



Original Research Paper

# A study on the presence of leucocytozoonosis in pigeon and chicken of hilly districts of Bangladesh

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Presence of Leucocytozoonosis in pigeon and chicken and the pathology it produced were investigated in four (4) selected districts of Bangladesh (Khagrachari, Bandarban, Sylhet and Sunamgonj) during the period of January to December' 2011. A total of 200 samples (live birds) were collected randomly. Both ante-mortem and post-mortem examinations were performed along with microscopy of blood and affected tissue samples and collection of data using well-designed questionnaire. A total of 100 pigeons and 100 chickens were examined of which overall 2% pigeon and 12% chicken were found to be infected by *Leucocytozoon* sp. Leucocytozoon belonging to two species were identified. Identified species were *Leucocytozoon marchouxi* in pigeons and *Leucocytozoon caulleryi* in chicken. Pathological lesions were also observed. The suspected organs such as liver, lungs, kidney, heart, bursa and intestine were apparently normal on gross observation. However, numerous microgametocyte and macrogametocytes of *Leucocytozoon* were seen in many blood vessels of the organs examined. Oval to irregular shape gametocytes were observed in vessels of the affected organs. Small necrotic foci were observed in the liver upon histopathological study.

**Key words:** Presence, leucocytozoonosis, pigeon, chicken, hilly districts

## INTRODUCTION

Bangladesh is a developing country where poultry industry is a rising sector. It plays an important role in the rural economy. This poultry sector employs about 5 million people & has experienced a long-term growth rate of about 4.50%, which is highest in the economy (BLRI report, 2009). It is an important component of farming system and plays a significant role to 80 % rural people of Bangladesh. Several health problems can affect chicken & pigeon but parasitic infections play a major role. Leucocytozoonosis is a protozoan disease of birds that affects the blood and tissue cells of internal organs. These parasites are assigned to the suborder Haemospororina of the phylum Apicomplexa. *Leucocytozoon* sp. use Simuliidae (black flies) as vectors. The life cycle includes reproduction by sporogony in insects with schizogony (merogony) in tissue cells and gametogony in erythrocytes or leukocytes. Initial development occurs in the liver and spleen, followed by the development of un-pigmented gametocytes in white or red

blood cells, depending upon the species (Soulsby, 1982).

The parasites have an almost worldwide distribution and occur in a variety of species, including pigeon and chicken. Although there are a very limited number of studies on blood parasites of avian species, very few study were done to describing the presence of *Leucocytozoon* sp. in pigeon and chicken in Bangladesh previously. The aim of this study is to report the presence of leucocytozoonosis and to examine pathological findings regarding illness of pigeon and chicken in hilly districts of Bangladesh.

## MATERIALS AND METHODS

To study the presence of Leucocytozoonosis of pigeon and chicken and the pathological changes produced by them, pigeon and chicken were selected randomly and collected from different local market and from farmer's house of

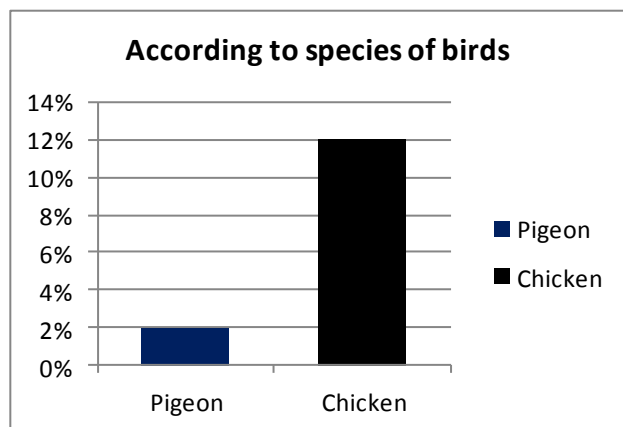


Figure 1: *Leucocytozoon sp.* According to species of birds

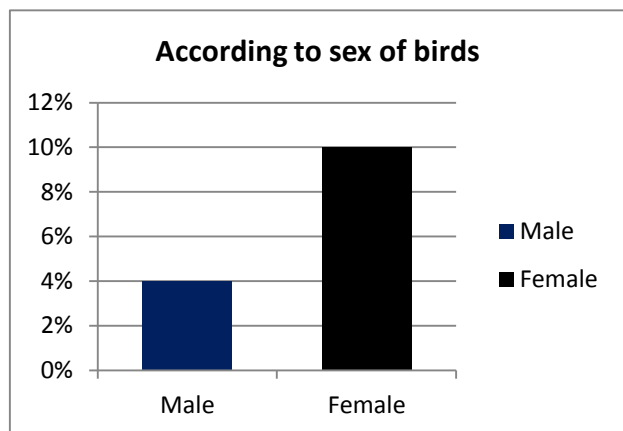


Figure 2: *Leucocytozoon sp.* According to sex of birds

different villages of four selected districts of Bangladesh such as Khagrachari (located at 23.0417°N 91.9944°E), Bandarban (located at 21°48' N 92°24' E) Sylhet (located at 24.8917°N 91.8833°E) and Sunamgonj (located at 25.030869°N 91.403761°E) districts during the period of January 2011 to December 2011.

#### Ante-mortem examination

After collection of birds, age, sex, breed and body weight were recorded. Pigeon and chicken were examined carefully by parting of feathers against their natural direction and simultaneous close inspection to detect clinical signs if any.

#### Collection and staining of blood and identification of protozoa

Blood samples were collected from the wing vein of pigeon and chicken with the help of syringe and needle. The thin

and thick smear was made immediately after the collection of blood for identification of blood protozoa. The smear was then air dried, fixed with absolute acetone free methanol, stained with Giemsa's stain and then air dried (Cable, 1957). The slides were then examined under microscope in higher magnification (40X, 100X) for the detection of blood protozoa. Protozoa were identified according to the keys and descriptions given by Levine (1985), Springer (1997) and Soulsby (1982).

#### Post-mortem examination

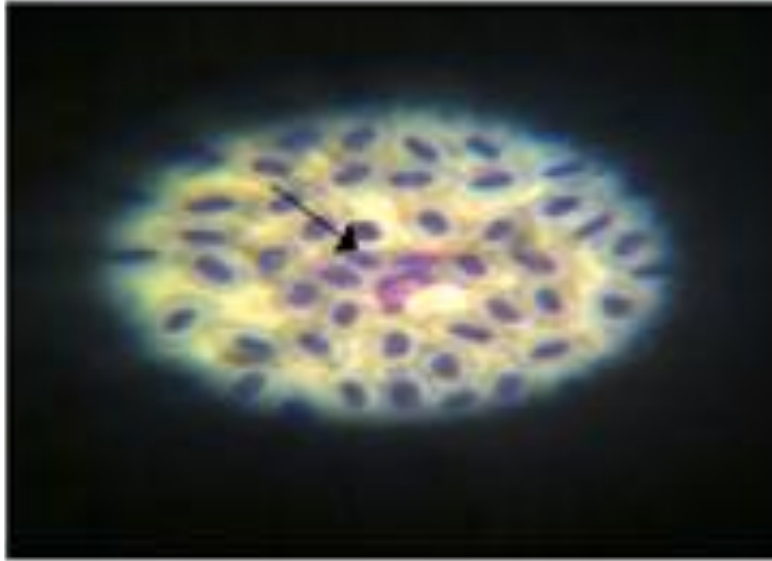
After ante-mortem examination, pigeon and chicken were euthanized and slaughtered and allowed to bleed completely and then post-mortem examination was performed as described by Fowler (1990). The skin overlying the breast and fluff were carefully removed. Then the abdominal wall was incised along the midline, starting from cloaca up to the posterior margin of the keel bone by a scissors. The ribs were then cut just lateral to the keel bone and keel bone was separated. After the opening of thoracic and abdominal cavities, the internal organs such as lung, heart, liver, spleen, kidney and ovary was examined carefully to detect pathological changes.

#### Histopathological examination

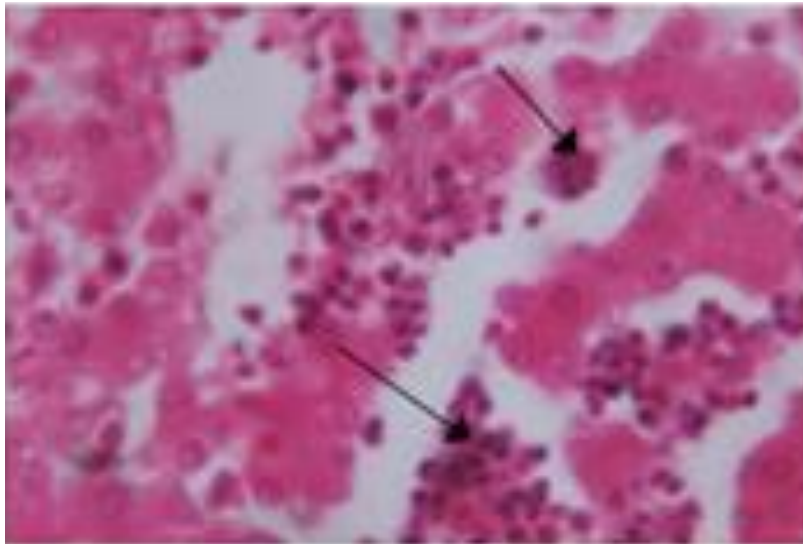
During necropsy suspected livers and kidney were collected and fixed in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed, embedded, blocked and sectioned and stained with Haematoxylin and Eosin (H&E) stain following standard method (Luna, 1968).

## RESULTS

In this study, 200 birds (100 Chicken and 100 Pigeon) were examined where 14 (7%) birds were affected with Leucocytozoonosis. A total of 12% chicken and only 2% pigeon were found to be infected by Leucocytozoon. (Figure 1 and Figure 2). In this study, the gametocytes of both *L. marchouxi* and *L. caulleryi* were found in lymphocytes. In *L. caulleryi* infection, the gametocyte, which was identified from the leucocyte were round and measuring 15.5 by 15.0  $\mu$ . The nucleus of the host cell formed a narrow dark violet coloured band extending about a third of the way around the parasite. These descriptions were in conformity to the keys and description given by Levine (1985). In *L. marchouxi*, the gametocytes were found in leucocyte and in Giemsa's stain these were more or less elongated measuring up to 12 to 24  $\mu$  in long in their host cells. The nucleus of the host cell was seen forming a very long, thin, dark band in one side of the cell. Gametocytes cause marked enlargement and distortion of the infected cells. The parasites nucleus was elongated, with a clear nucleolus



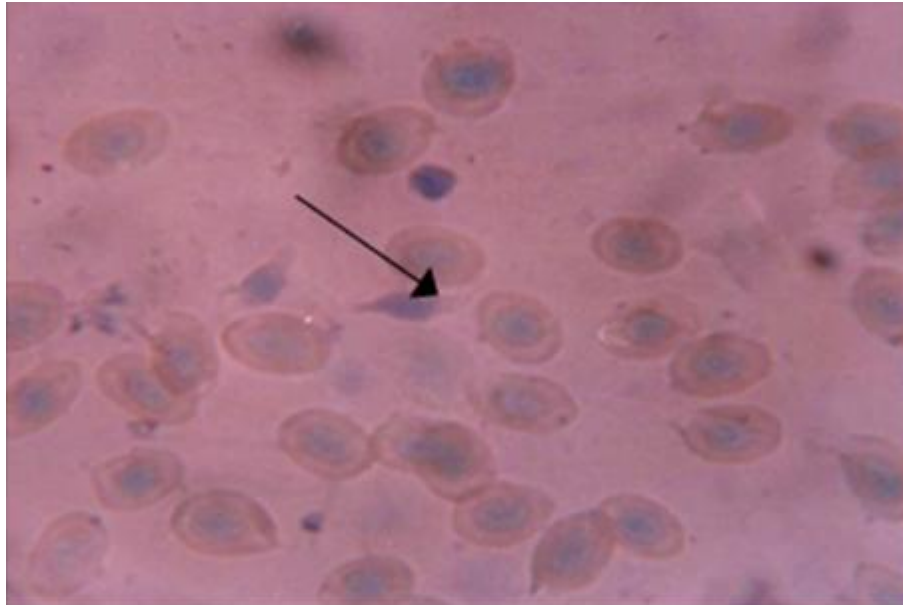
**Figure 3:** *Leucocytozoon sp.* (arrow) in chicken, stained with Giemsa stain (100X)



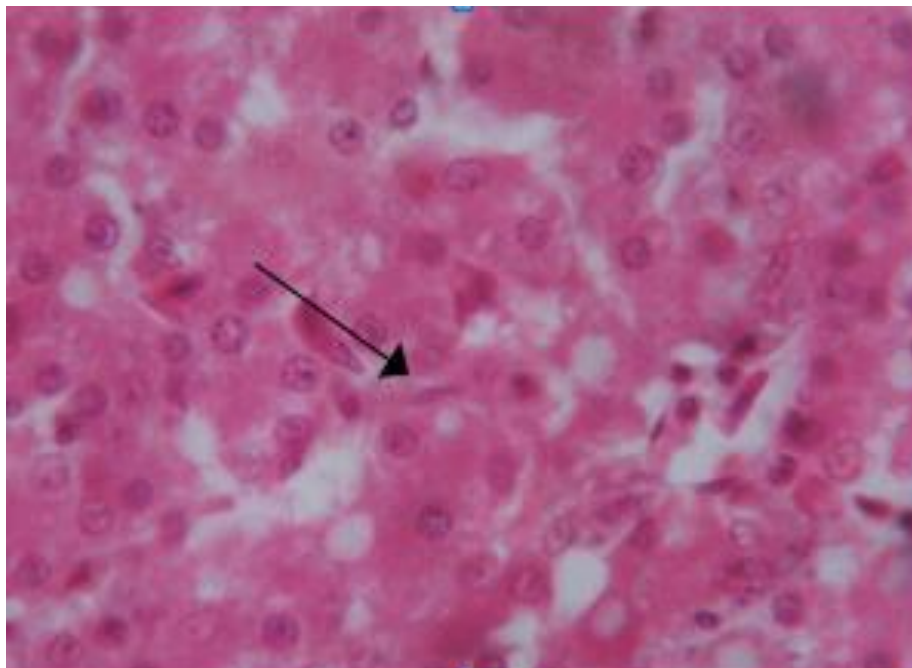
**Figure 4:** Section of a chicken liver stained with H&E. Hepatic cords were found to disappear in the affected area. The vascular lesion consisting of degeneration and dissolution of endothelial cell and it was impossible to detect endothelial cell lining in case of *Leucocytozoon* infection (100X).

close to the nuclear membrane. The host cell nucleus was wider at the ends and narrow at the centre. Pale cytoplasmic horns were seen in either sides, extending out beyond the parasite and the nucleus. This description was almost similar to the descriptions of Levine (1985). According to these characteristics, the parasite was morphologically identified as *Leucocytozoon marchouxi* in 2 pigeons and *Leucocytozoon caulleryi* in 12 Chicken. The overall presence for male bird was 4% while in female 10% (Figure 3).

Clinically the bird had seemed healthy before death. According to the owner, the bird had shown the signs of in-appetence, dullness and dyspnoea before death. Young birds manifest in-appetence, weakness, listlessness, dyspnoea. During the necropsy enlarged liver and swollen kidneys together with pale serosal membranes were the prominent findings. At histopathological examination, numerous microgametocyte and macrogametocytes of *Leucocytozoon* were seen in many blood vessels of the organs examined (Figure 4). Oval to irregular shape



**Figure 5:** *Leucocytozoon marchouxi* (arrow) infected leukocyte in the blood film of pigeon stained with Giemsa's stain (825X)



**Figure 6:** Section of pigeon liver stained with H&E. Comma shaped structure similar to leucocytozoon were seen in the liver parenchyma (825X)

gametocytes were observed in vessels. Merogony occurs in the parenchyma of liver. More than half of the parasites perimeter adhered to the host cell nucleus. Pieces of the host cell cytoplasm were often observed. There was no inflammatory reaction around the vessels. Megaloszizonts appear as grey-white nodules found in the liver. Ischemic necrosis and associated inflammation were recorded in the liver tissue. Small necrotic foci were observed in the liver

upon histo-pathology (Figure 5 and 6).

## DISCUSSION

*Leucocytozoon* sp. is arthropod-transmitted blood protozoa. Species of these protozoa appears to be widely distributed in many parts of the world. Despite the large

number of bird species known to be susceptible to infection with *Leucocytozoon*, very few records are described and disease due to infection is rarely mentioned.

Blood parasites are usually diagnosed in blood smears. *Leucocytozoon* infections are diagnosed by direct microscopic observation and identification of gametocytes in stained blood or schizonts in tissue sections. This study also showed that the best method for the diagnosis of blood parasites is examination of blood smears. However, if the birds are brought in dead it is possible to diagnose blood parasites by histopathological examination. The other possible method for the diagnosis of blood parasites in dead birds may be organ smears prepared before formalin fixation of the tissues. Hemoprotozoa are generally considered only slightly pathogenic to domestic birds. However, some evidence exists that they can cause subclinical and debilitating effects and depresses breeding rates. While the prevalence of *Leucocytozoonosis* is lower than that of other haemoprotozoa in pigeon and chicken. In the present study, *Leucocytozoon sp.* was observed in only 2 pigeons and 12 chickens out of 200 birds which were lower than the rate of infection of reported earlier by Gocok et al. (1999) in Ankara who recorded the prevalence of *Leucocytozoon sp.* on pigeon and chicken was 13% and 38% respectively. The variation between the results might be due to the differences among the geographical locations and climatic conditions of the study areas. Sex of birds had significant role to influence prevalence of *Leucocytozoon sp.* We found a higher prevalence rate of *Leucocytozoon sp.* in female birds in that of male. This was similar to the finding of Fallis et al. (1974) and Krone et al. (2001).

*Leucocytozoon* is the largest haemoparasite encountered in birds. The *Leucocytozoon* gametocyte does not contain refractile pigment and grossly distorts the host cell. Two forms are commonly encountered: an elongate form in which the host cell nucleus is pushed to the side and the cytoplasm is long with tapering tails; and a round form in which the host cell nucleus is eccentrically located and the cytoplasmic tails are obvious. The appearance of both round and elongated gametocytes is related to the pathogenicity of the *Leucocytozoon* strain, with the more pathogenic forms progressing to the elongate stage. Only the elongated form of the parasite was seen in pigeons in this study. Both elongated and round forms were observed in the chicken but elongated forms were numerous. These findings were supported by the findings of Krone et al. (2001).

## Conclusion

The present study indicates presence of *Leucocytozoonosis* in hilly districts of Bangladesh associated with species and sex of the birds and pathological findings related with it. However, this was an initial study; further study should be required to estimate the accurate prevalence of the disease

and to prevent the infections of birds and maximizing the poultry production in Bangladesh.

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