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Research Article

Histopathological and Molecular Detection of Lymphoid Leukosis in Sonali Chickens in Chattogram, Bangladesh

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ABSTRACT

Introduction: Lymphoid leukosis (LL), caused by the *Avian leukosis* virus (ALV), is a tumor-forming disease in poultry that causes considerable economic losses in commercial poultry farming. The present study aimed to evaluate the histopathological and molecular aspects of LL in Sonali chickens from several poultry farms in Chattogram, Bangladesh.

Methods: 200 dead Sonali chickens aged from 20 to 40 weeks that showed clinical signs of the disease, including gradual weight loss, loss of appetite, enlarged abdomen, pale comb and wattle with high morbidity and low mortality, were collected from poultry farms in Patiya, Anowara, Banshkhali, Chandanaish, Mirsarai, and the Chattogram metropolitan region, Bangladesh. Necropsy of these chickens was performed systematically, and the gross lesions were documented. Samples were collected from the affected organs, including the heart, liver, spleen, and intestine, for histopathological and molecular identification of LL. Histopathological examination of those samples was performed by the routine hematoxylin and eosin (H&E) procedure. A conventional PCR targeting the ALV *env* gene was performed with complementary DNA (cDNA) generated from extracted RNA.

Results: 120 (60%) chickens among the 200 demonstrated specific gross lesions of LL during necropsy examination, including disseminated nodular tumors in visceral organs such as the heart, liver, spleen, and intestines. Routine H&E procedure confirmed LL in 94 (78.33%) of cases. Moreover, 100 percent of the histologically confirmed samples indicated a distinct 220bp amplicon in PCR, confirming ALV infection.

Conclusion: The combination of histology and molecular detection successfully revealed ALV-induced lymphoid leukosis in Sonali chickens. The presence of ALV on several farms indicated the need for stronger biosecurity measures to prevent viral spread.

1. Introduction

Lymphoid leukosis (LL) in chickens is caused by the *Avian leukosis* virus (ALV), a retrovirus from the *Alpharetrovirus* genus within the *Retroviridae* family. The disease primarily affects the lymphoid system, causing B

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cells to proliferate neoplastically, notably in the bursa of Fabricius, spleen, and liver[1]. Globally, lymphoid leukosis causes considerable financial losses in commercial poultry production due to its slow onset, decreased productivity, and death in infected flocks[2]. Sonali chickens, a crossbreed between Rhode Island Red (RIR) cocks and Fayoumi hens, have gained popularity in the commercial poultry industry of Bangladesh due to their increased disease resistance, exceptional growth performance, and adaptability[3]. Nonetheless, the growing number of reports of neoplastic illnesses, such as LL, in Sonali chickens emphasizes the necessity of examining the disease's diagnosis and prevalence. One of the most accurate ways to diagnose LL is still by histopathological examination, which indicates consistent lymphoid tumor infiltration in visceral organs, including the kidney, liver, and spleen[4]. However, due to the latent nature and prolonged incubation period of ALV infection, molecular diagnostic methods such as polymerase chain reaction (PCR) are crucial for accurate detection[5]. Although ALV has been found in commercial and backyard poultry in Bangladesh, there has been no research conducted on Sonali chickens. As Sonali farming has grown rapidly throughout Chattogram, it is essential to utilize histopathological and molecular detection methods to determine the prevalence of LL. The present study aimed to investigate the current status of lymphoid leukosis in Sonali chickens and establish effective disease management programs in the poultry sector of Chattogram, Bangladesh.

2. Materials and Methods

2.1. Sample collection and processing

A total of 200 dead Sonali chickens, aged 20 to 45 weeks, were collected from poultry farms in Patiya, Anowara, Banshkhali, Chandanaish, Mirsarai upazilas, and the metropolitan areas of Chattogram City, which were suspected of having LL from January 2023 to January 2024. Tissue samples, including liver, spleen, kidney, and bursa of Fabricius, were collected from the chickens and immediately fixed in 10 percent neutral buffered formalin for 24-48 hours for histopathological observation. Then, the samples were sent to the Bangladesh Livestock Research Institute (BLRI) for molecular investigation and stored at -80 °C until further analysis.

2.2. Histopathological examination

Tissue samples from selected chickens were fixed and processed using standard paraffin embedding techniques. Dehydration was performed using graded ethanol solutions (70%, 80%, 95%, and 100%), followed by clearing in xylene and embedding in paraffin wax. Sections of four to five µm thickness were obtained using a microtome and placed on glass slides. The sections were then deparaffinized in xylene, rehydrated through a series of descending ethanol concentrations, and stained with hematoxylin and eosin (H&E) according to established protocols[6]. Stained tissue sections were examined under a photomicroscope (AmScope Trinocular Compound Microscope with 1.3 MP Camera, Model T490 B-MT) and AmScope image-measuring software (x86, 3.7.3036 version at various magnifications) to determine histological alterations. Lymphoid leukosis was diagnosed using characteristic histopathological features.

2.3. Polymerase chain reaction

The tissue samples were macerated using a mortar and pestle. The viral RNA was then isolated using the TIANamp Virus DNA/RNA Kit (TianGen, China), and complementary DNA (cDNA) was synthesized using an extraction kit (Qiagen, USA) according to the manufacturer's guidelines. The primers used in the study were forward-*GGTTGGTCTAGACAGGAAGC* and reverse-*CATTGCCACAGCGGTAC* with 35 cycles at 55°C for 30 seconds, 72°C for 40 seconds, annealing at 56°C for 40 seconds, and final extension at 72°C for 10 minutes. PCR products were separated on agarose gel 1.5%, along with ethidium bromide at a concentration of 0.5 µg/ml, following electrophoresis, which was performed in 0.5×TBE buffer at 5 V/cm for 60 minutes, according to established procedures[4].

2.4. Statistical analysis

The obtained data were stored in a Microsoft Excel 2016 spreadsheet. The data were sorted for errors and inconsistencies, coded, and checked for integrity in Microsoft Excel 2016. Afterward, the data were exported to STATA-IC-1 to analyse the percentage of positive cases in histopathological and molecular detection of LL.

3. Results

Necropsy of the suspected chickens indicated poor physical condition, including dehydration, emaciation, enlarged abdomen, and pale comb and wattle. Enlarged liver and spleen with disseminated nodular tumors in the heart, liver, and spleen. Nodules throughout the intestinal tract were also observed (Figure 1). Upon post-mortem examination, 120 chickens (60%) exhibited gross lesions suggestive of LL, such as hepatomegaly, splenomegaly, and tumorous growths in visceral organs.

Figure 2 demonstrates pleomorphic cells in the liver and spleen, indicating metastasis transformation of neoplastic cells, consistent with LL. Figure 3 depicts necrosis in visceral organs, as well as lymphocytic infiltration in the intestine and spleen. Out of 120 Sonali chickens determined to have LL by post-mortem investigation, a total of 94 chickens (78.33%) exhibited these histopathological changes.

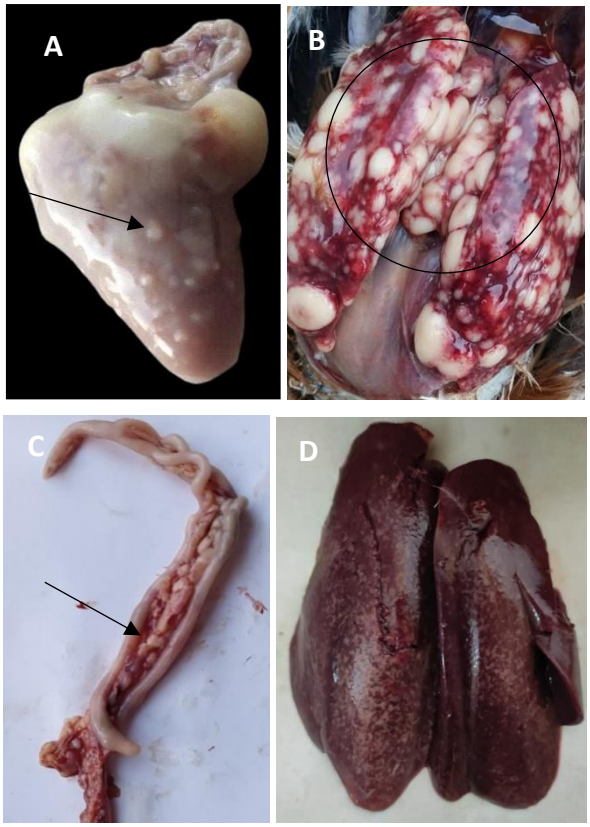

Figure 1. Necropsy findings of Lymphoid leukosis in Sonali chicken. A: Nodular tumor in heart, B: Nodular tumor in liver, C: Nodular tumor in intestine, and D: Swollen liver

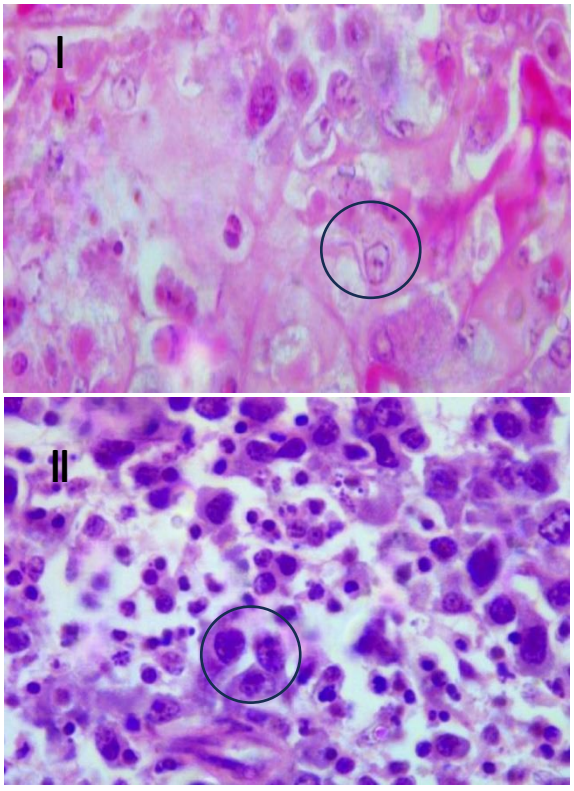

Figure 2. Accumulation of large pleomorphic cells in the liver (I) and spleen (II) in Sonali chicken. 40x, Haematoxylin and Eosin stain.

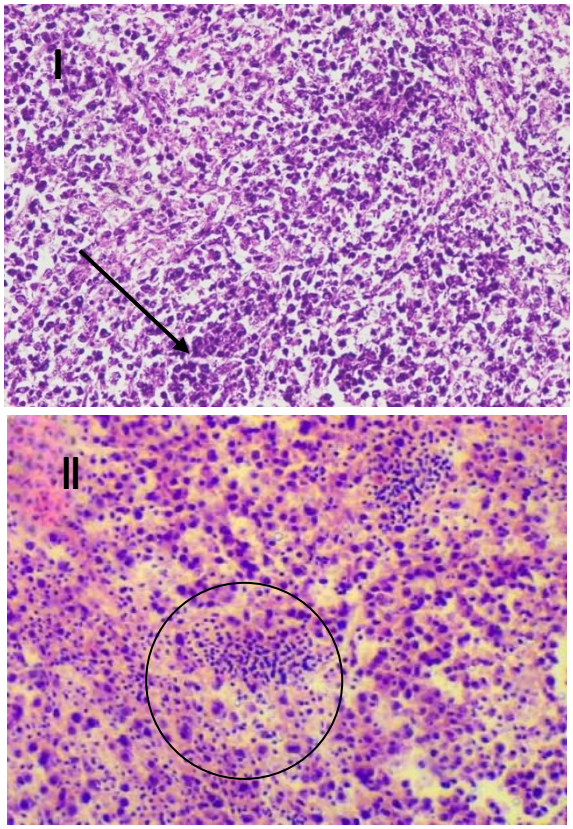

Figure 3. Accumulation of large pleomorphic cells in the intestine (I) and spleen (II) in Sonali chicken. 10x, Haematoxylin and Eosin stain.

In this investigation, all the samples (100 percent) identified as positive by histopathological examination (Figure 3) indicated a positive band at 220 bp in PCR (Figure 4).

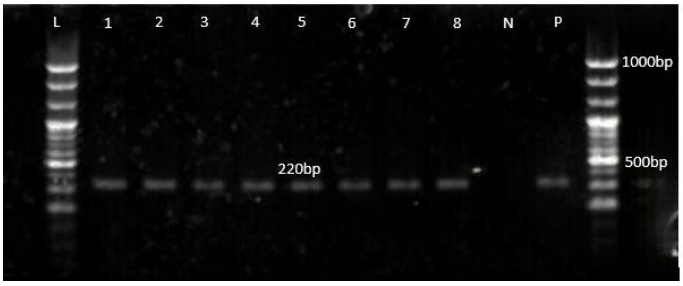

Figure 4. Polymerase chain reaction assay of the *Avian leukosis* virus **env** gene. Lane L: 100 bp DNA ladder, Lane P: Positive control, and N: Negative control, Lanes 1, 2, 3, 4, 5, 6, 7, 8 are Lymphoid leukosis positive samples (220bp).

4. Discussion

The *Avian leukosis* virus (ALV) causes LL, a neoplastic condition that predominantly targets chickens at or near maturity. The histopathological and genetic features of lymphoid leukosis in Sonali chickens from different poultry farms of Chattogram were examined in the present study. The presence of LL in symptomatic chickens was confirmed by gross and histological assessments, followed by PCR-based molecular identification[7]. In the present study, necrotic findings in Figure 1 revealed

poor physical condition, emaciation, dehydration, and pallor of the comb and wattle. Moreover, the development of nodular tumors in visceral organs aligned with the findings described by Payne and Nair[1] and Fadly and Nair[2].

The diagnosis of LL was further supported by histopathological analysis, which revealed widespread lymphocytic infiltration in the liver, spleen, and intestine along with necrosis in visceral organs. Pleomorphic cells in different organs indicated metastasis and neoplastic transformation (Figures 2 and 3), which are hallmarks of LL[8]. Similar histopathology abnormalities have been reported in flocks infected with LL, where normal organ architecture is disrupted by uniform lymphoblast infiltration[9].

Molecular identification using PCR was performed to validate the histopathological diagnosis of LL. The presence of the *Avian leukosis* virus (ALV) in the affected chickens was confirmed by the detection of a specific 220 base pair (bp) amplicon, which was consistently observed in all 54 samples that had previously shown histopathological lesions consistent with LL (Figure 4). This molecular finding provided strong evidence for the involvement of ALV in the pathogenesis of the disease. These results aligned with the findings of Zhang et al.[4], in which the amplification of a similar 220 bp fragment was also reported in ALV-infected chickens using PCR, thereby validating the use of this molecular approach for accurate and rapid virus detection.

However, a considerable prevalence of LL in Sonali chickens in the Chattogram area was demonstrated by the current findings. According to an earlier study, ALV can spread through both genetic and environmental means, which presents a significant problem for managing the health of poultry[10]. Effective biosecurity measures are essential for preventing the spread of ALV in commercial poultry. These methods include improved farm hygiene, vaccination plans, and strict breeder screening[11].

5. Conclusion

Post-mortem analysis, histology, and PCR confirmed the presence of ALV and its related neoplastic changes in the affected Sonali chickens. Considering the economic and health implications of LL in poultry. Further studies are crucial for understanding the genetic diversity of ALV strains in Bangladesh and for devising targeted preventive strategies.

Declarations

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Authors' contributions

Farnaz Kader Nova conceptualized the project, developed the methodology, conducted the investigation, curated the data, and wrote the manuscript. Homaira Pervin Heema was responsible for data collection, laboratory analysis, and manuscript editing. Bijoy Barua provided support for molecular analysis and reviewed the manuscript. Keya Ghosh performed data validation and edited the manuscript. Ummey Sahibunnesa served as the laboratory assistant and organized the data. ASM Lutful Ahasan supervised, guided, reviewed the manuscript, and granted final approval. All authors have read and approved the final version of the manuscript.

Competing interests

The authors declared that they have no conflict of interest related to this study. The research was conducted solely for academic and scientific purposes without any commercial, financial, or personal relationships that could be perceived as influencing the outcomes reported in this study.

Availability of data and materials

The manuscript contains all data sets described in the current study.

Ethical Considerations

The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification.

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