

**Etiological and histomorphological studies on enteritis in broiler chickens in
Kashmir, India**

Enteritis etiology and histomorphology in broiler chickens in India

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Etiological and histomorphological studies on enteritis in broiler chickens in Kashmir, India

Abstract

Enteric disorders comprise the most important groups of diseases affecting poultry birds and continue to be a major cause of high economic losses in many areas throughout the world, causing increased mortality rates, decreased weight gain, and increased feed conversion rates. This study was conducted to determine the prevalence and pathological investigations of various diseases affecting the intestines of poultry in the central part of the Kashmir Valley. 3 024 broiler chickens from 100 outbreaks of enteritis were necropsied and examined for the presence of lesions corresponding to different disease conditions. Various diseases were diagnosed based on history, clinical signs, gross pathology, and isolation of specific pathogens from the affected birds' organs. The study revealed 45.69 % of enteritis associated with various specific and nonspecific disease conditions. The highest prevalence was associated with mixed infections (12.4 %), followed by colibacillosis (10.78 %), coccidiosis (8.23 %), salmonellosis (7.8 %), New Castle disease (3.34 %), nonspecific causes (1.92 %), and necrotic enteritis (1.22 %). The intestines showed gross changes including congestion, thickened walls, mucosal hemorrhages, ballooning, and a velvety appearance of the mucosa. Microscopically the intestines showed mucosal sloughing and necrosis, mononuclear cell infiltration, tunica muscularis thickening, and goblet cell hyperplasia.

Keywords: Enteritis; Pathomorphology; Broiler; Colibacillosis; Coccidiosis.

Study contribution

This study makes a valuable contribution to the field of veterinary pathology and poultry health management by offering a detailed examination of the prevalence and pathological features of enteric disorders in broiler chickens in the Kashmir Valley. By identifying the high occurrence of these disorders, the research provides crucial epidemiological data that can guide more effective disease control strategies. The study's comprehensive description of both gross and microscopic intestinal lesions offers practical insights for veterinarians and researchers, enhancing the ability to diagnose and manage these diseases more accurately. The economic impact of these enteric diseases, reflected in increased mortality, reduced weight gain, and higher feed conversion rates, underscores the significant financial losses they cause to the poultry industry. This research also lays the groundwork for future studies on enteric disorders in the region, helping track disease trends and develop more efficient management practices to improve poultry health and productivity.

Introduction

The profitability of the global poultry industry, which has expanded into a multibillion-dollar sector, depends on a healthy gastrointestinal system as it leads to efficient feed conversions.⁽¹⁾ Enteric disorders are the most important groups of diseases affecting poultry birds, and they continue to be a major cause of high economic losses in many areas throughout the world, causing increased mortality rates, decreased weight gain, and increased feed conversion rates.⁽²⁾

The intestinal tract of chickens is heavily colonized with microbes, which directly interrelate with the host.⁽³⁾ There seems to be a correlation between animal performance and a “healthy” gut of animal.⁽⁴⁾ The basic purpose of the healthy gut is to modify physical homeostasis that delivers the host capability to resist pathogenic

stimuli.⁽⁵⁾ By maintaining the intestinal homeostasis, a healthy intestine also controls other systems of body as well that enable the animal to resist pathogenic stimuli.⁽⁶⁾

A variety of enteric bacterial diseases are recognized in poultry. Three of these bacterial diseases, necrotic enteritis, ulcerative enteritis, and spirochetosis, primarily infect the intestine, whereas other bacterial diseases, such as salmonellosis, colibacillosis, mycobacteriosis, erysipelas, and fowl cholera, affect a variety of organ systems in addition to the intestine.⁽⁷⁾ Besides bacterial diseases, coccidiosis is among the most common diseases of poultry caused by the protozoan parasite of *Eimeria* species that primarily affects the intestines of the birds. Coccidiosis is regarded as one of the domestic poultry illnesses with the greatest economic impact, causing enormous amounts of financial losses to the poultry industry throughout the world.⁽⁸⁾

The different viruses that affect the intestines include fowl adenovirus-I, chicken parvovirus, avian coronavirus, and chicken astroviruses. The combinations of two or more enteric viruses can also affect the intestines simultaneously.⁽⁹⁾ Mortality due to various enteritis-causing diseases poses a great economic challenge to poultry farmers and is a matter of great concern. To date, no systematic study has been undertaken to address mortality due to enteritis and its causative factors in the temperate areas of the Kashmir region. This study was undertaken to study the occurrence of enteritis, correlate enteritis in chickens with various etiological agents, and study the gross and histopathological changes in intestines affected by enteritis.

Materials and methods

Samples

A total of 100 outbreaks of enteritis were screened and correlated with various etiological agents based on isolation, history, clinical signs, and lesions, following a thorough postmortem examination of birds. A total of 3 024 broiler chickens were

necropsied and examined for the presence and nature of intestinal lesions. The carcasses were received from different private and government poultry farms of district Srinagar and its adjoining areas and were brought to the Division of Veterinary Pathology for postmortem examination.

Occurrence

The occurrence of enteritis was evaluated according to the number of birds that died due to the condition affecting the intestines out of the total number of birds in the flock. Case prevalence reflects the proportion of birds that died due to the condition out of total mortality in the flock.

Isolation and identification

Escherichia coli

Representative samples (heart, spleen, lung, liver, ceca, bursa, etc.) were inoculated into nutrient broth and incubated at 37 °C for 24 h. Bacterial growth in the nutrient broth was reinoculated on MacConkey agar plates (HI Media, Mumbai, India) and incubated at 37 °C for 24 h. Lactose-fermenting colonies on MacConkey plates were reinoculated on eosin methylene blue agar (HI Media, Mumbai, India). *Escherichia coli* colonies typically showing metallic sheen were transferred to nutrient agar slants and stored at 4 °C for further characterization. The identification of isolates was further carried out using standard morphological and biochemical tests, including Gram staining, Indole, Methyl Red, Voges–Proskauer, Citrate (IMViC) tests, and Triple Sugar Iron (TSI) agar test, and the characterization of Enterobacteriaceae was performed as described earlier.^(10, 11)

Salmonella

The representative samples from salmonellosis-affected chickens were inoculated into tetrathionate broth and incubated overnight. From the enrichment media, the cultures were streaked on Brilliant Green Agar (BGA) to produce small pink circular colonies of salmonella. Gram staining was performed on pure culture of BGA, and gram-negative, rod-shaped organisms were observed under a compound light microscope. Pink colonies identified as gram-negative rods were inoculated on Xylose lysine desoxycholate agar (XLDA) and MacConkey agar plates. On XLDA, the salmonella isolates produced red colonies with black centers and colorless colonies were produced on MacConkey agar. Isolates were identified using standard morphological and biochemical tests, including Gram staining, IMViC tests, and TSI test, as described earlier.⁽¹²⁾

Parasitological examination

The gut of the birds suspected of coccidiosis was collected and cut into different portions (small and large intestines) with the help of a sharp scissor, and the gut contents of respective portions were examined microscopically to detect the presence of coccidial oocysts as described earlier.⁽¹³⁾ For the non-infectious and viral diseases, the tentative diagnosis was made on gross and microscopic lesions found in intestines.⁽¹⁴⁾

Pathoanatomical studies

The carcasses were subjected to systematic necropsy examination. Based on gross lesions, the disease conditions were classified into various categories. Organs were thoroughly examined based on clinical conditions and gross pathological examination. Various organs were collected in 10 % formalin depending upon disease conditions to prevent autolytic changes. Tissue samples from various organs, especially the

intestines, were collected in 10 % formalin for histopathological examination and processed using the paraffin embedding technique. The sections were stained using the Harris' hematoxylin and eosin technique for routine examination.⁽¹⁵⁾

Results

Enteritis occurrence in broiler chickens

Gross intestinal lesions in broiler chicken carcasses due to various disease conditions are presented in **Table 1**. Necropsy was conducted on 3 024 broiler carcasses from 100 outbreaks. Gross intestinal lesions were observed in 1 382 birds, with a prevalence of 45.69 %. Colibacillosis was diagnosed in 326 out of 3 024 necropsied birds, giving an overall occurrence rate of 10.78 %. Salmonellosis was recorded in a total of 236 cases out of 3 024 necropsied carcasses, giving an overall occurrence of 7.8 % (236/3 024). Coccidiosis was recorded in a total of 249 cases out of 3 024 necropsied carcasses, giving an overall occurrence of 8.23 %. Newcastle disease was found in 243 cases out of 3 024 necropsied birds, giving an overall occurrence of 3.34 % (101/3 024). Mixed infections were recorded in 375 cases out of 3 024 necropsied birds, giving an occurrence rate of 12.4 %. Enteritis due to nonspecific conditions was recorded in 58 cases, giving an occurrence rate of 1.92 % (58/3 024). Necrotic enteritis was diagnosed in 68 of the 3 024 necropsied carcasses, giving an overall occurrence rate of 1.22 %.

Table 1. Enteritis occurrence due to various disease conditions

Disease condition	Outbreaks	Flock size	Mortality		Occurrence of enteritis		Proportionate distribution
			No	%	No	%	
Colibacillosis	20	21 512	857	3.98	326	10.78	23.59
Salmonellosis	17	18 218	383	2.10	236	7.80	17.08
Coccidiosis	21	18 551	278	1.50	249	8.23	18.02
Newcastle disease	10	9 294	243	2.61	101	3.34	7.31
Necrotic enteritis	03	4 561	68	1.49	37	1.22	2.68
Mixed infections	25	27 106	1 081	3.99	375	12.40	27.13
Non specific	04	5 960	114	1.91	58	1.92	4.19
Total	100	105 202	3 024	17.58	1 382	45.69	

Colibacillosis was diagnosed in 326 out of 3 024 necropsied birds, giving an overall occurrence rate of 10.78 %. The cases of *E. coli* infection causing enteritis were reported in the age group of 15–21 days (3.9 %) followed by 8–14 days (2.58 %) then 22–28 days (2.08 %) then 0–7 days (1.26 %), and > 29 days (0.96 %). Salmonellosis was recorded in a total of 236 cases out of 3 024 necropsied carcasses, giving an overall occurrence of 7.8 % (236/3 024). The infection mostly occurred in 0–7 days old birds (3.57 %) followed by 8–14 days old birds (2.74 %). Newcastle disease was found in 101 of the 3 024 necropsied birds, giving an overall occurrence of 3.34 % (101/3 024). Newcastle disease was mostly found in the age group of > 29 days

(1.29 %) followed by 22–28 days old birds (1.12 %) and then 15–21 days' age group (0.89 %). Coccidiosis was recorded in a total of 249 cases out of 3 024 necropsied carcasses, giving an overall occurrence of 8.23 %. Coccidiosis was mainly found in the age group 22–28 days old birds (3.17 %) followed by 15–21 days old (2.74 %), > 29-day-old group (1.72 %) and 8–14 days of age (0.56 %).

Nonspecific cases of enteritis were mostly reported in the age group of 22–28 days (1.03 %), followed by the age group of > 29 days (0.49 %) and 15–21 days (0.4 %). The nonspecific cases could have been due to different undetected and unidentified viral, bacterial, and other etiological agents. Necrotic enteritis was diagnosed in 68 of the 3 024 necropsied carcasses, giving an overall occurrence rate of 1.22 %. The disease was found mostly in 22–28 days age group (0.73 %), followed by the > 29-day age group (0.49 %).

Mixed infections were recorded in 375 cases out of 3 024 necropsied birds, giving an occurrence rate of 12.4 %. The mixed infections included two or more of the mentioned disease conditions as colibacillosis, salmonellosis, coccidiosis, New Castle disease, and other nonspecific enteritis conditions. Mixed infections were mostly reported in 15–21 days (3.11 %) followed by 22–28 days (2.91 %), then in 8–14 days (2.54 %), > 29 days (2.34 %) and in 0–7 days age group (1.51 %).

Pathomorphological studies

Colibacillosis

The intestines of *E. coli*-affected birds showed gross congestion and fibrin clot deposition throughout in some cases, particularly in birds aged > 1 week. Birds with enteritis had severe congestion and thickening of the intestinal wall (**Figure 1a**). In some birds, the mucosal surface was severely congested, velvety in appearance, and large amounts of catarrhal exudates were present. Congestion and severe

engorgement of mesenteric blood vessels were also observed in affected birds (**Figure 1b**).

Microscopically, the duodenum was characterized by hemorrhages in the mucosa, infiltration of heterophils and lymphocytes (**Figure 1c**), thickening of the tunica muscularis (**Figure 1d**), and necrosis and sloughing of the villi (**Figure 1e**). The jejunum showed sloughing of the mucosa (**Figure 1f**), infiltration of heterophils, necrosis of villi (**Figure 1g**), goblet cell hyperplasia (**Figure 1h**), and hyperplasia of the mucosal epithelium (**Figures 1i and J**).

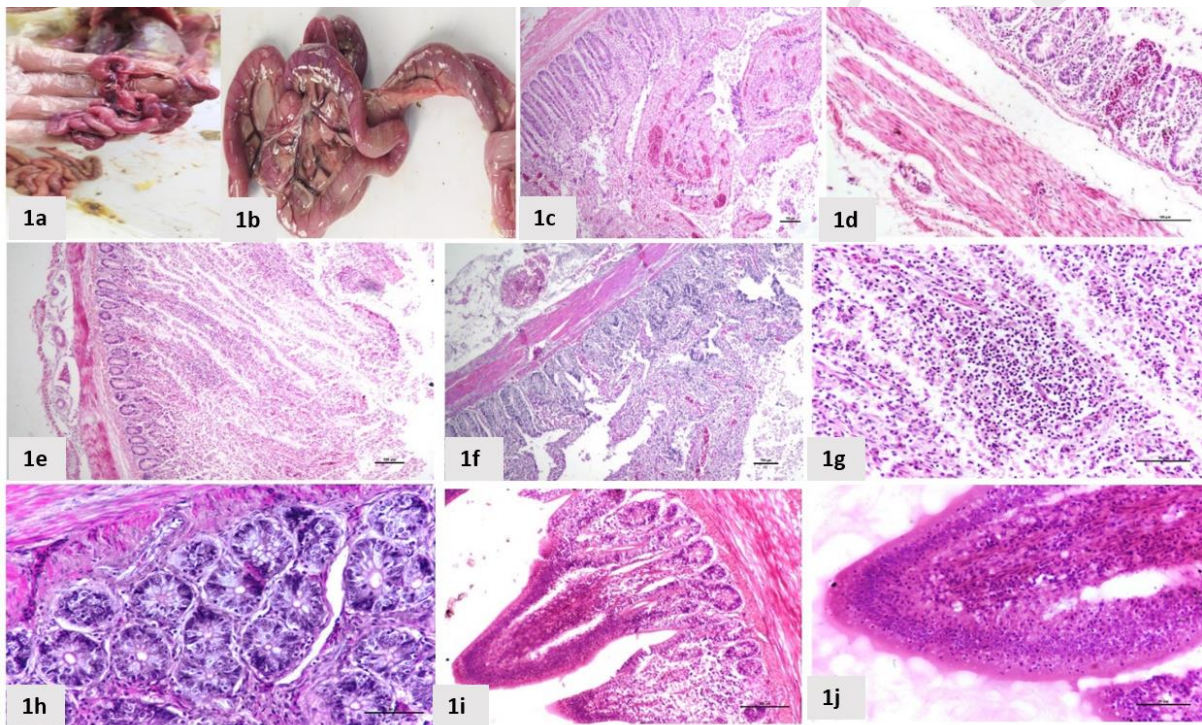


Figure 1. Intestines revealing gross and histopathological changes in colibacillosis. a) Small intestine with thick wall. b) Intestine showing congestion of mesenteric blood vessels. c) Duodenal section revealing hemorrhages, and d) Infiltration in the mucosa, thickened tunica muscularis. e) Necrosis and sloughing of villi. f) Jejunum showing mucosal sloughing, g) Mucosal infiltration, h) Goblet cell hyperplasia, and i, j) Mucosal epithelium hyperplasia. H&E.

Salmonellosis

Grossly intestines revealed severe congestion and hemorrhages on the mucosal surface of different segments (**Figure 2a**). The lesions in the ceca were more prominent than those in other segments, and these were inflamed and swollen. In a few chronic cases, the cheesy, dry, and necrotic material was observed in the lumen.

Microscopic examination of the duodenum revealed desquamation and degeneration of epithelial cell lining (**Figure 2b**). The jejunum revealed severe congestion and hemorrhages in the mucosa, submucosa, and even tunica muscularis (**Figure 2c**), thickened muscularis, hemorrhages and severe infiltration of heterophils in the mucosa, and disruption of the mucosa followed by fragmentation of villi. The ileum and jejunum revealed congestion and hemorrhage with degeneration and desquamation of the epithelium lining (**Figure 2d**) and disruption and necrosis of villi (**Figure 2e**) along with mononuclear cell infiltration (**Figure 2f**).

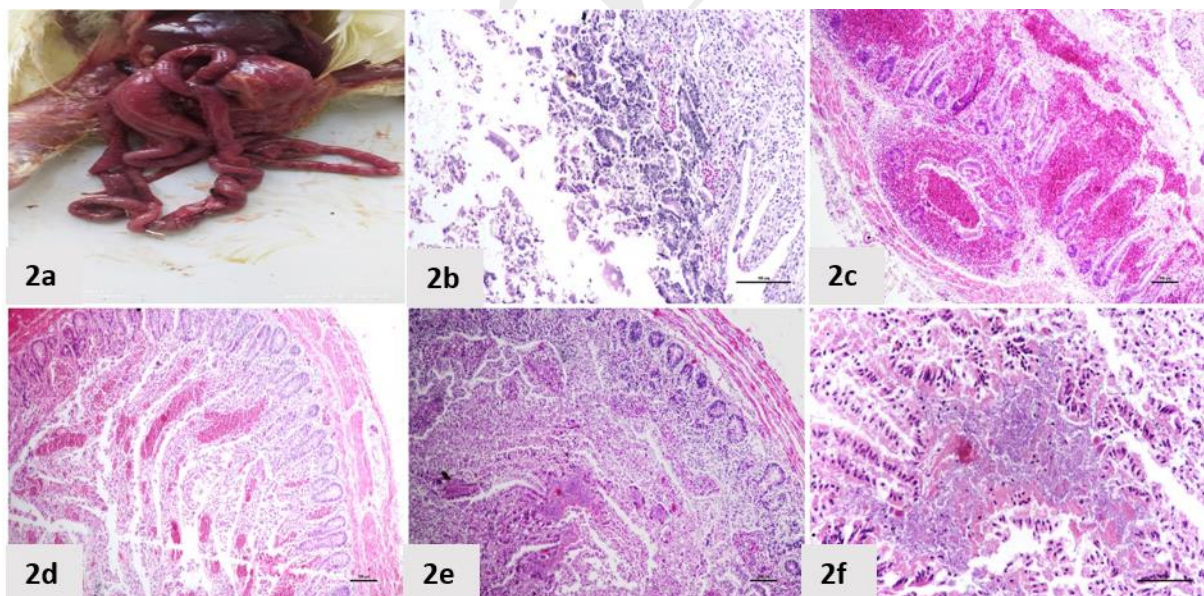


Figure 2. Intestines revealing gross and histopathological changes in salmonellosis.

a) Severely congested intestine. b) Duodenal section showing degeneration and desquamation of the mucosal epithelium. c) Ileal section showing severe vascular congestion and hemorrhage. d) section of jejunum revealing hemorrhages in the

mucosa e) Jejunal section revealing disruption and necrosis of villi. f) Jejunal section showing necrosis more clearly. H&E.

Coccidiosis

Grossly, the mucosa of the intestines was opaque and thickened with raised grayish-white necrotic areas. In the duodenum, the serosa was pale and contained watery fluid that turned purplish in severe cases. In mild infections, the gross lesions were limited to the duodenal loop, whereas in cases of heavy infections, the lesions extended to some distance through the small intestines and the plaques overlapped or coalesced. The jejunum was ballooned, the mucosa thickened, and the lumen was filled with blood, fluid, and tissue debris. The ceca were dark brown to reddish in color due to the presence of hemorrhages. The ceca were greatly enlarged, and the contents were in a consolidated form resembling a dark brown to blackish caseous mass containing feces (**Figures 3a** and **3b**). The underlying mucosa was congested. The mucosa of the lower small intestine was covered with small hemorrhages with some thickening and discoloration. The intestinal lumen was filled with coagulated blood and mucosal casts. Severe infections revealed edema, mucosal thickening, and petechial hemorrhage. The intestinal was filled with large quantities of sloughed necrotic material.

Histopathological examination of the intestines revealed the destruction of a larger number of epithelial cells due to the development of asexual stages of schizonts. The duodenum of diseased birds revealed marked congestion of blood vessels, mild to moderate infiltration of heterophils in the lamina propria of intestinal villi, and hemorrhages at the tips of the villi (**Figure 3c**). Sections of the ileum revealed sloughing of the mucosa (**Figure 3g**) and cellular infiltration with the presence of

numerous schizonts in the glandular epithelium and mucosa (**Figure 3d**). Developing schizonts in the glandular epithelium caused gland destruction and necrosis (**Figures 3e, f, h**). The ceca revealed loss of epithelial tissue, blood vessel congestion followed by disruption and hemorrhage, severe edema of muscularis layer and necrotic sub-mucosa and the lumen filled with hemorrhagic cecal core (**Figure 3i**). The cecal lumen was filled with a characteristic cecal core composed of disintegrated epithelium, cellular debris, fecal material, blood, and *Eimeria tenella* oocysts.

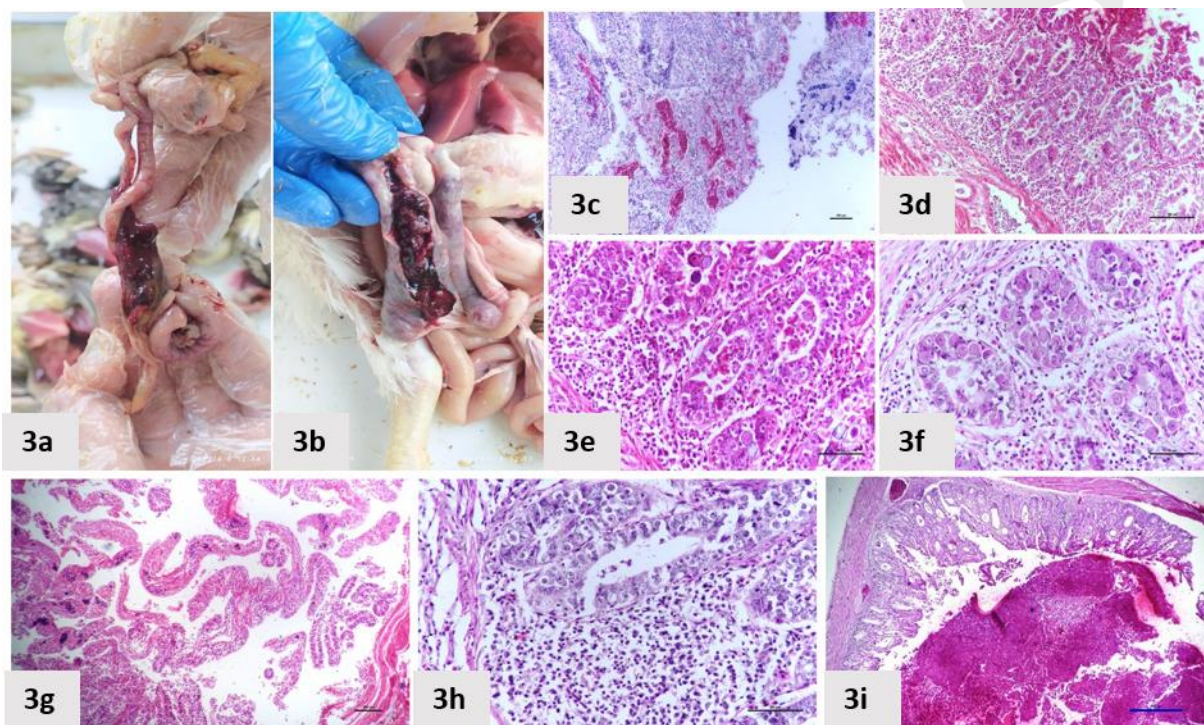


Figure 3. Intestines revealing gross and histopathological changes in coccidiosis. a) Cecum showing reddish contents. b) Enlarged cecum with dark-brown to blackish caseous mass-like contents. c) Duodenal section showing hemorrhages and matting of villi. d) Cecum section revealing thickened muscularis, schizonts in the glandular epithelium, and necrosis of glands. e, f) Destruction of glandular epithelium by developing schizonts. g) Ileum revealing sloughing of mucosa. h) Cecum revealing necrosis of glandular epithelium by developing schizonts and severe cellular infiltration. i) Lumen of cecum filled with hemorrhagic cecal core.

Newcastle disease

Grossly, multiple foci of necrosis were observed in the small intestine and cecal tonsils, revealing edema, necrosis, and hemorrhage. Multifocal hemorrhages were evident throughout the serosal surface. Hemorrhagic and necrotic lesions were also observed in the cecal tonsils, Peyer's patches, and the duodenum. "Button-like ulcers" consisting of dark red hemorrhages covered with yellow to brown or green fibrinous necrotic material involving the Peyer's patches were seen in the duodenum (**Figure 4a**).

Severe necrosis of the lymphoid-dependent areas was associated with focal to local extensive ulceration of the epithelium and accumulation of necrotic material within the intestinal lumen. The duodenum revealed severe congestion and hemorrhage of the mucosa (**Figure 4b**) and severe necrosis along with heterophil infiltration (**Figure 4c**). The jejunum was characterized by gut-associated lymphoid tissue ulceration (**Figure 4d**), villi disruption and necrosis (**Figure 4e**), and mucosal hemorrhage and sloughing (**Figure 4f**).

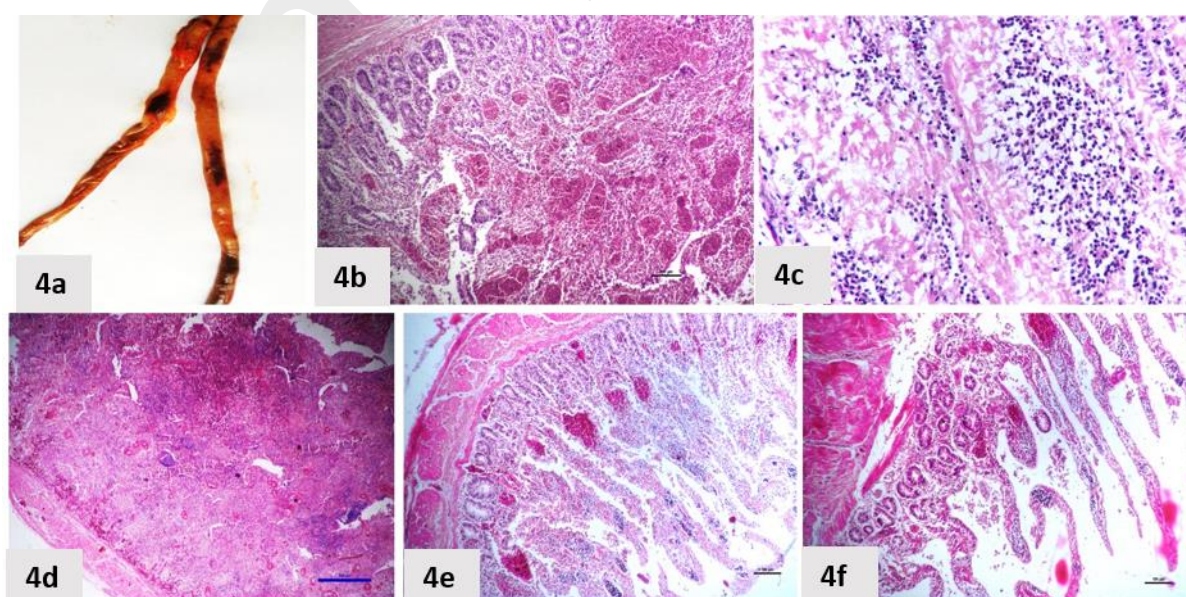


Figure 4. Gross and histopathological changes in the intestines of patients with Newcastle disease. a) Intestine showing characteristic ulcers. b) Duodenal section revealing hemorrhage and mucosal congestion, severe necrosis, and c) Mucosal infiltration. d) Intestinal section revealing ulcer formation. e) Jejunal section revealing heterophilic infiltration, villi necrosis, hemorrhage, mucosal sloughing, and f) Congestion.

Nonspecific conditions

In nonspecific cases of enteritis, the congestion typically involved more than one section of the intestine and was frequently accompanied by other lesions in one or more segments of the tract. Congestion differed in intensity, affecting a section partly or fully, with mucosa looking light to bright or dark pink in coloration. Congestion was obvious from the serosal surface of the intestines, and the mesenteric vessels were variably distended with blood (**Figures 5a** and **b**). The luminal fluids differed in consistency and were typical, watery, or mucoid in nature. Hemorrhages were detected in one or more segments of the intestine, involving a partially or fully enlarged section. Microscopically, congestion and hemorrhages, degeneration, and desquamation of the epithelial cell lining were observed (**Figures 5c** and **d**). The section of the intestines showed diffused hemorrhages, particularly in the duodenum and jejunum (**Figure 5e**). Severely affected cases showed necrosis of the mucosal layer and desquamation and disruption of villi (**Figure 5f**).

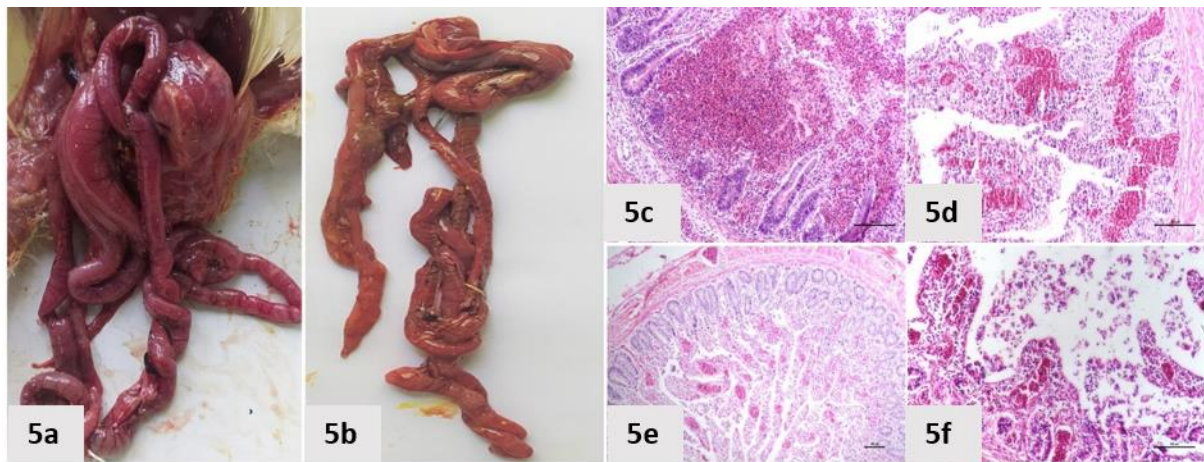


Figure 5. Gross and histopathological changes in intestines in nonspecific enteritis. a) Markedly congested intestine. b) Partially necrosed intestine. c) Section of intestine revealing very severe hemorrhage in mucosal layers. d) Section of intestine revealing hemorrhage in mucosa, necrosis, and heterophilic infiltration. e) Section of intestine showing diffused hemorrhage. f) Section of intestine revealing necrosis of mucosal layer, hemorrhages, and heterophilic infiltration.

Discussion

The gastrointestinal (GI) tract has the highest exposed surface area in the body and is continually exposed to various potentially hazardous chemicals. The GI tract serves as a selective barrier between the tissues of birds and their luminal environment. This barrier comprises physical, chemical, immunological, and microbiological components. Various variables linked with nutrition and infectious disease agents can negatively alter the delicate balance among the components of the chicken intestine, affecting the health condition and production performance of birds in commercial poultry operations.

The present study revealed that 10.78 % of patients had enteritis due to colibacillosis. Earlier studies also reported a mortality rate of 11.34 % due to

colibacillosis that was responsible for causing enteritis as 11.34 per cent in Parbhani, Maharashtra.⁽¹⁶⁾ However, a higher occurrence of the disease was reported earlier in some studies⁽¹⁷⁻¹⁹⁾ and lower in other studies.⁽²⁰⁾ Salmonellosis accounted for 7.8 % of all cases. These findings are similar with earlier findings.^(21, 22) In the present study, 8.23 % of patients had enteritis due to coccidiosis. These results are in agreement with earlier studies⁽²³⁾ who also reported a similar prevalence of the disease; however, a higher occurrence of this disease has also been reported by others.^(24, 25) The low occurrence of coccidiosis cases was due to the extraneous prophylactic and therapeutic use of anticoccidial drugs.

The present investigation revealed that the occurrence rate of Newcastle disease was 3.34 %. However, other studies^(26, 27) recorded a mortality rate of 8.1 % and 35 % in patients with Newcastle disease in Iraq and India, respectively. In the present study, the prevalence of non-specific enteritis was 1.92 %. These findings were similar with other studies⁽¹²⁾ who reported a case prevalence of non-specific enteritis of 1.027 %. The gross and histopathological lesions observed in the intestines of patients with colibacillosis were in concordance with earlier reports.⁽²⁸⁻³⁰⁾ The pathological findings in the intestines of patients with salmonellosis were in full agreement with the findings of other authors.^(22, 31) Similar gross and histopathological lesions in patients with New Castle disease were reported by other authors.⁽³²⁾ The gross and microscopic lesions in the intestines of patients with coccidiosis are in agreement with the findings of earlier studies.⁽³³⁾

Data availability

All data are provided in the manuscript.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contribution

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Project administration: SA Shah and MS Mir.

Resources: SA Shah and SA Kamil.

Writing-original draft: SA Shah and AB Kouchey.

Writing-review and editing: AB Beigh, ZA Wani, and KV Reddy.

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