HAEMOPROTOZOA INFECTION OF DOMESTIC BIRDS IN HILLY AREAS OF BANGLADESH

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ABSTRACT

The blood protozoa of two important domestic birds namely chickens (Gallus domesticus) and pigeon (Columba livia) reared in the hilly areas of Bangladesh were studied. A total of 400 birds (200 chicken and 200 pigeons) were examined of which 149 (37.3%) [95% CI] birds were found infected by one or more haemoprotozoan parasites. Haemoproteus belonging to three genera were identified. Pigeon 80 (40%) was recorded more susceptible to haemoprotozoa infection than chicken 69 (34.5%). 118 birds (29.5%) were found to be infected with single infection where as mixed infections were found in 31 birds (7.8%). The prevalence of blood protozoa in female birds (69.5%) was found significantly higher (p ≤ 0.0001) [95% CI] than male birds (5%). Within the study period, the prevalence rate of Haemoproteus was 60.6% in summer season, 36.7% in rainy and 23% winter seasons. This study has archived a high prevalence of haemoparasites, henceforth encourage further to determine the effect of contamination on the productivity and profitability of these birds, and evaluation of cost-benefit of various control strategies need to be undertaken.

Keywords: Prevalence, Haemoproteus, Plasmodium, Leucocytozoon
1. INTRODUCTION

Bangladesh is a developing nation where poultry industry is a rising sector. Being an integrated part of the livestock sector, poultry farming plays an important role in the agro-based economy of Bangladesh (MINISTRY OF LIVESTOCK; FISHERIES, 2012). The increasing demand for animal protein and the economic benefit obtained through raising of poultry in both scavenging and semi-scavenging condition have created a great deal of interest among the farmers in this country (LATIF, 2001). Poultry keeping is one of the most appropriate income generating activities for rural women especially for landless and marginal farmers which employs about 5 million people (BLRI, 2015).

Poultry production in Bangladesh specifically includes chickens, pigeon, ducks, quail, swan and guinea fowl. Chicken and Pigeon productions however, make up the main component of the commercial poultry. The production of backyard chicken and pigeon under the semi-scavenging system is found suitable to the villagers as an additional source of income and nutrient supplement (LATIF, 2001). The total population of poultry in our country is about 252.31 million where the chicken population is 256.78 million. However, there is no definite statistics in pigeon population (BLRI, 2015).

Several health problems can affect chicken and pigeon, but parasitic infections play a major role. Blood parasites have been the subject of extensive research since the beginning of the 20th century. Haemoproteozoa infection is common in the domestic birds but poorly studied in Bangladesh (THE RAFFLES BULLETIN OF ZOOLOGY, 2008). Some researchers reported haemoproteozoa of different species in poultry in Bangladesh (MOMIN, 2014; DEY et al., 2010).

But the number of work regarding distribution or prevalence of these parasites is very limited. Such information is crucial in the implementation of a disease control programme hence improvements in the productivity of indigenous free-ranging chicken and pigeon. This study is designed and conducted to investigate the type and prevalence of haemoparasites infection in indigenous free-ranging chickens, and pigeon in different hilly areas in Bangladesh, and the data will assist in identifying the host infectivity prevalence and contribute to a long term database on the occurrence of these parasites.
2. MATERIALS AND METHODS

A total of 400 birds (200 indigenous chickens and 200 pigeons) with matching for sex were purposively randomly selected from four (4) different hilly districts of Bangladesh (Sylhet, Hobigonj, Khagrachari and Bandarban) during January to December 2014.

Sex was determined subjectively based on the length of spur and flexibility of the xiphoid cartilage together with information from the farmers. Three (3) thin blood smears were prepared from each bird, processed and examined for haemoprotozoa. Blood collection samples from same chickens and pigeon were collected from the wing vein using a 1ml syringe. The skin was hosed with alcohol to disinfect the area and make the vein noticeable.

The blood was straightforwardly transferred into labelled test tubes containing anticoagulant (EDTA) and transported to the Laboratory of Parasitology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Bangladesh for staining and identification. In the laboratory, blood samples were processed using thin blood smear to detect and identify parasites. A drop of blood was put on a clean grease free glass slide.

A thin smear was made and allowed to dry. It was then fixed in alcohol and then stained with Giemsa stain. Slides were subjected to a microscopic examination and result was recorded. Haemoparasites were identified according to guidelines described by Levine (1985) and Soulsby (1982). The specific data was collected directly from the farmer by a structured questionnaire. The data was analyzed using STATA-13 statistical package. The association between sex and infection status was evaluated using the chi-squared test. A p value less than 0.05 was considered statistically significant.

3. RESULTS

The research showed that out of the 400 birds examined, 149 (37.3%) were infected with at least one out of three species of Haemoprotozoa. The study revealed a significant difference between haemoparasite species values either single or mixed infected in both chicken and pigeon (p ≤ 0.003) with no significant difference between species of birds (p ≤ 0.139) with the highest value for Pigeon 80 (40%) followed by Chicken 69 (34.5%). Some of the birds were singly infected while others
had multiple infections. Chicken higher prevalence of Leucocytozoon species whereas Pigeon had a higher infection with Haemoproteus species (table 1).

In chicken, three genera of Haemoparasites comprising Haemoproteus spp 5 (2.5%), Plasmodium spp 21 (10.5%), and Leucocytozoon spp 29 (14.5%) was found, while non-infected birds were 131 (65.5%) (Table-1). Mixed infection was recorded comprising Plasmodium spp and Leucocytozoon spp 13(6.5%) and Plasmodium spp and Haemoproteus spp 1 (0.5%).

No triple infection (Plasmodium spp + Heamoproteus spp + Leucocytozoon spp) was found in case of chicken. In pigeon, infection with Haemoproteus spp was more prevalent 49 (24.5%) followed by infection with Plasmodium spp 11 (5.5%). Only 3 (1.5%) cases of Leucocytozoon was recorded. For the double infection, Plasmodium spp and Haemoproteus spp 9 (4.5%), Haemoproteus spp and Leucocytozoon spp 2 (1%) and Plasmodium spp and Leucocytozoon spp 1 (0.5%) while for triple infection (Plasmodium spp + Haemoproteus spp + Leucocytozoon spp), was 5 (2.5 %) (Table-1).

Table 1: Prevalence of Haemoprotozoa in Chicken & Pigeon (single or mixed infection)

<table>
<thead>
<tr>
<th>Species</th>
<th>Haemoprotozoa occurrence in birds*</th>
<th>Number of birds infected with Haemoprotozoa</th>
<th>Percentage prevalence rate (%)</th>
<th>Overall Prevalence (n=400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken (n=200)</td>
<td>Only P</td>
<td>21</td>
<td>10.5</td>
<td>34.5%</td>
</tr>
<tr>
<td></td>
<td>Only L</td>
<td>29</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Only H</td>
<td>5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both P+L</td>
<td>13</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both P+H</td>
<td>1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both L+H</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triple H+P+L</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pigeon (n=200)</td>
<td>Only P</td>
<td>11</td>
<td>5.5</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Only L</td>
<td>3</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Only H</td>
<td>49</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both P+L</td>
<td>1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both P+H</td>
<td>9</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both L+H</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triple H+P+L</td>
<td>5</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

* P=Plasmodium spp., L=Leucocytozoon spp., H=Haemoproteus spp.

The results of haemoprotozoa infections of the chickens according to their sexes and season are presented in table 2. Regarding the sex of infected birds, results showed that female birds were more infected 139 (69.5%) than males 10 (5%) with significant difference (p \leq 0.0001). No significant difference was found between positive seasonal cases distribution (p \geq 0.067), which shows infections in
summer 91 (60.6%) was the highest than rainy 55 (36.7%) followed by winter 23 (23%) (Table-2).

**Table 2: Prevalence of Haemoprotozoa According to Sex and Seasonal Condition**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sex and Season</th>
<th>Number of birds infected with Haemoprotozoa</th>
<th>No. (%) infected with Haemoprotozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Male (n=100)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Female (n=100)</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Pigeon</td>
<td>Male (n=100)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female (n=100)</td>
<td>77</td>
<td>22</td>
</tr>
<tr>
<td>Overall</td>
<td>Male (n=200)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Female (n=200)</td>
<td>139</td>
<td>69.5</td>
</tr>
<tr>
<td>Season</td>
<td>Summer (n=150)</td>
<td>91</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>Rainy (n=150)</td>
<td>55</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Winter (n=100)</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

4. **DISCUSSION:**

Haemoprotozoa is continuously circulating around the universe. Prevalence of haemoparasites was reported high in human in hilly areas of Bangladesh but a review of the literature did not give prior information as regards the prevalence of haemoparasites in birds. Results obtained from this study exposed the presence of three haemoparasites (Plasmodium spp, Leucocytozoon spp and Haemoproteus spp) that were found to infect both chicken and pigeon in the study areas.

Mixed infections of these haemoparasites were also found. The findings of this study are supported by the study of Tiwari et al. (2012) in West Indies, Dey et al. (2010) in Bangladesh and Sadiq et al. (2003) in Nigeria, they reported the same three haemoprotozoa (Plasmodium spp, Leucocytozoon spp and Haemoproteus spp) during their study. Similar findings were also reported by Permin et al. (2002) in Zimbabwe, Bennett et al. (1975) in Nova Scotia and Gulanber et al. (2002) in Istanbul.

Prevalence of haemoparasites in birds in this study was found to be 37.3%. These findings are below than the findings of previous studies done by Valkiūnas et al. (2005) who documented the prevalence of avian blood parasites in Uganda to be 61.9%, and Njunga et al. (2003) in Malawi found the prevalence of haemoparasites in birds to be 71%. Their reported prevalence was higher, likely because they used molecular methods to detect parasites, which are known to be more sensitive. Another reason for this variation might be due to the variation in the geographical distribution, climatic condition, management system of poultry, and availability of vector.
Variations in the prevalence of infection in different bird families have been reported in this study. Pigeon harbors more haemoprotozoa than chicken. Mixed occurrence of blood parasite was also high in pigeons in compared to chicken. Momin (2014) also reported almost similar result during his investigation of blood protozoa in poultry in Tangail, Bangladesh. The difference in the prevalence may involve behavioral perspective or some physiological conditions characteristic for the species that may make the host more or less vulnerable to the parasites (Elahi et al, 2014; NATH et al, 2014).

In the present study, there were no significance differences in occurrences among chicken and pigeon but a significant association between sex and haemoprotozoa infection was detected. Senlik et al. (2005) were unable to detect a significant difference in the infection rate of this parasite in terms of host sex. However, the reason for different prevalence across bird’s sexes was not clear because there were no documented studies on the comparisons between sexes, hence should be studied further.

In this study, the prevalence of Leukocytozoon was found higher in chicken than pigeon. Very few data is available to explain the prevalence of Leukocytozoon in chickens as well as pigeons. Mamud et al. (2012) in Nigeria reported the prevalence of Leukocytozoon in pigeon was 3% while Mbuthia et al. (2011) in Kenya reported 31.6% of Leucocytozoon in chicken.

The high prevalence of Plasmodium in Chicken was detected in compare to Pigeon which are supported by the study carried out by Mamud et al. (2012) in Nigeria, Mbuthia et al (2011) in Kenya, Akinpelu (2008) in Africa, Sehgal et al. (2006) in Ivorycoast and Valkiunas et al. (2004) in Northwestern Costa Rica. Mamud et al. (2012) in Nigeria reported 30% prevalence of Plasmodium in pigeon while Mbuthia et al. (2011) in Kenya reported 29.8% of Plasmodium in chicken. The higher prevalence of Plasmodium was possibly due to the high abundance of its vectors (mosquito) in the study areas.

The prevalence of Haemoproteus found in this study was higher in pigeon than chicken. These findings are supported by the findings of Tiwari et al. (2012), Mamud et al. (2012), Mbuthia et al. (2011), Permin et al. (2002) and Sehgal et al. (2005).
This study was also conducted throughout the year where higher prevalence was recorded during the summer season which provides a very conducive environment for the breeding and proliferation of the arthropod vectors (OLAYEMI et al. 2014). The mosquito breeding rate generally is high during summer season in Bangladesh. This important role of seasonal impact on vector and the haemoparasite spread could be used as a vital tool in the institution of preventive and control measures for both chicken and pigeon.

Apart from rainfall and differences in habitat composition, differences in prevalence may be influenced by proximity to breeding 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).

5. CONCLUSION

Three genera of blood parasites were present, which include Haemoproteus, Plasmodium, and Leucocytozoon. This study documented that there is a high prevalence of haemoprotozoa infection in apparently healthy looking indigenous chicken and pigeon. However, further study with a greater sample size is necessary to assess the intensity of the infection more accurately.

Predisposing factors to haemoproteozoa infection also need to be examined. What's more, the only tests used in this study was microscopy which has a very limited specificity, and therefore more obtrusive tests need to be used. Identification of different species of this blood protozoon among chicken and pigeon not only will generate knowledge but also help in developing strategies for successful control programs.

6. ETHICAL APPROVAL

Approval for this study was obtained from the Department of Parasitology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Bangladesh before the implementation of the study. Birds were extracted from the traps as soon as possible after collecting blood sample. All the samples were collected by the corresponding author (registered veterinarians). Blood collected from each bird was less than 1% of the body weight.
7. ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Shafiul Alam, Associate Scientist, International Centre for Diarrheal Disease Research, Bangladesh for his guidance, contribution and logistic supports during the course of this study. Local guides and smallholder farmers from various villages in Chittagong and Sylhet division where the samples were collected are appreciated for their indefatigable support and cooperation while sourcing for the study material.

8. COMPETING INTERESTS

There is no conflict of interest declared by any of the authors.

9. AUTHOR CONTRIBUTIONS

The study was supervised by Md. Jamal Uddin Bhuiyan while the design of the study, field experiments, data analysis and writing of the manuscript was performed by Tilak Chandra Nath.

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