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Research Article

Pathological and Pharmacochemical Evaluation of Broiler Chicken Affected Naturally with Colibacillosis in Kashmir Valley

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Abstract

Background and Objective: Avian colibacillosis is associated with heavy economic losses to the poultry industry and generally indicative of immunosuppression in the birds. It thus warrants rapid and accurate diagnostic approaches. Therefore, the present study was aimed to assess the pathological and pharmacochemical status of broiler chickens affected naturally with colibacillosis in Kashmir valley. **Methodology:** Commercial broiler chickens reared in Ganderbal District of Kashmir valley were used in this study. The samples included the diseased or dead birds received from various poultry farms. Gross pathological, histopathological, histoenzymatic and pharmacochemical observations were recorded. **Results:** On necropsy, among lymphoid organs, the major changes were observed in spleen and bursa. Histopathologically, lymphoid depletion was observed in bursa, thymus, spleen and caecal tonsils. No significant change in enzymatic activity in terms of acid phosphatase and alkaline phosphatase was observed in the lymphoid organs, with the exception of very mild enzyme activity in spleen. Pharmacochemical analysis showed that the caecal glandular epithelium, bursal plica epithelium and alveolar epithelium of harderian gland were positive for acid mucopolysaccharides with no signs of acid mucin in any other lymphoid organ. **Conclusion:** In conclusion, laboratory diagnosis is an important asset and proper lab diagnosis would serve as an important tool in preventing the economic losses incurred as a result of colibacillosis in poultry production.

Key words: Broiler chicken, colibacillosis, diagnosis, pathology, pharmacochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The poultry industry has an important position in the provision of animal protein to man and plays a vital role in the national economy as a revenue provider[1]. In India, within a span of 25 years, the broiler chicken production has gone to 3.8 million ton from nowhere, with growth rate of 10-12% year^{-1} against the growth of agriculture as a whole which is around 2.5%[2]. The local native broiler meat production of Jammu and Kashmir State of India is 543 lac kg[3].

In spite of the enormous potential of the poultry industry, the inherent nature of production system predisposes birds to various diseases leading to direct and indirect losses in terms of mortality, morbidity and decreased production performance[4,5]. In majority of the farms in Kashmir being small and medium scale, the impact of disease is overtly serious[6]. Among the various diseases, the rank of immunosuppressive diseases is high among factors that threaten the broiler production in terms of secondary bacterial, parasitic and fungal infections and vaccination failures.

In India, avian colibacillosis is a major problem encountered in poultry especially broiler chickens[7]. It is caused by *Escherichia coli* and is an important bacterial disease associated with heavy economic losses to the poultry industry[8]. It initially involves respiratory tract (airsacculitis), followed by generalized infection manifested by peri-hepatitis, peri-carditis and septicemia[9]. *Escherichia coli* is normally a commensal organism present in the intestinal tract of poultry; however under certain adverse conditions viz., poor ventilation, overcrowding and immunosuppression, it becomes pathogenic[10]. Pathogenicity of *E. coli* strains has been attributed to the presence of one or more virulence factors like invasiveness factors; invasins, heat labile, heat stable enterotoxins, verotoxins and colonization factors or adhesins[11]. Mortality in colibacillosis may reach up to 94% in severe outbreaks[12,13]. Avian colibacillosis has been reported to be a major infectious disease affecting birds of all age groups[8].

The clinically apparent *E. coli* infection is generally indicative of immunosuppression in poultry birds which could be assessed by the examination of various lymphoid organs[14] The present study was undertaken because of utmost importance of early diagnosis of immunosuppressive diseases and lack of literature regarding histoenzymatic and pharmacochemical study of lymphoid organs of colibacillosis affected broiler chicken. The aim was to evaluate the broiler birds affected with colibacillosis by gross examination, histopathological, histoenzymatic and pharmacochemical methods.

MATERIALS AND METHODS

Study area and sampling: Commercial broiler chickens reared in Ganderbal District of Kashmir valley were used in this study. Ganderbal is a newly formed district of the state of Jammu and Kashmir (India). It is located at 34°14′ N 74°47′ E/34.23° N 74.78° E. It has an average elevation of 1,619 m (5,312 ft) a.m.s.l. The district was divided into 3 strata representing only 3 tehsils namely Ganderbal, Lar and Kangan of the said district. The samples included the diseased or dead broiler chickens received from various poultry farms in the Division of Veterinary Pathology. Complete history with regard to population, age group, signs of the disease, along with its morbidity and mortality were noted. The birds were then subjected to thorough post-mortem examination and the materials were collected for examination. All procedures performed in this study involving birds were approved by Institutional Animal Ethics Committee.

Pathological examination

Gross pathology: Broiler chickens were systematically subjected to detailed macroscopic examination with special emphasis on the lymphoid organs (bursa of fabricius, thymus, spleen, bone marrow, caecal tonsils and harderian gland) and the lesions were recorded.

Histopathology: Representative tissue samples from the lymphoid organs like bursa of fabricius, spleen, thymus, caecal tonsils and harderian gland were collected in 10% formalin. The tissue samples were processed for routine paraffin embedding technique and 5 µ thin sections were stained with Haris' haematoxylin and eosin as described by Luna[15].

Histoenzymatic studies: Histoenzymatic study was carried out for the demonstration of any alkaline phosphatase and acid phosphatase in tissue sections. Fresh tissue specimens from the affected lymphoid organs were cut into thin slices of approximately 3 mm in thickness and chilled in cold acetone or kept at a temperature of -20°C. The chilled tissues pieces were fixed in two to three changes of acetone at 4°C for a total period of 24 h. Alkaline phosphatase was demonstrated by using Gomori's calcium cobalt method and acid phosphatase by Gomori's lead phosphate method[16].

Pharmacochemistry: Parallel tissue sections selected on the basis of histopathological examination were stained for pharmacochemical observations. Demonstration of acid and

neutral mucin was done by combined alcian blue PAS stain[17]. Tissue sections were immersed in alcian blue (pH = 7), heated in microwave at 900 W power for 45 sec and allowed to stand in solution for 5 min. After washing in running tape water for 5 min followed by rinsing in distilled water, sections were immersed in 0.5% periodic acid for 5 min. The slides were dipped in Schiff's reagent, heated in microwave at 900 W power for 45 sec and allowed to stand for 5 min. Again the slides were washed in running tape water for 5 min followed by rinsing in distilled water. Counter staining was performed with haematoxylin and differentiation and bluing was performed with acid alcohol and ammonia water, respectively. The sections were dehydrated, cleared and were mounted in DPX.

RESULTS

Gross pathology

Group I: In this group, 18 days old broiler chickens from a farm of 6000 birds were diagnosed to have colibacillosis. Clinically, the affected birds sneezed, coughed and were depressed. On necropsy, severe perihepatitis and pericarditis were observed (Fig. 1). Samples of lymphoid organs were collected from the birds which died on the day of clinical examination. Grossly, marked atrophy of bursa of fabricius was observed. The spleen and caecal tonsils were enlarged but no lesions were observed in other lymphoid organs.

Group II: In this group, the samples were collected from 21 days old broiler chickens in a farm having flock strength of 5000. These were diagnosed for colibacillosis. On necropsy, the lymphoid organ which showed prominent lesion was spleen, which was congested and enlarged (Fig. 2). However, no significant gross lesions were observed in other lymphoid organs.

Group III: In this group 23 days old broilers in a farm of 3500 birds were diagnosed for colibacillosis. On necropsy the bursa was found atrophied. The spleen was found slightly enlarged and congested. However, no significant changes were observed in other lymphoid organs.

Group IV: In this group, lymphoid organs were collected from the mortality taken out from a broiler farm having a strength of 3500 .These were 25 days old and the gross lesions included slight congestion and enlargement of spleen. No significant gross lesions were observed in other lymphoid organs except slight enlargement of bursa and thymus.

Fig. 1: Yellowish membrane in pericarditis and perihepatitis in colibacillosis affected broiler chicken

Fig. 2: Enlarged and congested spleen in colibacillosis affected broiler chicken

Group V: The samples were collected from a broiler farm of 3000 birds. They were 27 days old broilers; reluctant to move and were having soiled vent, ruffled feathers and closed eyes. The affected birds sneezed, coughed and were depressed. The lesions were mostly confined to the spleen. The enlarged spleen showed petechial hemorrhages on the surface. The bursa and caecal tonsils were slightly enlarged. Catarrhal exudate was observed in bursa (Fig. 3). In other lymphoid organs no significant changes were observed.

Histopathology

Group I: Normal follicles were observed in bursa (Fig. 4). Congestion and inflammatory changes were observed in spleen but with no significant changes in other lymphoid organs.

Group II: In this group, mild to moderate lesions were observed in spleen which included congestion and necrotic areas (Fig. 5). The other lymphoid organs revealed no significant change.

Fig. 3: Bursa enlarged, oedematous with catarrhal exudate in colibacillosis affected broiler chicken

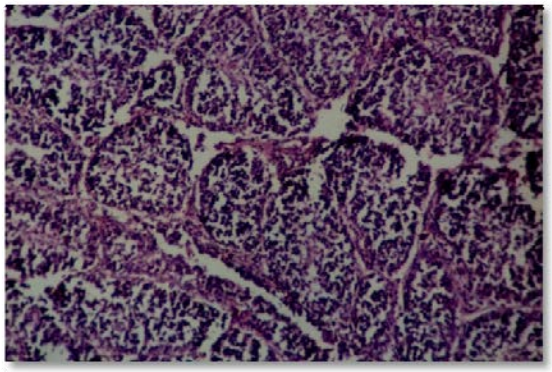

Fig. 4: Section of bursa of fabricius of a broiler chicken affected with colibacillosis revealing normal follicles. HE. X400

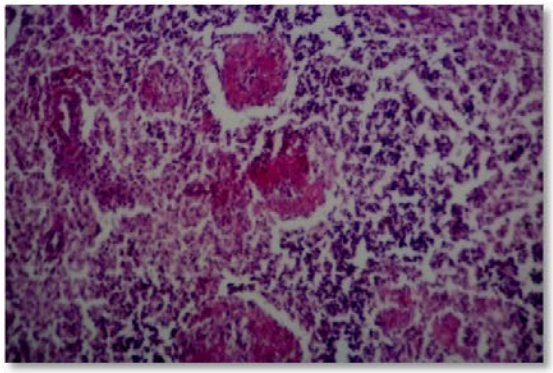

Fig. 5: Section of spleen of a broiler chicken affected with colibacillosis revealing inflammatory and necrotic changes. HE. X400

Group III: Mild lymphoid depletion was observed in bursa (Fig. 6). Medullary congestion and mild cortical depletion

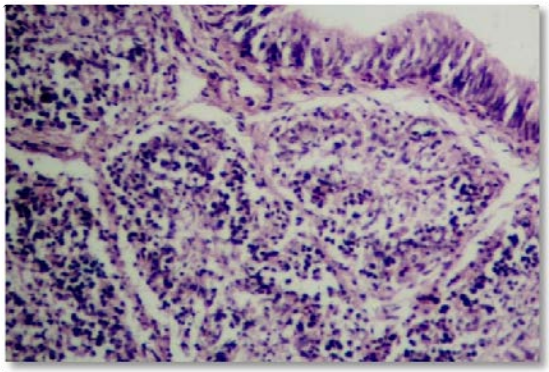

Fig. 6: Section of bursa of fabricius of a broiler chicken affected with colibacillosis revealing mild lymphoid depletion. HE. X800

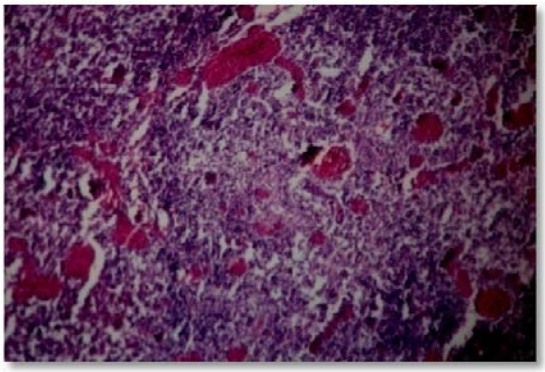

Fig. 7: Section of thymus of a broiler chicken affected with colibacillosis revealing congestion and depletion in cortex. HE. X300

were observed in thymus (Fig. 7). Depletion of lymphocytes and areas of congestion and necrosis were recorded in spleen.

Group IV: In this group, no specific changes were recorded in bursa, thymus and other lymphoid organs. However, congestion, lymphoid depletion, necrotic areas were observed in spleen.

Group V: The spleen and caecal tonsils were most affected in this group. Lymphoid depletion and necrotic patches were observed in caecal tonsils (Fig. 8). However in bursa and thymus, mild depletion was recorded. No significant effect was seen in harderian gland.

Histoenzymatic study: No significant change in enzymatic activity in terms of acid phosphatase and alkaline phosphatase

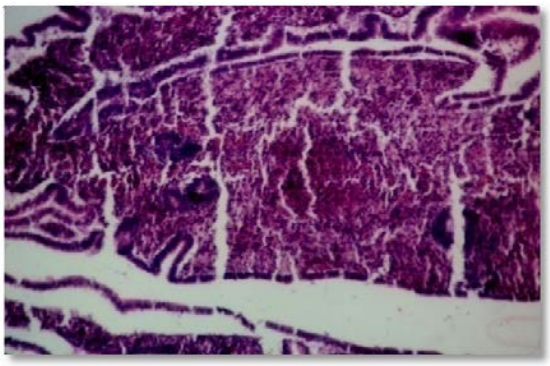

Fig. 8: Section of caecal tonsil of a broiler chicken affected with colibacillosis revealing mild lymphoid depletion and necrotic patches. HE. X400

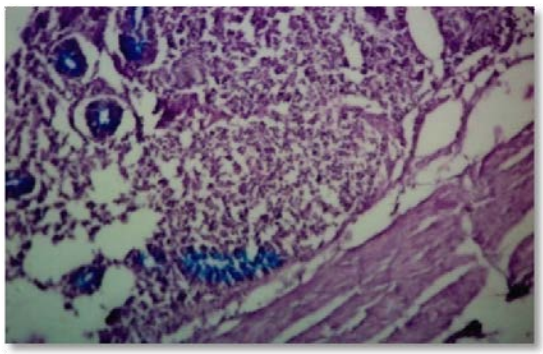

Fig. 9: Section of caecal tonsil of a broiler chicken affected with colibacillosis revealing the glandular epithelium positive for acid mucopolysaccharides. Combined alcian blue PAS. X300

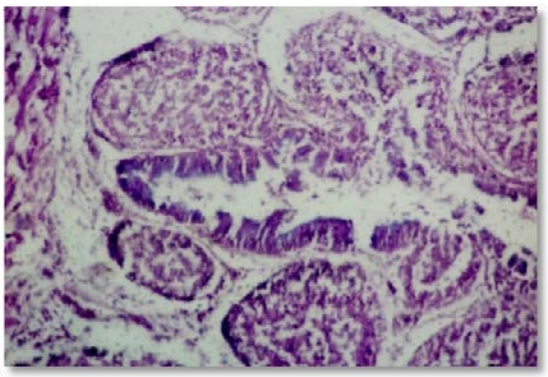

Fig. 10: Section of bursa of fabricius of a broiler chicken affected with colibacillosis revealing the plica epithelium positive for acid mucopolysaccharides. Combined alcian blue PAS. X600

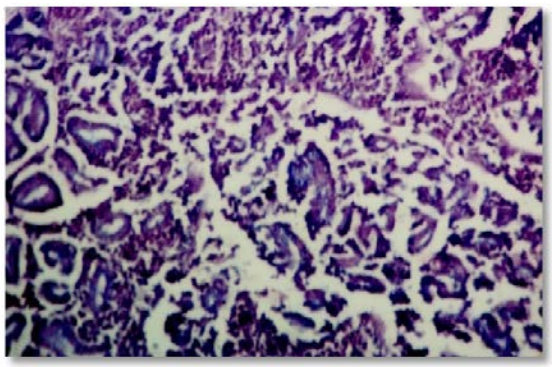

Fig. 11: Section of harderian gland of a broiler chicken affected with colibacillosis revealing the alveolar epithelium positive for acid mucopolysaccharides. Combined alcian blue PAS. X500

was observed in the lymphoid organs, with the exception of very mild enzyme activity in spleen.

Pharmacochemistry: In all the cases of colibacillosis, it was observed that the caecal glandular epithelium (Fig. 9), bursal plica epithelium (Fig. 10) and the alveolar epithelium of harderian gland (Fig. 11) were found to be positive for acid mucopolysaccharides with no signs of acid mucin in any other lymphoid organ.

DISCUSSION

These lymphoid organs are sometimes rendered non-functional or their function is reduced to a minimum level in certain cases of viral, bacterial, fungal and parasitic infections, resulting in reduced resistance of the birds, due to which they become susceptible to other infections causing huge economic losses in terms of morbidity, mortality, treatment and management. Therefore, the rational assessment of the immunosuppression in the poultry warrants rapid and accurate diagnostic approaches. This has remained a challenge and rather a weak link in any conceivable surveillance and control program. The present study therefore, formed a meagre attempt to assess the immunological status of broilers affected with various commonly encountered diseases in Kashmir valley.

The gross lesions were more prominent in spleen which included enlargement and congestion. Further, the bursa was found atrophied. These findings are in accordance with Zanella *et al.*[18], Tonu *et al.*[19] and Kumari and Gupta[20]. No significant gross changes were observed in other lymphoid

organs. Histopathologically, the spleen was the most severely affected organ which revealed depletion of lymphocytes, areas of congestion and necrosis of lymphocytes from the white pulp. The common histological findings in this study like focal necrosis, congestion and lymphocyte depletion in the spleen supported an active role for Chick Lethal Toxin (CLT) in the pathogenesis of *E. coli* disease[21]. In bursa of fabricius, lymphoid depletion observed in follicles was similar to the findings mentioned by earlier authors[22,23]. In thymus, the lesions like medullary congestion and mild cortical depletion were like those reported earlier[21,23]. The possible cause of the lesions in case of *E. coli* infection could be attributed to its virulence factors like adhesions, which play an important role in its colonization in the host[24]. Toxins like cytolethal distending toxin, cytotoxic necrotizing factor, Vacuolating Autotransporter Toxin (VAT) causing cytotoxic effects in cells had been reported by La Ragione and Woodward[25]; Parreira and Gyles[26] and Rodriguez-Siek *et al.*[27]. The ability of iron acquisition mechanisms to obtain iron is important in the pathogenesis of avian colibacillosis[28,29]. Similarly the ability of protectins to resist complement is a common characteristic in the pathogenesis of colibacillosis[30]. The invasins contribute to the invasion of bacteria into the deeper tissues of internal organs and thus play a critical role in the pathogenesis of *E. coli* infection[31]. The lymphoid depletion found in various lymphoid organs in the present study could be the result of toxins liberated by *E. coli*[21].

In the present study, no considerable change in enzyme activity in terms of acid phosphatase and alkaline phosphatase was observed in the lymphoid organs, with the exception of very mild enzyme activity in spleen. Phosphatases are present in a wide variety of animal tissues. They are responsible for the hydrolysis of organic phosphate esters. Alkaline phosphatase exhibits optimal activity at high pH values while acid phosphatase exhibit optimal activity at low pH values[32]. Acid phosphatase, a lysosomal enzyme is usually found within neutrophils and macrophages[33]. The increased acid phosphatase in spleen might have been an outcome of increased neutrophil infiltration. Further, more neutrophilic lysosomal enzymes could have been released on immune complex phagocytosis, by dead neutrophils, by leakage during formation of phagocytic vacuoles and by reverse endocytosis[34]. The alkaline phosphatase belongs to a class of enzymes with restricted tissue distribution. It has been found to catalyzed phosphorylation of adenosine triphosphate (ATP) and plays an important role in the absorption and transportation of ions across the membrane[35,36]. The enzyme is also present in the larger and more numerous specific granules (secondary) of neutrophills[33], which might the reason for increased alkaline phosphatase found in spleen.

Upon pharmacochemical examination, the caecal glandular epithelium, bursa plica epithelium and the alveolar epithelium of harderian gland were positive for acid mucopolysaccharides. Various authors have opined that hypersecretion of mucopolysaccharides in and around the lesions may be attributed to prolonged irritative action of insults. Qualitative increase in both acid and neutral mucopolysaccharides has been attributed to inflammatory process[37,38]. Darzi *et al.*[37] reported that the inflammatory exudates revealed qualitative increase in acid mucopolysaccharides and the basement membrane was positive for neutral mucopolysaccharides in spontaneous hepatic coccidiosis in rabbits and opined that it constitutes a component of local defense reaction. The present study was also supported by Dezfuli *et al.*[39] who found number of mucous cells close to the site of parasite attachment within the intestine significantly higher than the number detected in uninfected individuals and in infected individuals at sites 1 cm or greater from the point of parasite attachment staining positively for acid glycoconjugates. Increased production of mucopolysaccharides might also be attributed to their probable role in the inflammation and prolonged irritative action of different insults, which are believed to determine hypersecretion of these substances[40].

CONCLUSION

In conclusion, the marked pathological changes were observed in various lymphoid organs of broiler chickens affected with colibacillosis. The histoenzymatic and pharmacochemical methods were found to be helpful in the diagnosis of the disease. Further, extensive systematic studies need to be made on immunosuppression in avian species to determine the factors acting as immunosuppressive agents. Moreover, the immunodiagnostics need to be developed further for early diagnosis of the diseases in order to cut down the economic losses incurred as a result of colibacillosis in poultry production.

SIGNIFICANT STATEMENTS

The colibacillosis is one of the important diseases of broiler chicken in terms of the economic losses incurred. Its main effect is related to the immunosuppression in the birds which in turn make them susceptible to other secondary infections, thereby multiplying to the miseries of the owner. If

this disease is diagnosed early in terms of new laboratory techniques, it would certainly help a lot in preventing the economic losses a farmer suffers. Since there is lack of literature regarding histoenzymatic and pharmacochemical diagnosis of colibacillosis, the present study was conducted. The results of the present study evaluated carcasses in terms of histoenzymatic and pharmacochemistry, thus adding to the already known diagnostic methods regarding colibacillosis. The present study thus would be helpful for future researchers in combating the ill-effects of lethal colibacillosis disease in broiler chickens.

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