Bangladesh. Furthermore, Momin *et al*. (2014) reported 45.8% infection with blood protozoa in domestic poultry in Tangail district.

The prevalence of malaria parasites was higher in the rainy season (18.3%) followed by summer (10.0%) and winter season (9.3%). This finding was almost similar to Okanga *et al.* (2013) who detected higher prevalence in weaver birds of South Africa during late summer (16.0%) than winter (10.0%) season with significant seasonal difference. In India Gupta *et al.* (2011) recorded highest infectivity of pigeon malaria during the summer season (82.9%) followed by spring season (59.4%) and least in the winter season (42.3%). Moreover, Senlik *et al*. (2005) found highest infection rate in autumn (44%) while the lowest in spring in Iran. These variations might be due to the abundance of insect vector population during summer and rainy seasons. The mosquito breeding rate is generally high during late summer and early of rainy season in Bangladesh. Apart from rainfall and differences in habitat composition, differences in prevalence may be influenced by proximity to breeding site for vectors, relative levels of host resistance, local temperature differences, time of collection during the day and age of host among the others (Olayemi *et al*., 2014; Nath *et al*., 2014).

Conclusion

The present study provides data on the prevalence of haemoprotozoa in indigenous poultry in Bangladesh with an emphasis on village chickens. It is envisaged that the data will contribute in maintaining database on the occurrence of these pathogens among wild and domestic avifauna in the country. Further studies may be undertaken to characterize the malaria parasites in indigenous poultry of Bangladesh.

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