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Pathological attributes of naturally occurring salmonellosis in broilers reared in Ganderbal district of Kashmir: A series of case study

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Abstract

A survey on naturally occurring cases of Salmonellosis was carried out in various commercial broiler farms of Ganderbal District of Kashmir. During this period, 10 outbreaks were documented with clinical signs commonly observed included ruffled feathers, crowding near heat or light sources, loss of appetite, increased water intake, reluctance to move, pasting of the vent, diarrhoea, prostration, stunted growth and occasionally lameness. The gross pathological changes were characterized by hepatomegaly with bronze discoloration, splenomegaly, congestion and necrotic foci in the liver with greyish-white nodules on the heart. The Pulmonary lesions include congestion, haemorrhage and oedematous changes while the bursa of Fabricius was atrophied and the caeca contained firm caecal cores. The histopathological alterations were mainly observed in the liver, spleen, heart, kidney and bursa. The liver and spleen showed congestion, haemorrhages, necrotic areas, reticuloendothelial hyperplasia and infiltration by mononuclear cells and heterophils. The heart tissue section revealed congestion, infiltration of inflammatory cells, muscle fibre atrophy and necrosis. Both the spleen and bursa exhibited lymphocyte depletion. The intestinal changes were marked by congestion of mucosal vessels, goblet cell hyperplasia and infiltration of heterophils and mononuclear cells in the lamina propria of villi.

Keywords: Salmonellosis, broiler, chicken, gross pathology, histopathology

Introduction

The Indian poultry industry is one of the largest and fastest-growing in the world, ranking third in egg production and fourth in chicken meat production globally (FAO, 2023). The rapid modernization of poultry farming systems and the globalization of breeding stock have facilitated the transboundary spread of pathogens, including food-borne bacteria such as *Salmonella* (Chakraborty *et al.*, 2020) [8]. Despite its substantial contribution to the national economy, poultry production in India is hindered by several infectious diseases, among which *Salmonella* infection is one of the most significant, causing severe economic losses through mortality, reduced growth and lowered productivity (Begum *et al.*, 2021) [7].

Salmonella infection in poultry remains one of the most widespread foodborne pathogens worldwide, associated with diarrhea, fever, vomiting and in severe cases, death. The World Health Organization has estimated that Salmonella infection accounts for approximately 78 million foodborne illnesses annually, resulting in over 59,000 deaths (WHO, 2018). In India, reports of salmonellosis remain relatively limited, largely due to inadequate field-level diagnostic facilities and underreporting (Balasubramanian et al., 2021) [3]. This infection in poultry also pose a public health risk, as mishandling and contamination of poultry products can lead to human outbreaks (Akter et al., 2020) [1].

Avian salmonellosis represents a group of acute or chronic diseases caused by one or more members of genus *Salmonella* (Lutful Kabir *et al.*, 2010) ^[19]. The two major host-adapted, non-motile serovars *Salmonella enterica* subsp. *enterica* serovar Gallinarum and *S. enterica* subsp. *enterica* serovar Pullorum are of particular concern, being responsible for fowl typhoid and pullorum disease, respectively (Barrow *et al.*, 2011) ^[5].

These diseases are associated with high morbidity and mortality in poultry, frequently resulting in substantial economic losses. Although cases of *S. Gallinarum* passing to humans are rarely reported, *Salmonella* infections in general continue to present a serious challenge to food safety and public health (Antunes *et al.*, 2016) ^[1]. In India, *Salmonella* infection continues to be a leading cause of mortality and morbidity in commercial poultry flocks, contributing heavily to financial losses.

Keeping in view the paucity of information regarding these challenges, this study was undertaken to investigate the pathology of natural outbreaks of *Salmonella* infections in commercial broiler farms of Ganderbal District of Kashmir with the aim of developing effective therapeutic and preventive strategies for future disease control.

Materials and Methods

This study was undertaken at the Division of Veterinary Pathology, SKUAST-Kashmir, with the objective of studying the pathology associated with salmonellosis in broiler chickens. During the study period, regular visits were conducted to both organized and unorganized poultry farms in order to monitor and document the patterns of morbidity and mortality. A total of 100 broiler chickens were included in the study. During this study period, 10 outbreaks were documented with clinical signs and pathological lesions. Among these outbreaks, a total of 100 broiler chickens were screened out of which 40 live birds exhibiting typical clinical manifestations of salmonellosis such as ruffled feathers, depression, reduced feed intake, diarrhea and prostration which were selected for clinical and laboratory investigation. In addition, 30 dead birds from affected flocks were submitted to the Division of veterinary pathology for detailed postmortem examination and constituted the primary study group along with the clinically affected live birds. The diagnosis of salmonellosis in these cases was confirmed on the basis of characteristic gross pathological lesions and histopathological alterations observed in various organs. A group of 30 apparently normal broiler chickens, free from disease symptoms and gross lesions, was kept as the non-infected controls. These birds provided a baseline for assessing pathological changes associated with the disease. During necropsy, special attention was given to the liver, spleen, bursa of Fabricius, thymus, kidneys, and intestines, where gross pathological changes were carefully observed and recorded in detail. From the confirmed cases, 15 representative tissue samples from each of the aforementioned organs were collected, particularly those showing distinctive salmonellosis-related lesions such as hepatomegaly, necrotic foci, splenomegaly, or bursal atrophy. The collected tissues were preserved in 10% neutral buffered formalin to ensure proper fixation and maintenance of cellular architecture. Following fixation, samples were processed by the routine paraffin embedding technique, sectioned at 4-5 µm thickness and subsequently stained with hematoxylin and eosin (Bancroft et al., 2014) [4]. These prepared histological slides were examined microscopically for detailed evaluation of cellular and tissue-level changes associated with salmonellosis.

Results

The clinical picture in broilers affected by spontaneous outbreaks of salmonellosis was highly variable, making it difficult to establish firm diagnostic conclusions solely on the basis of clinical observation. The disease course varied between flocks and even among individual birds within the same outbreak. The affected broilers displayed nonspecific signs of illness, including dullness, pronounced depression, anorexia and listlessness. Many birds preferred to remain inactive and motionless, often adopting a characteristic posture with the head drooped against the chest and eyes closed, suggesting systemic weakness and discomfort. As the infection progressed, a significant proportion of the chicks developed progressive debility, accompanied by complete in appetence, pronounced thirst and drooping of wings. The plumage appeared rough and ruffled, reflecting poor body condition and reduced grooming activity. The most prominent and consistent clinical feature noted in acute cases was the occurrence of watery, greenish-yellow diarrhea, which frequently soiled the feathers around the vent region. In some outbreaks, lameness was also recorded and in a limited number of cases, birds exhibited mild respiratory distress characterized by laboured breathing and occasional gasping. The severity of clinical signs appeared to correlate with the age of the broilers. Birds above 25 days of age generally manifested less severe illness, possibly due to improved resilience or partial immune development, whereas younger chicks showed more pronounced and fatal disease progression. In some cases, mortality often occurred suddenly and without any obvious warning signs, emphasizing the aggressive nature of the disease. In more typical outbreaks, affected broilers exhibited a recognizable set of signs, including severe depression, half-closed eyes, ruffled plumage, fecal-soiled vent feathers and persistent watery diarrhea. In some of the young chicks, failure of yolk absorption was also observed, further contributing to weakness and higher mortality. Thus, the clinical manifestations of salmonellosis in broilers provided auxiliary evidence, but their variability demanded confirmatory gross and histopathological evaluation.

Gross Pathology

The gross lesions in the infected birds may include bronze discoloration of the liver which was considered the most consistent and diagnostic feature (Fig:1). This distinctive lesion was particularly evident in chicks aged 7-15 days, suggesting a predilection of the disease for younger birds. On gross examination, the livers often revealed a mottled appearance due to the presence of multiple grevish-white necrotic foci interspersed with reddish hemorrhagic areas, which were evenly distributed across the surface (Fig:2). These lesions indicated both hepatocellular necrosis and vascular damage within the organ. The affected chicks that succumbed at an even earlier age often displayed pale, enlarged and congested livers, accompanied by gall bladder distension. In several cases, diffuse areas of necrosis were also observed, pointing toward severe hepatic dysfunction and systemic toxemia. Grossly, heart revealed congestion and thickening of the pericardium, suggestive of pericarditis. In a proportion of cases, especially in chicks older than two weeks, the myocardium exhibited small, raised, greyish-white nodular lesions, most prominent in the ventricular region. These lesions corresponded to focal necrosis and infiltration, indicating myocardial involvement in the disease process. The spleen displayed age-dependent variations. In chicks aged 1-7 days, it was typically enlarged and congested, whereas in older birds, multiple necrotic foci were common. These appeared hemorrhagic and reflected

advanced disease progression with widespread reticuloendothelial involvement. The lungs of young affected broiler chicks showed mild pulmonary congestion, whereas those older than one week often developed moderate congestion and in some, focal areas of consolidation were recorded. These findings indicated localized pneumonic changes and a secondary compromise of respiratory function. The kidneys frequently exhibited enlargement, congestion and swelling, which could be attributed to systemic septicemia and circulatory disturbances (Fig:3). The affected birds may also defecate watery diarrhoea, occasionally tinged with blood. The intestines of the affected broiler chicks also showed marked congestion and hemorrhages (Fig:4). These intestinal changes emphasized the role of salmonella infection in enteric pathology and subsequent dehydration of affected chicks. The bursa of Fabricius of affected birds revealed moderate atrophy, indicating the immunosuppressive effects of salmonella infection. In acute cases of disease, vent pasting with loose whitish fecal material and cloacal impaction were consistently recorded. In addition, unabsorbed yolk and yolk sac infections were frequently noted in chicks during the first week of life, often contributing to early chick mortality and poor performance. Some severe revealed most prominent gross lesions included necrotic foci in the liver, pericarditis with fibrinous exudation, perihepatitis and intestinal congestion. The greyish-white nodules on the ventricular myocardium were also observed in the affected birds. The other common changes comprised renal congestion and yolk sac infections, both of which occurred with considerable frequency. The caeca of the affected birds were markedly inflamed and swollen with cheesy, dry, necrotic material within the lumen. Thus, these lesions emphasis the systemic nature of salmonella infections in broiler chickens, with consistent multi-organ involvement affecting the liver, spleen, heart, lungs, kidneys, intestines and bursa. The characteristic hepatic discoloration, necrotic foci, splenic lesions and cecal cores emerged as the most important diagnostic indicators, reinforcing the necessity of gross pathological examination as a key tool in field diagnosis of salmonellosis.

Gross Pathology

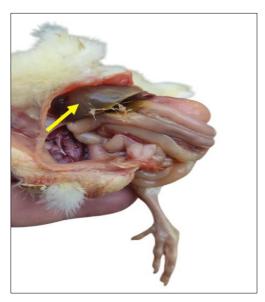


Fig 1: Photograph of the affected carcase revealing bronze discoloration of the liver



Fig 2: Photograph of the affected liver revealing necrotic foci interspersed with reddish haemorrhagic areas

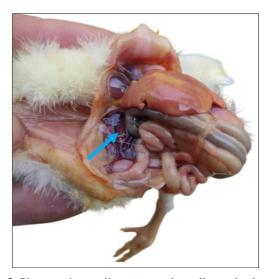


Fig 3: Photograph revealing congested, swollen and enlarged kidneys in the affected birds

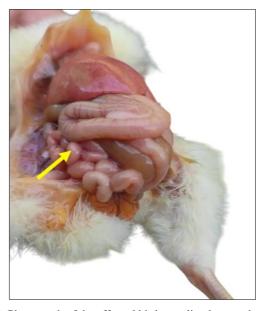


Fig 4: Photograph of the affected bird revealing haemorrhages in the intestines

Histopathology

Liver: Microscopically, the liver of affected broiler chicks, especially those in early age groups, frequently exhibited pronounced vacuolar degeneration fatty changes and infiltration of inflammatory cells, indicating impaired hepatocellular metabolism and functional compromise (Fig: 5a). The vascular congestion and multifocal hemorrhages were consistently observed across all age groups, reflecting systemic circulatory disturbances caused by infection. The broiler chicks aged 7-15 days typically displayed isolated foci of hepatocellular necrosis, accompanied by dense infiltration of leukocytes, predominantly mononuclear cells. These inflammatory cells were primarily located around portal triads and perivascular areas, emphasizing the predilection of salmonella organisms for vascular and periportal regions. The microscopic examination of the affected liver revealing a mixed inflammatory infiltrate composed of mononuclear cells and heterophils, occasionally forming nodular aggregates. In some instances, reticular cell hyperplasia disrupted the normal hepatic cord architecture, further compromising liver integrity and function.

Heart: Heart involvement was consistent in this infections in case of affected birds. The pericardium frequently showed thickening with mononuclear cell infiltration, while congestion and haemorrhage beneath the epicardium were particularly prominent in younger chicks. The focal myocardial necrosis along with mononuclear infiltration was a common finding. In older birds above 2 weeks of age presenting with gross nodular lesions with extensive infiltration of mononuclear cells, often resulting in atrophy, necrosis and replacement of cardiac muscle fibres (Fig: 5b). In severe cases, entire muscle fibres were replaced by inflammatory cells, illustrating the destructive potential of the infection on myocardial tissue.

Histopathology

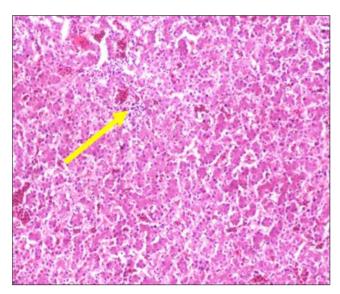


Fig 5a: Photomicrograph of the liver revealing hepatitis as evidenced with massive influx of inflammatory cells with sinusoidal congestion (H&E, stain)

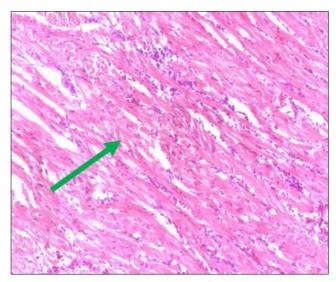


Fig 5b: Photomicrograph of the Heart revealing separation & disruption of muscle fibres (H&E, stain)

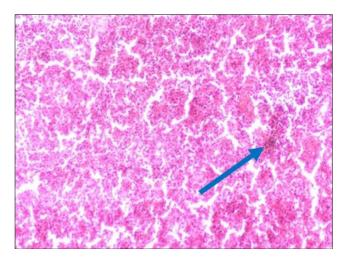


Fig 5c: Photomicrograph of the spleen revealing spleenitis as evidenced with inflammatory cell infiltration (H&E, stain)

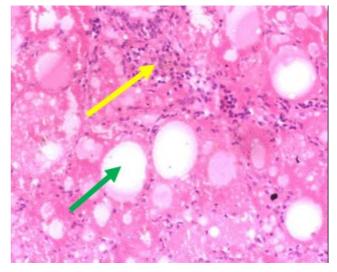


Fig 5d: Photomicrograph of the Lung revealing edema with presence inflammatory cell infiltration (H&E, stain)

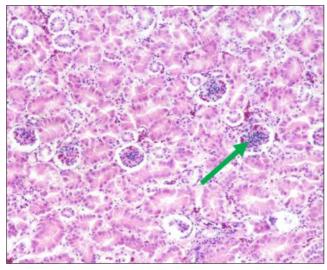


Fig 5e: Photomicrograph of the Kidney revealing glomerular nephritis with denudation of renal tubular epithelium (H&E, stain)

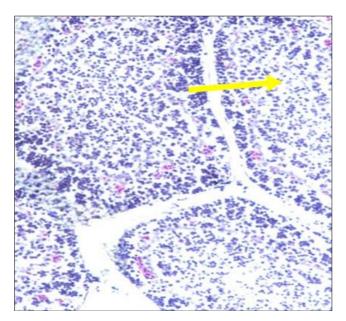


Fig 5f: Photomicrograph of the bursa revealing lymphoid depletion in the bursal lymphoid follicles (H&E, stain)

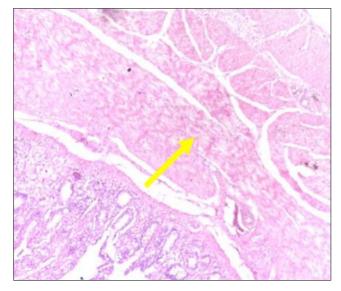


Fig 5g: Photomicrograph of the intestine revealing thickening of the serosa muscularis (H&E, stain)

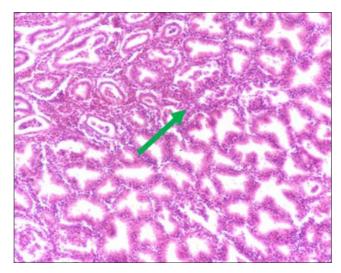


Fig 5h: Photomicrograph of the proventriculus revealing degeneration with necrosis (H&E, stain)

Spleen: The spleen showed age- and stage-dependent changes. Early-stage lesions included congestion, thickened blood vessels sub capsular hemorrhages and spleenitis (Fig: 5c). As the disease progressed, lymphoid depletion and focal necrotic areas became evident. In some birds, reticular cell proliferation accompanied with lymphoid depletion and disrupting normal splenic architecture, reflecting significant immunosuppression associated with systemic *salmonella* infection.

Lungs: Pulmonary lesions included congestion of interlobular septae and parabronchial hemorrhages. The edema and mild mononuclear cell infiltration were also observed in the lungs of affected birds (Fig:5d). In more severe infections, some birds developed suppurative bronchopneumonia, characterized by heterophilic exudates filling the parabronchi. These findings not only reflected localized respiratory involvement but also suggested secondary bacterial colonization as a complication of systemic infection.

Kidneys: Histologically, the kidneys of affected broiler chicks showed variable degrees of congestion, ranging from mild to severe, reflecting systemic circulatory disturbances associated with salmonellosis. The affected kidneys of the birds revealing glomerular nephritis (Fig: 5e). The interstitial hemorrhages were frequently observed, often scattered throughout the renal parenchyma, suggesting vascular compromise and increased capillary fragility. In addition to these vascular changes, some cases exhibited focal infiltration of inflammatory cells, primarily heterophils, indicating an active immune response targeting the renal tissue. These inflammatory foci not only disrupted normal tubular and interstitial architecture but also pointed toward direct renal parenchymal involvement by the pathogen. Such lesions suggest that the kidneys are secondary target organs in systemic salmonella infections, reflecting hematogenous dissemination of bacteria. These pathological lesions like congestion, haemorrhage and cellular infiltration emphasizes both the hemodynamic stress and localized immune response within the kidneys, which may contribute to impaired renal function and morbidity in affected broiler chickens.

Bursa of Fabricius: The histopathological examination of the bursa of Fabricius in affected broiler chicks revealed depletion of lymphocytes within the bursal follicles, which is a finding that aligns with the immunosuppressive nature of salmonellosis (Fig: 5f). This reduction in lymphocyte population suggests compromised humoral immunity, which may predispose the birds to secondary infections and exacerbate disease severity. In some birds, the follicles exhibited marked atrophy, with shrinkage of follicular size and loss of structural integrity, reflecting a more severe impact of infection. The microscopic examination further showed degenerative changes, such as cytoplasmic vacuolation or disorganization of follicular architecture, indicating variability in the extent of bursal involvement among different cases. These histopathological changes accentuate the role of the bursa as a primary target of systemic salmonella infection, emphasizing its susceptibility to both direct bacterial effects and the inflammatory response. The degree of lymphoid depletion and follicular atrophy can serve as a reliable indicator immunosuppression in broiler chicks which provides valuable diagnostic grasp when correlated with clinical and gross pathological findings.

Intestines and Caeca

Intestinal lesions were marked by congestion of mucosal blood vessels, goblet cell hyperplasia, infiltration of heterophils and thickening of the muscularis (Fig: 5g). The caeca were particularly affected in this infections, showing hemorrhages, congestion, epithelial degeneration and desquamation. The dense mononuclear infiltration in the mucosa and submucosa often led to pressure atrophy of intestinal glands, and in advanced cases, the lumen contained cheesy necrotic material, which is a hallmark of chronic infection.

Proventriculus

Histopathological examination of the proventriculus in affected broiler chicks generally revealed minor lesions, indicating that this organ is lightly impacted in systemic salmonellosis. In a few cases, there was focal infiltration of mononuclear cells within the mucosal layer, suggesting a localized inflammatory response. These pathological changes disrupt the architecture of the proventricular glands or mucosa. The affected proventriculus may also reveal degeneration and necrosis (Fig: 5h). The limited involvement of the proventriculus indicates that, compared to other organs such as the liver, spleen, heart, or bursa of Fabricius, this region is less susceptible to direct pathological damage during salmonella infection. However, the presence of mild cellular infiltration may reflect transient immune activity in response to circulating pathogens, providing supporting evidence of systemic infection when considered along with lesions in more heavily affected organs.

Thus, histopathological evaluation confirmed the systemic impact of *salmonella* infections in broiler chicks, affecting multiple organs including the liver, spleen, heart, lungs, kidneys, intestines, bursa and caeca. The most diagnostic features included hepatocellular necrosis with inflammatory infiltration, myocardial degeneration with inflammatory replacement, lymphoid depletion in the spleen and bursa and characteristic cecal lesions. These microscopic observations complemented gross pathological findings and served as critical indicators for the definitive diagnosis of *salmonellosis* in broiler chickens.

Discussion

This study provides an in-depth analysis of the pathological attributes associated with naturally occurring salmonellosis in broilers reared in the Ganderbal district of Kashmir. The Salmonellosis remains one of the most significant bacterial infections affecting poultry worldwide, leading to considerable economic losses due to mortality, reduced growth performance and condemnation of carcasses. The findings of this case series reveal the clinical and postmortem manifestations that characterize the disease under field conditions in this specific geographic region. The affected broilers exhibited non-specific clinical signs such as lethargy, ruffled feathers, anorexia and diarrhea, which are indicative of the systemic nature of Salmonella infections. The Post-mortem examinations revealed marked organ involvement, with prominent lesions observed in the liver, spleen and intestines. The hepatomegaly with necrotic foci, splenomegaly and intestinal congestion were frequently observed, reflecting the tropism of Salmonella for reticuloendothelial tissues and the gastrointestinal tract. These pathological findings align with earlier studies that emphasize the characteristic lesions associated with Salmonella infection in broilers, although variations in lesion severity and distribution were noted, likely influenced by factors such as age, immune status and environmental conditions specific to the Ganderbal district.

The results of this study are in agreement with previous reports identifying Salmonella outbreaks causing severe economic loses in India. The gross pathological lesions recorded further corroborate earlier findings. The bronze discoloration of the liver, which is a distinctive feature of this disease, was prominent in the affected birds and is attributed to the affinity of S. Gallinarum for bile canaliculi, resulting in bile stasis (Basnet et al., 2008) [6]. The hepatic changes may include liver mottling and necrotic foci in the affected birds (Chisti et al., 1995) [9]. The Cardiac lesions in the affected birds may include hemorrhages and caseous nodules on the myocardium (Hafeeji et al., 2001) [13]. The splenic lesions in the affected birds, including mottling, necrosis and hemorrhagic foci (Chisti et al., 1995) [9]. The Pulmonary congestion and consolidation, along with renal swelling and congestion, reflected findings with the available online literature (Kaura et al., 1990, Hafeeji et al., 2000, and Freitas et al., 2007) [17, 12, 11]. The intestinal mucosal congestion with hemorrhages and moderate atrophy of the bursa of Fabricius also mirrored with earlier observations (Mohammadi et al., 1996) [20].

Microscopic examination revealed cellular alterations underpinning these gross changes. The liver exhibited severe vacuolar degeneration, fatty changes, vascular congestion, hemorrhages, and necrotic foci, accompanied by infiltration of mononuclear cells and heterophils. The mild reticular cell hyperplasia in some cases disrupted hepatic architecture, emphasizing the cytopathic effects of S. Gallinarum, (Freitas et al., 2007) [11]. The Perihepatitis and myocarditis indicated systemic dissemination of infection (Kumar et al., 2002) [18]. The nodular myocardial lesions with extensive mononuclear infiltration and replacement of muscle fibers were in agreement with previous studies (Hafeeji et al., 2010) [14], emphasizing the pathogen's destructive impact on cardiac tissue. The lymphoid organs showed evidence of immunosuppression, with lymphocyte depletion and focal necrosis in the spleen and bursa, occasionally accompanied by reticular cell proliferation

(Freitas et al. 2007) [11]. The microscopic examination of the affected lungs revealed bronchopneumonia, congestion and hemorrhages, which confirmed respiratory involvement (Hafeeji et al., 2000) [12]. The histopathological changes in the affected kidneys revealed interstitial congestion, hemorrhages and focal infiltration of inflammatory cells, reflected systemic septicemia (Deshmukh et al.., 2007) [10]. The affected intestinal lesions, including congestion of mucosal vessels, goblet cell hyperplasia and infiltration of heterophils and mononuclear cells (Prasanna et al., 2001) [21]. Thus, this study demonstrates that naturally occurring salmonellosis in broilers is a multisystemic disease, involving the liver, heart, spleen, lungs, kidneys, intestines and lymphoid organs. The clinical signs, gross pathology and histopathological changes observed closely align with findings, emphasizing the characteristic pathological patterns associated with this disease. These results emphasize the importance of integrating clinical observations, gross examinations and microscopic analyses for accurate diagnosis and effective management of this disease in commercial broiler operations.

The poultry industry of Kashmir valley has been consistently threatened by major infectious diseases, particularly salmonellosis, Newcastle disease (ND), infectious bursal disease (IBD), colibacillosis, coccidiosis and fowl cholera, which collectively contribute to high morbidity, mortality and economic losses. The recent studies on salmonellosis, ND and IBD have emphasized significant prevalence and associated clinico-pathological alterations in broiler flocks of the region (Rafiq et al., 2024; Yaqub et al., 2024) [22, 26]. The Clinico-pathological assessments of naturally occurring salmonellosis and Colibacillosis further revealed severe digestive, respiratory and neurological signs, accompanied by characteristic hemorrhagic and necrotic lesions in lymphoid tissues, emphasizing the virulent nature of organism in the northern Himalayan belt (Shafi et al., 2023) [24]. The investigations into salmonellosis and IBD demonstrated profound lymphoid depletion, immunosuppression and secondary bacterial complications, making poultry more vulnerable to concurrent infections (Wani et al., 2021; Yaqub et al., 2023) [25, 27]. The other bacterial diseases such as colibacillosis and fowl cholera have also been reported to cause hepatopathy, nephropathy, septicemia and high mortality rates, thereby compounding the burden of infections in broiler production systems (Rafiq et al., 2023) [23]. The parasitic infestations, especially coccidiosis caused by Eimeria spp., continue to remain endemic in both broiler and layer flocks of Central Kashmir, with significant intestinal pathology and production losses (Yaqub et al., 2023; Shafi et al., 2023) [27, ^{24]}. The Long-term epidemiological records also suggest that environmental and seasonal variations in temperate climates exacerbate disease occurrence, particularly salmonellosis, pulmonary hypertension syndrome and gout-related complications in broilers (Janwari et al., 2019; Kashani et al., 2018) [15, 16]. Thus, these studies highlight the multifactorial etiology of poultry mortality in Kashmir, emphasizing the urgent need for integrated disease surveillance, improved vaccination strategies strengthened biosecurity measures to ensure sustainable poultry health and productivity.

Conclusion

This investigation emphasizes that naturally occurring salmonellosis in broilers of Ganderbal district is a multisystemic disease characterized by distinctive gross and histopathological lesions in different organs. The consistent occurrence of hepatic discoloration with necrotic foci, splenic enlargement, cardiac nodules and cecal cores emerged as important diagnostic indicators under field conditions. The microscopic findings of hepatocellular degeneration, myocardial necrosis, lymphoid depletion, and mucosal infiltration further confirmed the systemic and immunosuppressive nature of the disease. The effective control and preventive strategies are essential to reduce economic losses and safeguard poultry health in commercial farms

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