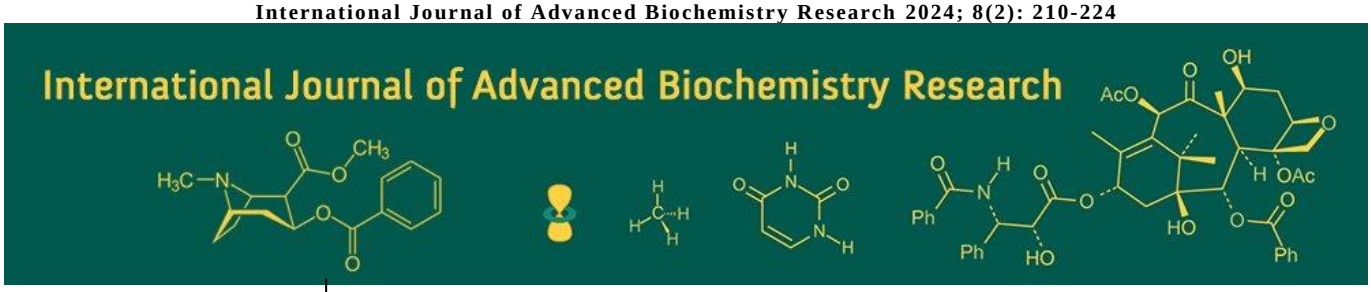

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(2): 210-224
www.biochemjournal.com
Received: 23-12-2023
Accepted: 24-01-2024

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In vivo pathological evaluation of naturally occurring infectious Bursal disease in broiler chicken reared under temperate climatic conditions of Kashmir

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DOI: https://doi.org/10.33545/26174693.2024.v8.i2c.555

Abstract
A case compilation study was conducted to assess the clinical signs, hematological and pathomorphological changes observed in the Infectious bursal disease in broiler chicken reared in Ganderbal district of Kashmir valley during the period of May to August, 2019 in commercial broiler farms at Ganderbal district of Kashmir. During this study, a total of 50 sick birds showing characteristics clinical signs of Infectious Bursal Disease and 15 death birds that were brought to the Division of Veterinary pathology for detailed postmortem examination. The confirmation of cases was performed by detection of gross and histopathology of the organs. The blood samples from 20 randomly selected sick birds were tested to routine hematology. The birds showed lymphocytes were highest and basophils were lowest percentages in IBD suspected broiler chicken. The disease was found to occur all around the year and highest incidence was observed during winter. The clinical signs included dullness, depression, anorexia, ruffled feathers and yellow diarrhea. On necropsy, birds showed darkened discolouration of thigh and breast muscles with paint brush like hemorrhages and enlarged bursa with accumulation of thick mucoid, creamy or bloody exudates. Some birds showed congestion and hemorrhages at the junction of bursa and proventriculus. Microscopically, lymphoid depletion, formation of cysts filled with necrotic debris, heterophils and diffused hemorrhages were observed in bursa. This disease remains an economical threat to the poultry industry and require regular monitoring in order to prevent occurrence of disease in poultry farms

Keywords: Broiler, infectious bursal diseases, hematology, histopathology, outbreak, diagnosis

Introduction
Rural poultry production is an important agricultural activity in the *Union Territory of Jammu and Kashmir*. India ranks third in egg production and fifth in poultry production in the world. Poultry is one of the vibrant sub-sectors of agriculture that plays a significant role in the development of agro-based economy of Kashmir Valley. Poultry farming, also known as aviculture, refers to the raising and breeding of domesticated birds for various purposes, such as meat (broilers), eggs (layers), and feathers (for down or other products). Poultry farming can be done on a small scale in backyard setups or on a larger scale in commercial operations. There are two main types of poultry farming namely conventional and organic. The conventional poultry farming involves raising birds in confinement systems such as battery cages or free-range systems. In contrast, organic poultry farming uses methods that promote natural behaviors and animal welfare, such as access to outdoor space and organic feed.

Mortality is a daily consideration for poultry farming which affects the economic condition of the poor farmers. There are a variety of methods of disposal which may include burial, composting, incineration and rendering. Burial is a common method of carcass disposal to manage mortalities but it may pose contamination threat to the groundwater. Composting is the method of disposal in which organic material is broken down and decomposed with the help of the bacteria in order to reduce it to stable humus.

Incineration is a costly method of disposal in which the carcass is burned with the help of fuel energy. In the process of rendering, the carcasses are exposed to high temperatures of nearly about 130 C using pressurized steam to ensure destruction of most pathogens. Mortality records in poultry plays an important role in determining the prevalence of diseases and strategies in order to control the disease (Kumar, 2018).

There are many bacterial and viral diseases in the poultry in which Infectious bursal disease causes severe economic losses to the poultry farmers. Infectious bursal disease also known as Gumboro disease and was first recognized in the 1960s as a highly contagious viral disease affecting young chickens. It is caused by the infectious bursal disease virus (IBDV), a member of the Birnaviridae family, which primarily targets the B cells in the bursa of Fabricius. The disease is spread mainly through direct contact with infected birds or their droppings, and can also be transmitted indirectly through contaminated equipment, clothing, or feed. Infectious bursal disease has a worldwide distribution and is prevalent in many Asian countries, particularly in India. The disease can cause significant economic losses due to mortality, reduced growth rates and immunosuppression, which can lead to increased susceptibility to other infections.

Materials and Methods

Study area and study period

The study was conducted at Division of Veterinary Pathology, SKUAST-KASHMIR during the period of May to August, 2019.

Selection of the cases

Both organized and unorganized poultry farms were visited regularly during the study period and the morbidity as well as mortality was rerecorded. A total number of 100broiler chickens were screened in which 50 sick birds showing characteristics clinical signs of infectious bursal disease and 20 death birds that were brought to Division of Veterinary Pathology for postmortem examination were considered as target study cases. The Confirmation of cases was performed by detection of gross and histopathology of the organs. The other thirty birds are normal and served in non-infected group

Hematological examinations of sick birds

The blood samples were randomly collected from the suspected sick birds to evaluate the hematological parameters during Infectious bursal disease. The Blood samples were kept in sterile vials with anticoagulant (EDTA). The blood samples collected with anticoagulant were analyzed for routine examination of blood within 24 hours as per Weiss and Wardroom.

Postmortem and Histological examination

Post mortem examination was performed according to protocol described by Calnek (1997) and lesions were recorded. Among the different cases 15 samples from each of bursa of Fabricius, spleen, thymus, kidney and liver with gross typical lesions of IBD were taken for histopathology. The Collected samples were fixed in 10% formalin and the tissues were processed routinely and stained with Haematoxylin and Eosin as recommended by Bancroft *et al*., (2014) [1]. All the chemicals and reagents utilized in this study were obtained from Erba and glass wares used in this study were obtained from Borosil (India). The glass wares were properly cleaned and sterilized before use. All the plastic wares used in this study were obtained from Tarsons (India). The plastic wares were scientifically sterilized by autoclave prior to use.

Statistical Analysis: All data are presented as mean (M) ± standard deviation (SD). Unpaired t-Test was applied to determine the significant differences among different groups.

Results

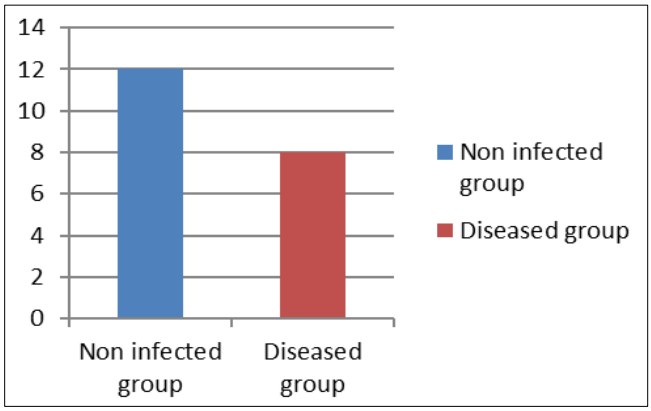
(A) Hematology

The results showed that the hemoglobin level, packed cell volume, Total red blood cell (RBCs) count and total white blood cells (WBCs) count differ significantly between the groups under this study as shown in Table 1 &Table 2.

Table 1: Hematological parameters in different groups of Broiler Chickens.

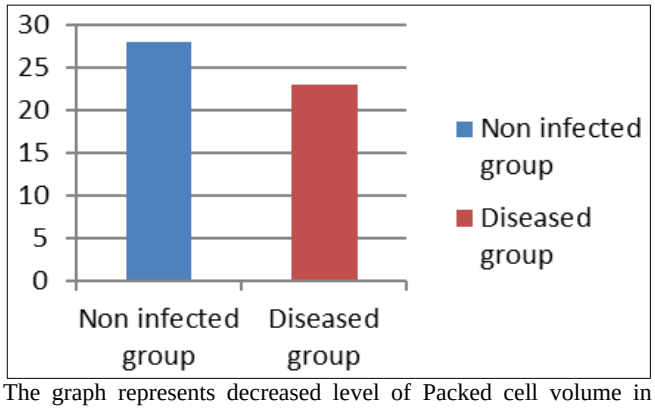
S. No.	Group	Haemoglobin concentration (g/dl)	Packed cell volume (%)
1.	Non infected group	12.00±0.67 [a]	28.32±1.72 [c]
2.	Diseased group	8.00±0.42 [b]	23.21±1.25 [d]

Means of various Hematological parameters differ significantly



The graph represents decreased level of haemoglobin in infected group

Graph 1A: Haemoglobin alteration in Infectious Bursal Disease in Broiler Chickens



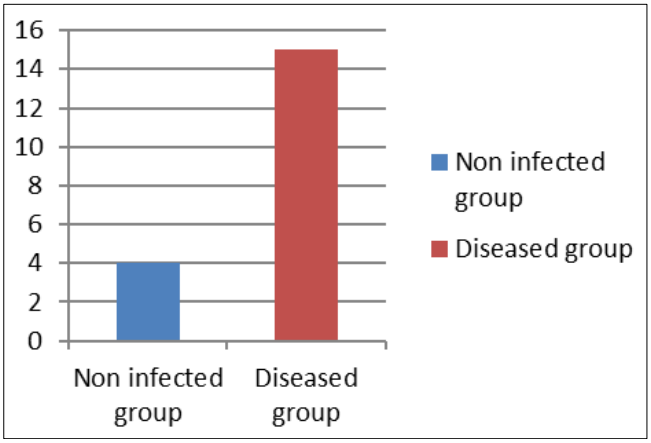
The graph represents decreased level of Packed cell volume in infected group

Graph 1B: Packed cell volume alteration in Infectious Bursal Disease in Broiler Chickens

Table 2: Hematological parameters in different groups of Broiler Chickens

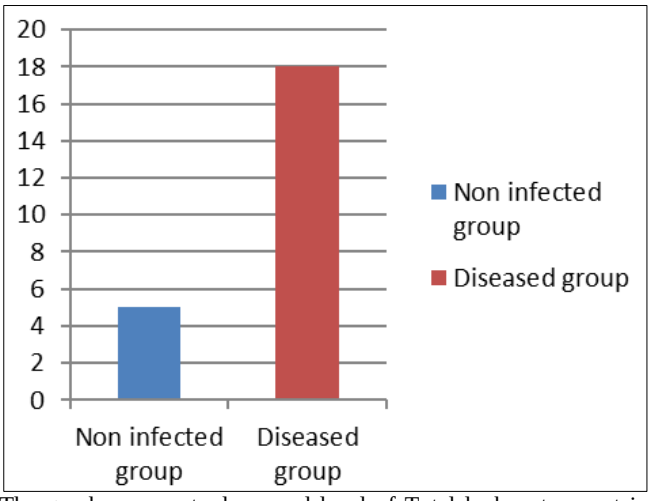
S. No.	Group	Total erythrocyte count (M/mm^3)	Total leukocyte count (Th/mm^3)
1.	Non infected group	4.00±0.24^a	5.00±0.21^c
2.	Diseased group	15.83±1.62^b	18.62±1.53^d

Means of various Hematological parameters differ significantly



The graph represents decreased level of Total erythrocyte count in infected group as compared to control group

Graph 2A: Total erythrocyte count alterations in Infectious Bursal Disease in Broilers



The graph represents decreased level of Total leukocyte count in infected group as compared to control group

Graph 2B: Total leukocyte count alterations in Infectious Bursal Disease

B) Biochemical Analysis

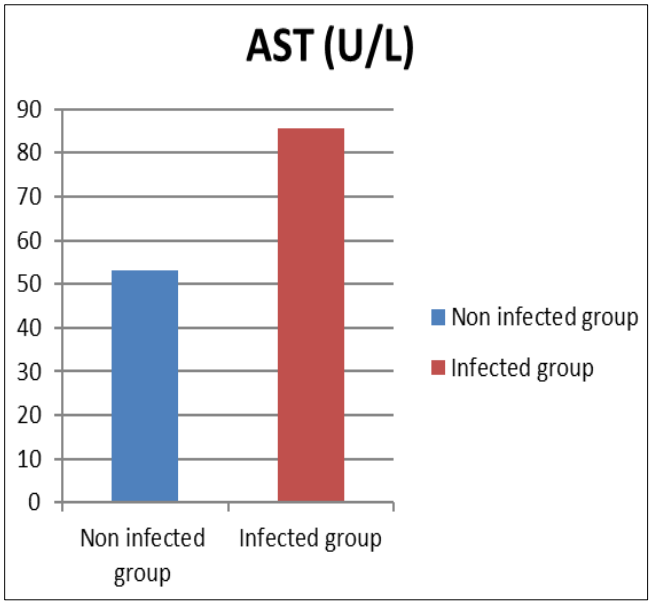
The overall mean AST ALT and creatinine value in infected chicken was significantly higher when compared with non infected chickens.

Table 3: Serum biochemical alterations in broiler chicken affected with Infectious Bursal Disease

S. No	Group	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)
1.	Non infected group	53.03±1.27^a	62.27±1.35^c	5.37±0.25^e
2.	Infected group	85.56± 1.43^b	86.62±3.49^d	8.72±0.27^f

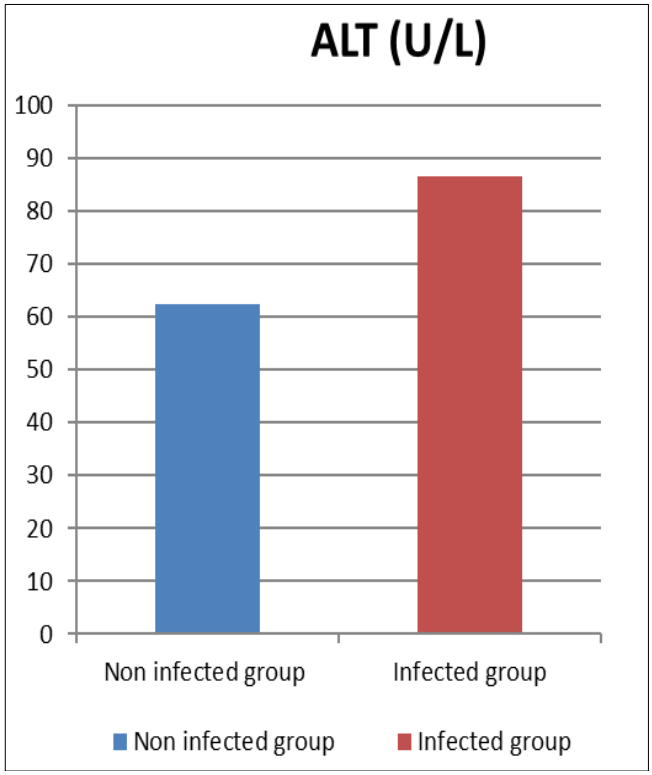
Means of various biochemical parameters differ significantly

ALT and AST are primarily found in the liver, and an elevation in its blood levels is a strong indicator of liver damage. It is often used as a specific marker for liver injury. Creatinine levels are commonly used to estimate the glomerular filtration rate (GFR), which is a measure of how well the kidneys are filtering waste from the blood. An alteration in the glomerular filtration rate indicates hampered kidney function, and it is often categorized into different stages of chronic kidney disease based on the severity of impairment. Thus the altered level of creatinine in the blood is a valuable indicator of kidney function.



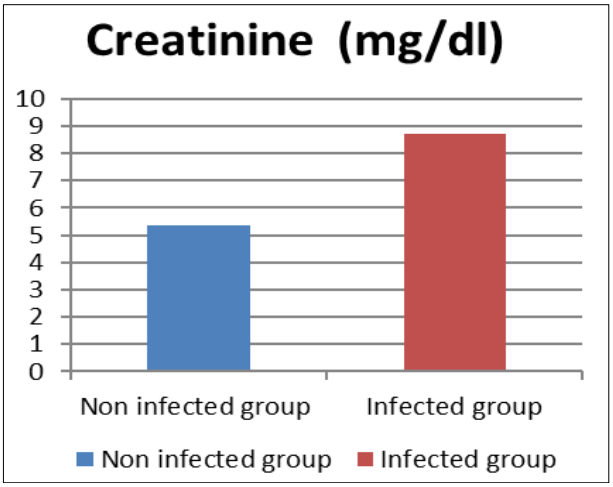
The graph represents AST (U/L) level alteration in infected and Non infected birds

Graph 3A: AST (U/L) level alteration in infected Broiler Chickens



The graph represents ALT (U/L) level alteration in infected and Non infected birds

Graph 3B: ALT (U/L) level alteration in infected Broiler Chickens



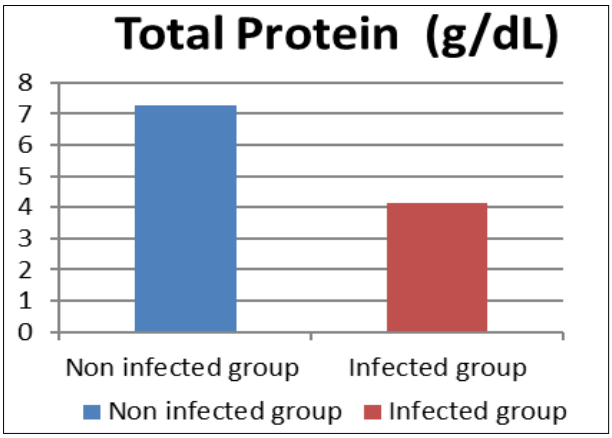
The graph represents Creatinine (mg/dl) level alteration in infected and Non infected birds

Graph 3C: Creatinine (mg/dl)level alteration in infected Broiler Chickens

Table 4: Serum biochemical alterations in infected broiler chicken

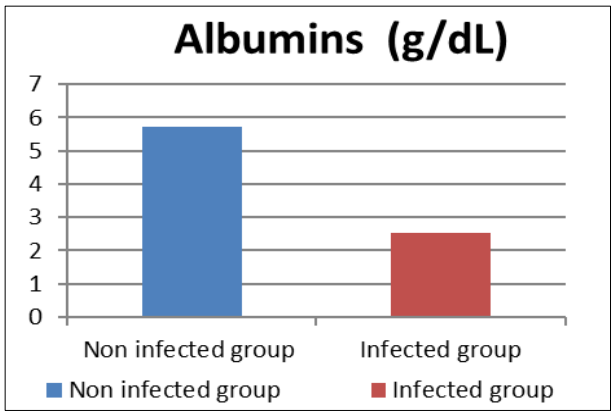
S. No	Group	Total Protein (g/dL)	Albumins (g/dL)	Globulins (g/dL)
1.	Non infected group	7.26 ±0.04 [a]	5.72± 0.07 [c]	3.63±0.03 [e]
2.	Infected group	4.15±0.03 [b]	2.53± 0.05 [d]	2.68± 0.02 [f]

Means of various biochemical parameters differ significantly



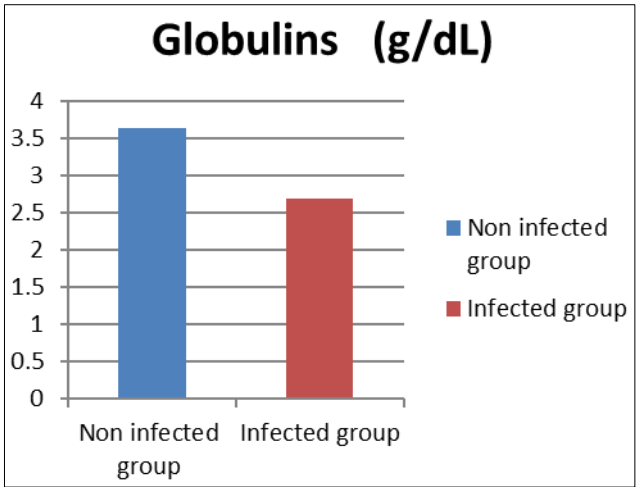
The graph represents Total Protein (g/dL) level alteration in infected and Non infected birds

Fig 4A: Total Protein (g/dL) level alteration in Infected Broiler Chickens



The graph represents Albumin (g/dL) level alteration in infected and Non infected birds

Fig 4B: Albumins (g/dL)level alteration in infected Broiler Chickens



The graph represents Globulins (g/dL) level alteration in infected and Non infected birds

Fig 4C: Globulins (g/dL)level alteration in infected Broiler Chickens

Clinical Signs

Infectious bursal disease is a highly contagious viral infection that primarily affects young chickens and causes immunosuppression. The clinical signs of Infectious bursal disease can vary depending on the severity of the infection and the age of the birds. The foul-smelling diarrhea is a common symptom of Infectious bursa disease. The affected chickens often appear lethargic, depressed, huddle together and may exhibit decreased appetite with reduced water consumption. Due to decreased water intake and diarrhea, chickens with Infectious bursal disease can become dehydrated. This can be manifested by sunken eyes, dry mucous membranes and poor skin elasticity. The emaciation and dehydration were severe in later stage of Infectious bursal disease. Infected birds may experience poor weight gain despite adequate feeding. The affected chickens often have ruffled feathers, giving them a disheveled appearance. The chickens infected with Infectious bursal disease may exhibit delayed feathering or poor feather quality, leading to a ragged appearance. In severe cases, Infectious bursal disease causes high mortality rates, especially in young birds as compared to adults. The mortality rate may vary depending on the virulence of the virus strain. In addition to these signs, birds infected with infectious bursal disease may show neurological symptoms, such as tremors, convulsions and paralysis. The bursa of fabrics becomes swollen initially but later undergoes atrophy due to destruction of B lymphocytes. In laying hens, Infectious bursal disease can cause a temporary decrease in egg production or even a complete cessation of egg lying. The significant effects of Infectious bursal diseases immunosuppression which makes birds more susceptible to secondary infections. The secondary complication in the affected birds may include respiratory signs such as coughing, sneezing, labored breathing and nasal discharge. The birds infected with Infectious bursal disease may have poor feather quality with feathers appearing dull, ragged or even feather loss. Some birds may show signs of uncoordinated movements, such as stumbling or difficulty walking. The affected chickens tend to sit with drooping wings and have a hunched posture. They may also huddle together for warmth and comfort. The virus can produce a pale appearance of the comb and wattles, which are

normally red in healthy chickens. The mucus membranes of the affected birds were pale and thin in appearance. Some cases of Infectious bursal disease revealed tibial dyschondroplasia, abnormal bending of the tibial bones, enlarged hock joints, sternal recumbency, convulsion, lacrimation from eyes, profuse salivation, restlessness and ataxia. The keel bone appeared sharp due to emaciation of the affected birds.

Gross lesions of bursa of fabricius

The Bursa of Fabricius is a primary lymphoid organ found in birds, located at the base of the cloaca. It is a key component of the avian immune system, playing an important role in the development and maturation of B lymphocytes, a type of white blood cell that produces antibodies and is responsible for the adaptive immune response.

The bursal lesions were most prominent in the all affected flocks. Birds from three flocks had enlarged hemorrhagic bursa having blood mixed with cheesy exudate in the lumen whereas few of the birds from different flocks had smaller and atrophic changes of bursa with necrotic and hemorrhagic changes. Along with swollen and hemorrhagic bursa, the other prominent gross lesions from dead birds included mottled appearance with pale discoloration on surface

During this study congestion and hemorrhage of the pectoral and leg muscles are the main gross lesion in the birds other than the bursal lesions. The birds suspected for Infectious Bursal Disease strains revealed swollen cloacal bursa edematous, pale and occasionally hemorrhagic. Chickens that have recovered from this infection have small, atrophied, cloacal bursas due to the destruction and lack of regeneration of the bursal follicles.

Gross lesions in the bursa of Fabricius are commonly seen in birds affected by Infectious Bursal Disease. These lesions can range from mild to severe which include hemorrhage, necrosis and edematous. The extensive hemorrhage throughout the bursa. The bursa may also appear enlarged and the presence of fluid and fibrin in the bursal cavity. The bursa of the affected birds appeared swollen with a pale coloration and may be soft and friable. The mild affected cases of Infectious Bursal Disease revealed Swollen bursa with a pale discoloration and friable in nature. The affected birds may also have a watery discharge from the cloaca. In severe cases, the bursa may rupture, leading to peritonitis and death.

Histopathology of bursa of Fabricius

The histopathology of the bursa of Fabricius in Infectious Bursal Disease is characterized by lymphocyte depletion, necrosis and hemorrhage. The virus infects the B-lymphocytes in the bursa of Fabricius, leading to their destruction and causing a decrease in the number of lymphocytes. This results in atrophy of the bursa of Fabricius and a decrease in antibody production, leading to increased susceptibility to secondary infections. The microscopic examination further revealed that the bursa of Fabricius in Infectious Bursal Disease appears as a shrunken and discolored organ, with a depletion lymphoid tissue from the lymphoid follicles and infiltration of inflammatory cells (Fig.1a).

Histologically, in the acute phase of Infectious Bursal Disease, the bursal follicles become congested, edematous and necrotic with an influx of inflammatory cells. The bursal epithelium becomes disrupted, and the underlying lymphoid cells undergo programmed cell death. These changes lead to a significant reduction in the size of the bursal follicles (Fig. 1b) and a loss of its immunological function.

In the subacute phase, there is a marked reduction in the size of the bursa with the persistence of necrotic foci, fibrosis and inflammatory infiltrates. There is a progressive loss of lymphocytes and germinal centers, which are replaced by fibroblasts and connective tissue. The microscopic examination further revealed degenerative changes in lymphoid follicles (Fig. 1c), interstitial oedema along with the congestion. Histopathological examination of bursa of Fabricius revealed necrosis of the lymphoid follicles (Fig. 1d) and lysis out of lymphoid cells characterized by presence empty spaces which were surrounded by thick band of the connective tissue. The chronic phase of Infectious Bursal Disease, the bursal follicles become fibrotic, with a decrease in lymphoid cells (Fig.1e) and the replacement of the bursal parenchyma with connective tissue. These changes can persist for several weeks after the initial infection and the bursa was never seen to fully recover its normal structure or function. In the chronic phase, the bursa becomes severely atrophic, with the presence of fibrosis, lymphoid depletion and glandular atrophy. The microscopic examination further revealed edematous and necrotic bursal follicles with an influx of inflammatory cell (Fig.1f). The bursal epithelium is replaced by fibrous tissue, and the remaining lymphocytes are mainly plasma cells and macrophages.

As the disease progresses, the bursa undergoes further degenerated, small and irregular in shape, and the remaining lymphoid tissue may be replaced by adipose tissue or connective tissue. The severity of the lesions can vary depending on the age of the bird, the virulence of the virus, and the immune status of the host. The severity of the lesions depends on the virulence of the virus strain, age of the bird and the immune status of the bird.

Patho morphology of spleen

Grossly, the affected spleen may appear swollen, mottled and heavier than normal. The spleen of the affected birds can exhibit changes in color which may include pale or white. The hemorrhagic lesions observed in the spleen might be due to rupture of congested blood vessels. These lesions appear as pinpoint petechial hemorrhages or even larger ecchymotic areas. This hemorrhage can result in a dark or bluish appearance of the spleen. The spleen appeared enlarged due to lymphoid hyperplasia, which is a response to ongoing inflammation and immune stimulation. Infectious Bursal Disease can lead to congestion of blood vessels within the spleen which is a result of increased blood flow to the affected organ. The consistency of the spleen may be softer than normal due to the destruction of lymphoid tissue and infiltration of inflammatory cells. The loss of lymphocytes results in a reduction in the overall size of lymphoid follicles within the spleen. In some cases, nodular lesions or focal areas of necrosis are also observed on the surface or within the parenchyma of the spleen. The gross pathological changes observed in the spleen can vary depending on the stage and severity of the infection, as well as individual host factors.

Histopathology of spleen

In the acute phase of the disease, the microscopic examination of the affected spleen revealed necrosis and congestion in the red pulp with erythrocytes. The histopathological changes further revealed destructed lymphoid tissue in the splenic follicles (Fig. 2a). The severity of these pathological changes depends on the stage of the disease, virulence of the virus, age of the bird and immune status of the bird. As the disease progresses, cellular infiltration particularly macrophages and heterophils were also observed in the spleen. This can result in the formation of nodules and aggregates of inflammatory cells, which is surrounded by fibrosis. In severe cases, the lymphoid depletion leads to decrease in the overall size of the spleen (Fig.2b). This occurs because the spleen is primarily composed of lymphoid tissue and its depletion from the lymphoid follicles results in the atrophy of the spleen. The histopathological examination further reveals degenerated both red and white pulp of the spleen which contributes to the immunosuppressive effects of Infectious bursal disease virus in birds.

In sub-acute cases of Infectious bursal disease, the histopathological changes in spleen are generally characterized by necrosis, splenic atrophy and congestion (Fig. 2c). The white pulp of spleen is composed of lymphocytes and is responsible for the immune response to blood-borne antigen. This white pulp gets severely depleted in this disease due to loss of lymphoid tissue resulting in the immunosuppression inside the body of the host (Fig. 2d). The red pulp, which is responsible for the removal of old and damaged red blood cells, also show hemorrhage and hemosiderosis. The microscopic examination of the affected spleen further revealed scattered lymphocytes throughout the red pulp and reduction in the size of the spleen. The pathomorphological changes also include significant reduction in the weight of spleen of IBD infected birds. The histopathological examination further revealed massive influx of inflammatory cells into the affected areas of spleen. These inclusion bodies are eosinophilic and can be visualized by using hematoxylin and eosin (H&E) stain. In addition to this, other histopathological changes observed in the spleen include edema, degeneration and fibrin deposition. These changes are indicative of an inflammatory response and may contribute to the overall pathogenesis of the disease.

In chronic cases of Infectious bursal disease, the pathomorphological changes include germinal center disruption within the spleen as these germinal centers are important for B-cell maturation and antibody production. In few cases, hemorrhagic and degenerative changes are also observed within the spleen (Fig. 2e). These hemorrhages can result from vascular damage caused by the viral infection. In advanced cases, the spleen exhibits atrophy which characterized by a reduction in size and weight. This atrophy is a consequence of ongoing lymphoid depletion and destruction of lymphoid follicles. Chronic IBD often causes a significant reduction in lymphocytes within the spleen. This depletion primarily affects the white pulp, which is responsible for immune responses. The white pulp appeared diminished or even absent in severe cases. In response to the viral infection, heterophils infiltrate the spleen which is typically seen in the periarteriolar lymphoid sheath (PALS). The microscopic examination of the affected spleen further revealed deposition of fibrous tissue within the spleen. Fibrosis is a reparative process that occurs in response to tissue damage and can affect the normal architecture of the organ. This fibrotic response is a result of chronic inflammation. In severe cases, areas of necrosis are observed in the spleen which can occur due to the direct viral cytopathic effect (Fig. 2f). The histopathological examination further revealed Intracellular inclusion bodies formed by the birna virus within the affected spleen. These intracellular inclusions are often referred to as Bursal Disease Inclusion Bodies (BDIBs) and are a characteristic feature of Infectious bursal disease.

Gross pathology of thymus

In Infectious bursal disease (IBD), the gross pathology of the thymus can exhibit characteristic changes associated with the disease. The severity and specific findings can vary depending on the stage of the disease and the individual bird's response to the infection. Grossly, Thymic atrophy must be noticed in the affected birds. Thymic atrophy is a hallmark of IBD and reflects the destruction of lymphocytes, particularly in the thymic cortex. The thymus exhibits a pale and mottled appearance compared to its normal color. This discoloration can be due to decreased blood supply and lymphoid depletion within the lymphoid follicles of the thymus. In severe cases of IBD, hemorrhagic areas may be observed within the thymus. These areas indicate bleeding into the thymic tissue and can be associated with the destruction of blood vessels caused by the virus. The thymus may exhibit a soft consistency. This change in texture is due to the loss of lymphoid cells and disruption of normal thymic architecture. In severe cases of Infectious bursal disease, hemorrhagic areas may be observed in the thymus. These hemorrhages can occur due to the direct cytopathic effects of the virus or as a result of the compromised vascular integrity due to the destruction of lymphoid cells. The thymus appeared smaller in size as compared to a healthy thymus. This is due to lymphoid depletion and atrophy caused by the infection. The thymus exhibit swelling and edema, especially in more severe cases. This can be attributed to the inflammatory response and fluid accumulation within the organ. The thymus in few cases of this disease may become soft and fragile to the touch. This softening or friability is a result of tissue damage caused by the virus and the associated immune response.

Histopathology of thymus

In acute cases of Infectious Bursal Disease (IBD), the histopathological changes observed in the thymus are typically indicative of the severe impact of the disease on the organ. Acute form of this disease is characterized by a rapid onset and progression of the infection, leading to significant damage to the immune system, including the thymus. The microscopic examination of acute cases of this disease revealed necrosis within the thymus (Fig. 3a). These necrotic regions represent localized cell death caused by the cytopathic effects of the virus. The extent and severity of necrosis can vary, but it is generally more pronounced in acute cases. In addition to this, the main effect of IBDV on the thymus is lymphoid depletion, resulting in a reduction in the number of lymphocytes. The loss of lymphocytes and disruption of normal thymic architecture contribute to this atrophic appearance. The histopathological changes observed in the thymus are consistent with the severe

immunosuppression seen in acute IBD. The destruction of lymphoid cells in the thymus compromises the ability of the immune system to mount an effective response against the virus and other pathogens. In response to the viral infection and tissue damage, the thymus may exhibit infiltration of inflammatory cells, including macrophages and lymphocytes. This inflammatory response is part of the host's immune reaction to the infection.

In subacute cases of Infectious Bursal Disease (IBD), the histopathological changes observed in the thymus may exhibit a range of histopathologic features. Subacute cases typically represent an intermediate stage between acute and chronic forms of the disease. The severity and nature of the lesions can vary depending on factors such as the duration of the infection, host immune response and the virulence of the Infectious Bursal Disease Virus (IBDV) strain. The atrophy of the thymus and lymphoid depletion are the characteristic feature of this form of disease (Fig. 3b).The extent of lymphoid depletion can vary depending on the stage and progression of the subacute infection. The thymic cortex may appear hypocellular, and there may be a reduction in the number of lymphocytes. In response to the viral infection, the thymus may exhibit infiltration of inflammatory cells such as macrophages and lymphocytes. The extent of infiltration may be less severe as compared to acute cases. The subacute Infectious Bursal Disease can also exhibit signs of lymphoid hyperplasia in the thymus. This refers to an increased proliferation and accumulation of lymphocytes. It represents an attempt by the immune system to compensate for the ongoing infection and the associated lymphoid depletion. In some cases, regenerative changes may be observed in the thymus during sub-acute IBD. This can include the presence of small lymphoid follicles, the proliferation of thymic epithelial cells, and the restoration of some thymic architecture.

In chronic cases of Infectious Bursal Disease (IBD), the histopathological changes observed in the thymus may differ from those seen in acute or subacute cases. The chronic cases of IBD typically indicate a persistent or prolonged infection with the Infectious Bursal Disease Virus (IBDV). The microscopic examination of thymus revealed severe lymphoid depletion and atrophy of thymic follicles in chronic cases of Infectious Bursal Disease. The thymic cortex revealed hypocellularity and reduction in the overall size of the thymus (Fig. 3c). The chronic inflammation persists in the thymus, leading to the infiltration of inflammatory cells such as macrophages, lymphocytes and plasma cells. These infiltrates must be present in the interstitial spaces and may contribute to ongoing tissue damage. The prolonged infection and tissue damage can lead to the development of fibrosis, scarring and degeneration with severe lymphoid depletion from thymic follicles (Fig.3d). Fibrous connective tissue may replace the normal architecture of the thymic lobules. This fibrosis can disrupt the function and structure of the thymus, further impairing immune responses. In few cases of chronic Infectious Bursal Disease, thymic cysts get appeared a result of persistent inflammation and necrosis, leading to the accumulation of fluid-filled spaces within the thymus. The chronic form of this disease can disrupt the normal process of lymphocyte maturation in the thymus which can lead to a decrease in the production of functional T-lymphocytes, further compromising the immune system. The formation of germinal centers within the thymus was observed in few chronic cases of this disease. The germinal centers are specialized areas where B lymphocytes undergo proliferation and antibody production. Their presence within the thymus suggests ongoing immune stimulation and attempts at antibody production in response to the persistent viral infection.

Gross pathology of kidney

In case of Infectious bursal disease (IBD) in chickens, the kidneys exhibit different gross pathological changes which are caused due to the persistent infection produced by the Birna virus inside the body of the host. The gross pathological changes include kidney enlargement which is commonly known as nephromegaly. In this disease, the kidneys appear swollen and have an increased weight as compared to normal. The affected kidneys also exhibit mottled appearance due to changes in the renal tissue caused by inflammation and renal tubular necrosis. The prolonged infection and renal damage can lead to thinning of the renal cortex which can be

Observed upon gross examination. The development of fibrous tissue in the kidneys can be observed in severe infections resulting in fibrosis and scarring. These areas of fibrosis may appear as white regions on the surface of the kidney tissue. The surface of the kidneys may appear rough and irregular due to the presence of fibrotic areas, scarring or other pathological changes.

Histopathology of kidney

In an acute case of Infectious bursal disease, the histopathological examination of the kidney exhibits congestion which can occur due to inflammation and increased blood flow associated with the immune response to the viral infection. The microscopic examination further revealed glomerular congestion (Fig.4a) and interstitial nephritis which can be characterized by infiltration of immune cells such as lymphocytes, plasma cells and macrophages (Fig. 4b). The severity of interstitial nephritis can vary depending on the stage and intensity of the infection. The virus may directly or indirectly affect the glomeruli resulting into the Glomerular congestion, thickening of basement membranes and infiltration of inflammatory cells. The histopathological examination of acute cases of kidney reveals degenerative changes in the renal tubules, such as swelling, vacuolation and disruption of the cellular structure. In severe cases, there might be renal tubular necrosis which can impair function of kidney and the inability to properly filter waste products from the blood. In addition to this, the affected kidney tissue sections also reveal perivascular infiltration of inflammatory cell around blood vessels in the kidney which contribute to the overall inflammatory response and may be associated with vascular damage.

The histopathology of the kidney in sub-acute cases of Infectious bursal disease typically shows interstitial nephritis, which is an inflammatory condition affecting the spaces between the renal tubules. This inflammation is characterized by infiltration of inflammatory cells, such as lymphocytes and plasma cells into the interstitial spaces of the kidney. The microscopic examination of the kidneys also exhibit congestion which is characterized by dilated blood vessels and an increased blood supply. In addition to interstitial nephritis, sub-acute cases of Infectious bursal disease may also show renal tubular necrosis (Fig. 4c),

characterized by the loss of renal tubular cells and the presence of necrotic debris in the tubular lumen. This can lead to impaired kidney function and renal failure. The microscopic examination of few cases of this disease reveals hemorrhagic areas with renal tubular degeneration. The tubules may contain cellular debris including sloughed off epithelial cells and proteinaceous casts. The other histopathological findings in sub-acute cases of this disease include glomerulonephritis and peritubular fibrosis, which is the deposition of fibrous tissue around the renal tubules. This can manifest as immune cell infiltration, endothelial cell swelling, thickening of the glomerular basement membrane and glomerular atrophy (Fig. 4d). The histopathology of the kidney in sub-acute cases of this disease reflects the systemic immunosuppression that occurs as a result of the virus's effects on the bursa of Fabricius, leading to damage in other organs, including the kidney.

In chronic cases of infectious bursal disease (IBD), the kidney may continue to exhibit severe histopathological changes as a result of persistent viral infection and prolonged immune responses. The chronic cases of this disease can cause various pathological changes including vascular damage in the kidney, endothelial cell proliferation, narrowing of the vascular lumens and thickening of blood vessel walls (Fig.4e). These changes can affect blood supply to the kidney and contribute to renal dysfunction. The affected glomeruli show sclerosis of the glomerular basement membrane, leading to decreased glomerular filtration rate and impaired kidney function. In addition to this, the microscopic examination of the affected kidneys tissue sections revealed dilatation of the renal tubules (Fig.4f). The histopathological examination of kidneys revealed inflammatory changes in the interstitial tissue of the kidney which is characterized by the infiltration of immune cells, such as lymphocytes and plasma cells. The prolonged inflammation and tissue damage may lead to calcification in the kidney. These calcium deposits can accumulate in the renal tubules or other areas of the kidney, impairing normal function. In severe cases, inflammation and tissue damage can trigger the deposition of excessive collagen fibers, leading to fibrosis in the kidney. This can result in the formation of scar tissue, which may disrupt normal renal architecture and impair kidney function.

Gross Pathology of Liver

The affected liver appeared swollen, rounded and larger as compared to normal. The degree of hepatomegaly can vary depending on the severity of the infection. The liver may appear engorged with blood, giving it a reddish appearance. This congestion is a result of the viral infection and associated inflammatory response. In severe cases of Infectious bursal disease, hemorrhages can also be observed in the liver which may appear as small red spots on the liver surface or within the liver tissue. They occur due to the fragility of blood vessels caused by the viral infection. The focal areas of necrosis can be observed on the surface of liver. These areas represent localized tissue death and may appear as pale regions within the liver tissue. The necrosis can occur due to direct viral damage to the liver or secondary to compromised blood supply caused by the viral infection.

Histopathology of Liver

In acute cases of Infectious bursal disease, the liver often shows signs of congestion in the blood vessel which is characterized by an increased presence of blood within the liver sinusoids (Fig. 5a).This congestion is a result of the inflammatory response and increased blood flow to the liver. The hepatocytes present in the liver show varying degrees of degeneration and necrosis. This is a consequence of the viral infection and the associated immune response. The affected hepatocytes may exhibit swelling, vacuolation, loss of cellular integrity and thickening of the wall of blood vessel (Fig. 5b). The inflammatory cells such as lymphocytes and heterophils might get infiltrated the liver tissue. These infiltrates are a result of the immune response to the viral infection. The inflammatory cells can be observed around blood vessels or within the liver parenchyma. The normal hepatic architecture may be disrupted due to the inflammatory response and hepatocellular necrosis. The liver tissue may appear disorganized with loss of normal lobular patterns. In acute cases of Infectious bursal disease, Kupffer cells can become activated and increase in number in order to eliminate damaged hepatocytes from the body of the host. Infectious bursal disease can cause hemorrhage around the blood vessels within the liver. In some cases of Infectious bursal disease, cholestasis may occur due to liver cell damage and inflammation which can manifest as the accumulation of bile pigments within hepatocytes or might be due to bile duct proliferation.

In subacute cases of Infectious bursal disease, the histopathological findings in the liver may vary which depends on the stage and severity of the disease. The hepatocytes exhibit degenerative changes which may include vacuolar degeneration, congestion and cellular infiltration in the affected liver tissue (Fig. 5c). The extent and severity of these changes may depend immune status of the affected bird. These degenerative changes reflect cellular injury and dysfunction. The microscopic examination further revealed bile duct hyperplasia which is characterized by the proliferation of bile duct epithelial cells. This response may be related to the inflammatory process or a direct effect of the virus on the biliary system. In subacute cases of Infectious bursal disease, there may be lymphocytic infiltration in the sinusoids of the liver and perivascular infiltration (Fig. 5d). This infiltration occurs due to the systemic spread of the virus and the subsequent immune response. This infiltration is often associated with the immunological response triggered by the viral infection. The lymphocytes may be present diffusely throughout the liver parenchyma or form lymphoid aggregates. The histopathological examination of the liver revealed necrotic areas characterized by the death of hepatocytes. The extent and severity of necrosis depend on the stage of the disease and the health status of the bird. The severe cases of this disease lead to the development of fibrosis which is characterized by the deposition of collagen fibers in the liver parenchyma. This can lead to the development of cirrhosis, which is a severe form of liver disease. In some cases, subacute form of Infectious bursal disease may lead to the activation of regenerative processes in hepatocytes.

In chronic cases of Infectious bursal disease, the histopathological examination of the liver may reveal certain characteristic finding in which the hepatocytes exhibit chronic degenerative changes. These changes can include vacuolar degeneration, nuclear alterations and cytoplasmic changes. Some cases of this disease may reveal degeneration of the hepatocytes around the congested central vein (Fig. 5e). The severity of these changes may

depend on the stage of the disease and the overall condition of the affected bird. The prolonged inflammation and tissue damage in the liver can trigger a fibrotic response in which there is excessive deposition of collagen fibers and the formation of scar tissue. The histopathological examination of the liver revealed degeneration with necrosis of hepatocytes (Fig. 5f). Fibrosis can result in the disruption of normal liver architecture and impair liver function. In chronic cases, there may be an ongoing inflammatory response within the liver in which there is Infiltration of inflammatory cells, including lymphocytes, plasma cells, and macrophages within the hepatic tissue. The distribution and severity of the inflammatory infiltrate may vary, reflecting the persistence of the immune response against the viral infection

Discussion

Poultry farming is the practice of raising domesticated birds, such as chickens, ducks, turkeys and geese for the purpose of producing meat, eggs and other poultry products Singh *et al.* (2018) [11]. It is a significant branch of the agricultural industry and plays a crucial role in meeting the global demand for animal protein. Poultry farming has a long history and has evolved with advancements in technology and breeding techniques. Initially, poultry farming was primarily focused on egg production, but now it has been expanded to meat production as well. Poultry farming is a highly specialized and efficient industry with various production systems and management practices. One of the key advantages of poultry farming is its high productivity and relatively short production cycle compared to other livestock. Additionally, poultry farming requires less land and capital investment compared to larger livestock operations, making it accessible to a wide range of farmers, including small-scale and backyard producers. Poultry farming has a significant economic impact, providing employment opportunities, income generation and food security.

This work was performed to assess the clinical and pathomorphological changes observed in the affected broiler chickens. The clinical signs like depression, reluctant to move, watery diarrhea, dehydration, ruffled feather, trembling, gasping and vent picking were recorded in IBD affected birds. The Hb, PCV, TEC and TLC in the affected broiler chickens were low as compared to normal broiler chickens in this study, which is accompanied with work of Panigrahy *et al*. (1996) [9] and Mahmood *et al*. (2018) [7]. The increase in AST, ALT and Creatinine activity might be attributed due to hepatopathy and nephropathy observed in the infected birds which were previously reported by Muller *et al*. (2012) [8] and Cortes *et al*. (2005) [3]. The decrease in total protein, albumin and globulin may be attributed to severe hepatic damage, as seen in infectious bursal disease which were in concurrence with the reports published in various scientific reports by Balamurugan *et al.*, (2014) [2] who additionally found large decrease in values of overall protein, albumin and globulin in inflamed birds. The Gross pathological lesions were hemorrhages and atrophy observed in the bursa of Fabricius in the affected birds which was more prominent at later stage of study period. Histopathologically, Bursa revealed vascular congestion, hemorrhages, interstitial edema and severe depletion of lymphoid cells. These changes are in accordance with the observations of Sohini *et al.* (2019) [13] in infectious bursal disease in poultry. Grossly, Spleen revealed pinpoint necrotic spots with varying degrees of vascular congestion and hemorrhages in the birds affected with infectious bursal diseas. Microscopically, depletion of lymphoid cells was more prominent during later periods of the experiment. The histopathological examination further reveals degenerated both red and white pulp of the spleen which contributes to the immunosuppressive effects of Infectious bursal disease virus in birds. These changes in the Spleen are in accordance with those reported by Rautenschlein *et al.* (2016) [9] in infectious bursal disease in broiler chickens. Grossly, thymic atrophy is a hallmark of IBD and reflects the destruction of lymphocytes, particularly in the thymic cortex. The affected thymus reveals a pale and mottled appearance compared to its normal color. This discoloration can be due to decreased blood supply and lymphoid depletion within the lymphoid follicles of the thymus. Microscopically, hemorrhages with mild to moderate depletion of lymphoid cells were consistently observed in affected birds. The thymic cortex revealed hypocellularity and reduction in the overall size of the thymus. The chronic inflammation persists in the thymus, leading to the infiltration of inflammatory cells such as macrophages, lymphocytes and plasma cells. These infiltrates must be present in the interstitial spaces and may contribute to ongoing tissue damage. The prolonged infection and tissue damage can lead to the development of fibrosis, scarring and degeneration with severe lymphoid depletion from thymic follicles. More or less similar changes have been reported by Jung *et al.* (2014) [5] in infectious bursal disease in broiler chickens. Microscopically, lymphoid depletion was observed in all the lymphoid organs like spleen, bursa and thymus of the affected birds which were more pronounced in chronic cases. These lesions observed in this study are in line with those observed by Mardani *et al*. (2018) [6] in the affected birds. The kidneys on gross examination appeared enlarged and congested with varying degrees of haemorrhage. The affected kidneys also exhibit mottled appearance due to changes in the renal tissue caused by renal tubular necrosis. The prolonged infection and renal damage can lead to thinning of the renal cortex which can be observed upon gross examination. The development of fibrous tissue in the kidneys of the affected birds. Histopathologically, the kidneys of affected birds revealed vascular congestion and nephritis associated with fibroplasia. The kidney also revealed degeneration, desquamation and coagulative necrosis. The other pathological changes observed in affected kidneys include cellular swelling, vacuolar change and denudation of epithelial lining of convoluted and collecting tubules which with lesion observed by Zekarias *et al.* (2018) [14] in broiler chickens. Grossly, the liver of affected birds revealed congestion with petechial hemorrhages on the surface which can be due to the fragility of blood vessels caused by the viral infection. The focal areas of necrosis can be observed on the surface of liver which might be due to direct viral damage to the liver or secondary to compromised blood supply caused by the viral infection. Microscopically, the liver of the affected birds showed sinusoidal congestion and hepatocellualar degeneration with diffused areas of necrosis characterized by pyknosis and karyolysis in hepatocytes. The other pathological changes observed in the affected liver include coagulated necrosis, degeneration hepatic hemorrhage, hepatocellualar swelling and cytolysis.. Similar changes have been reported in infected birds by Jackwood *et al.* (2017) [4] in infectious bursal disease in broiler chickens.

Gross lesions of broiler chickens infected with Infectious Bursal Disease

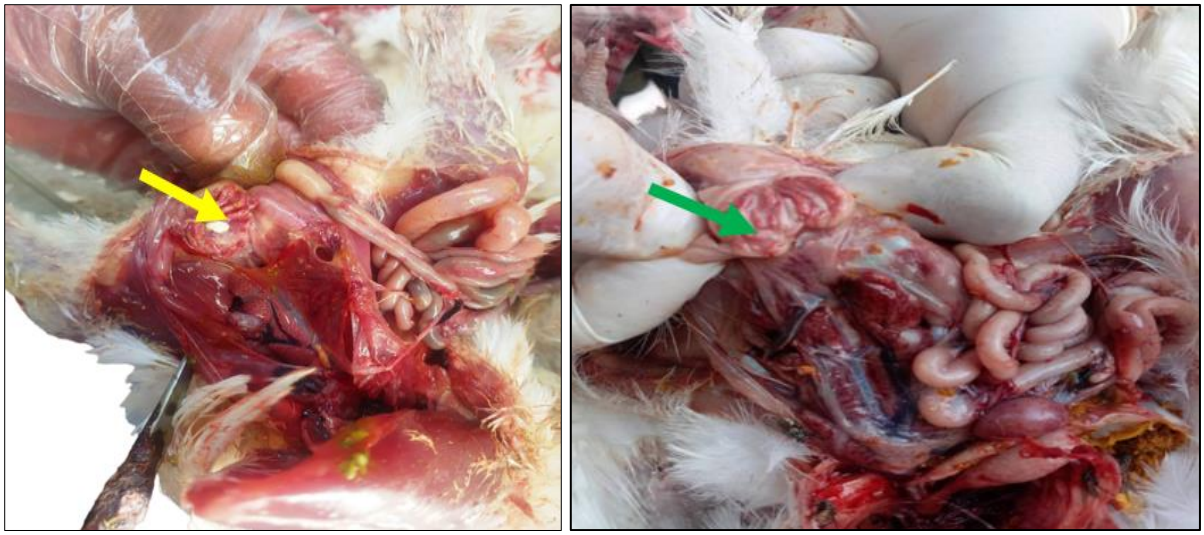


Fig A: Infectious Bursal Disease affected bird showing hemorrhagic bursa of fabricus

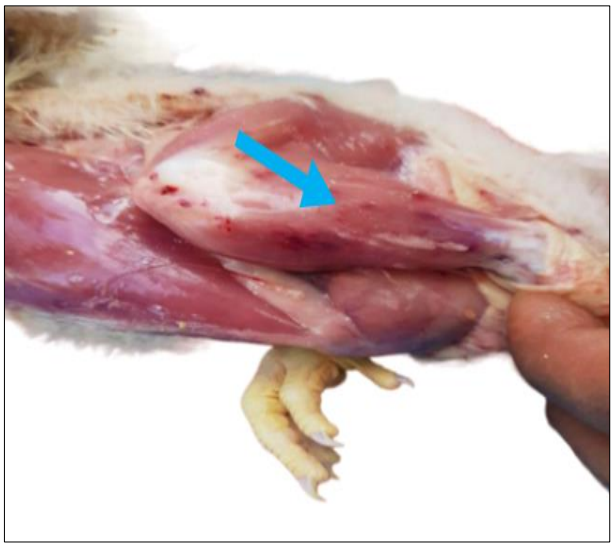


Fig B: IBD affected bird showing haemorrhages on thigh muscles

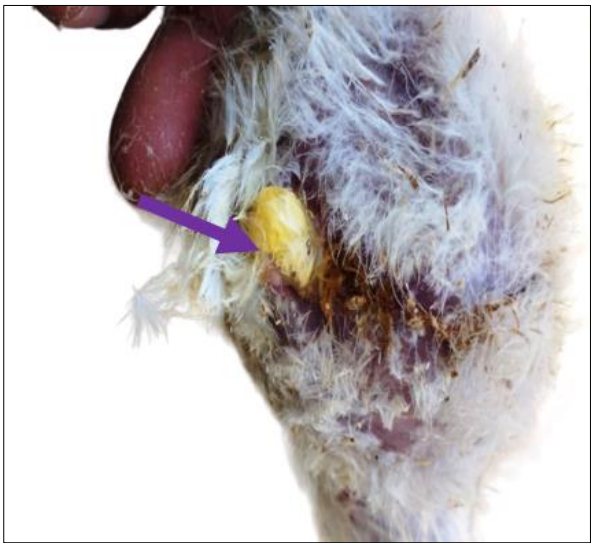


Fig C: Birds with a clinical signs of vent picking had gross lesion suggestive of Infectious bursal disease

Plate 1: Histopathology of Bursa of Fabricius

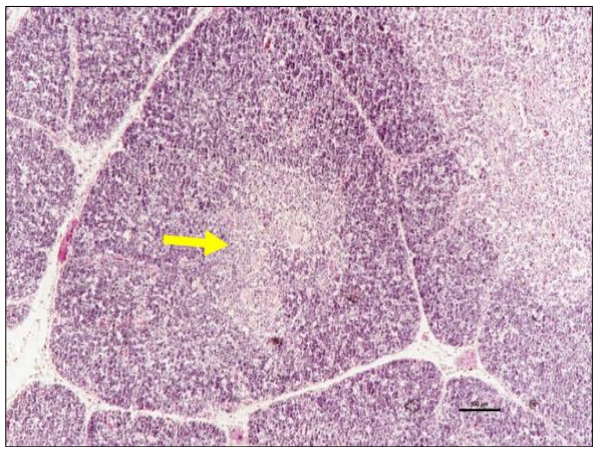


Fig 1a: Photomicrograph revealing depletion of lymphoid tissue from the lymphoid follicles of Bursa of Fabricius

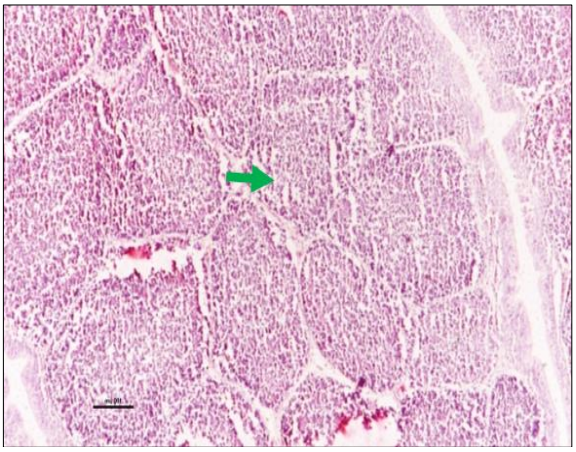


Fig-1b: Photomicrograph revealing reduction in the size of the bursal follicles

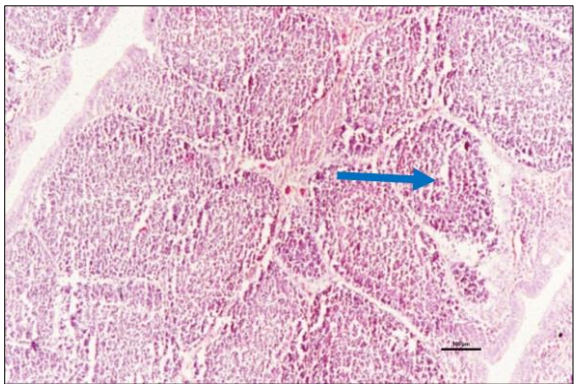


Fig 1c: Photomicrograph revealing degenerative changes in lymphoid follicles of Bursa of Fabricius

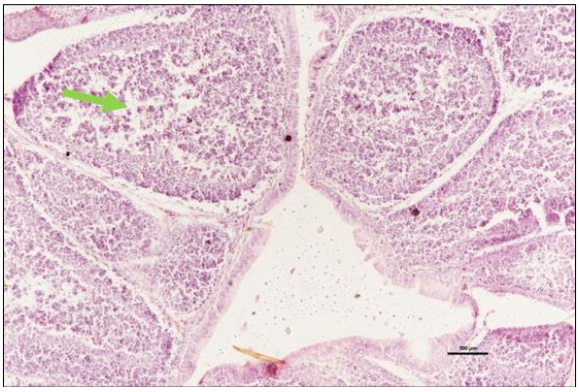


Fig 1d: Photomicrograph revealing necrotic changes in lymphoid follicles of Bursa of Fabricius

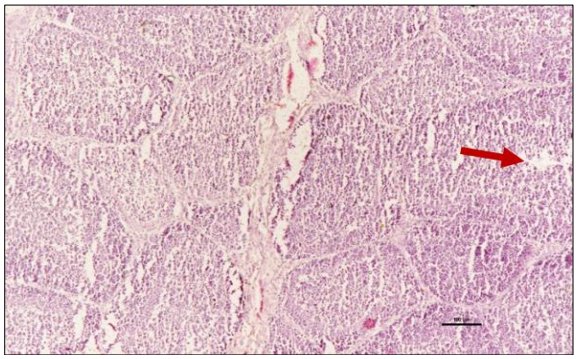


Fig 1e: Photomicrograph revealing decrease in the lymphoid cells with fibrotic bursal follicles

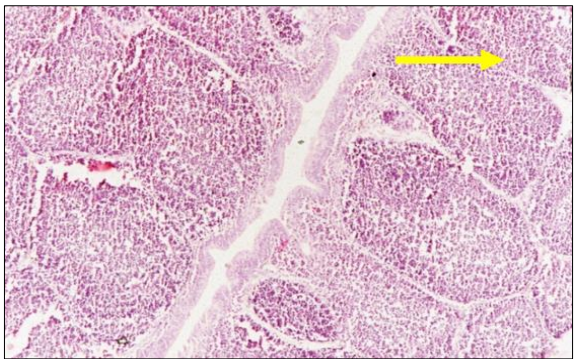


Fig 1f: Photomicrograph revealing edematous and necrotic bursal follicles with an influx of inflammatory cell

Plate 2: Histopathology of Spleen

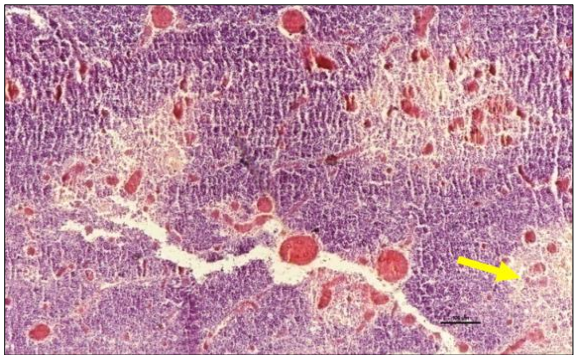


Fig 2a: Photomicrograph revealing depleted lymphoid tissue in the splenic follicles

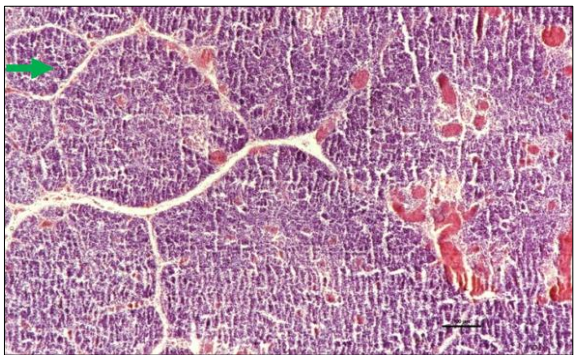


Fig 2b: Photomicrograph revealing reveals splenic atrophy with follicular degeneration

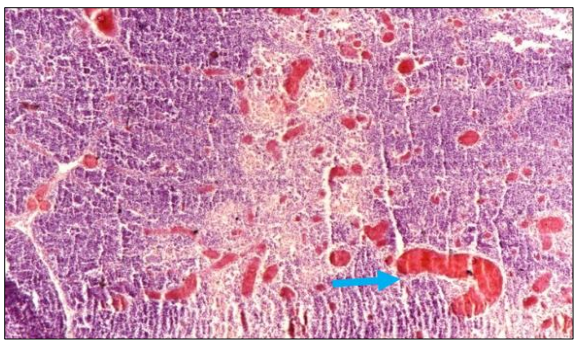


Fig 2c: Photomicrograph revealing congestion in the red pulp with erythrocytes

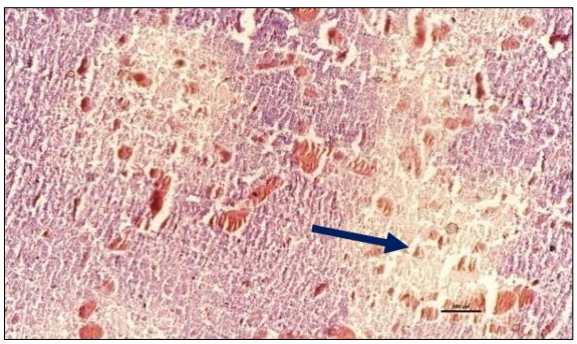


Fig 2d: Photomicrograph revealing severe lymphoid depletion in the white pulp of spleen

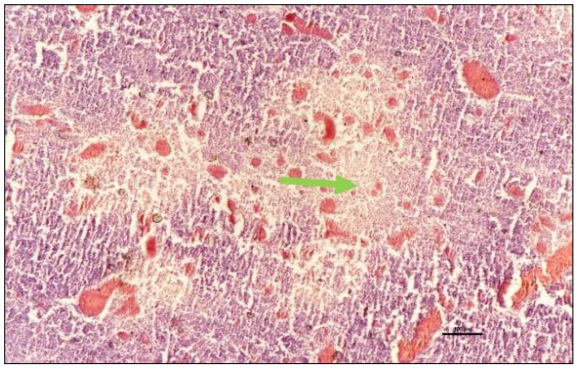


Fig 2e: Photomicrograph of spleen revealing degeneration and destructed lymphoid tissue in the white pulp

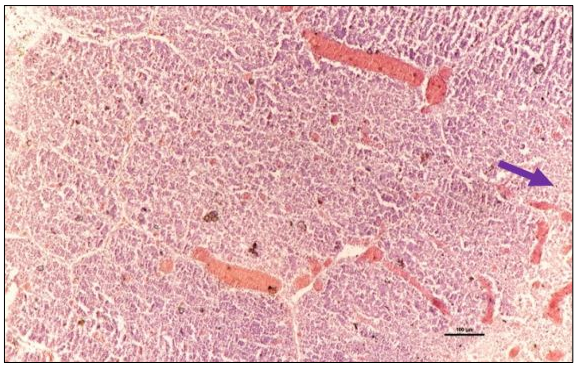


Fig 2f: Photomicrograph revealing necrotic changes in the white pulp of the spleen

Plate 3: Histopathology of Thymus and Lungs

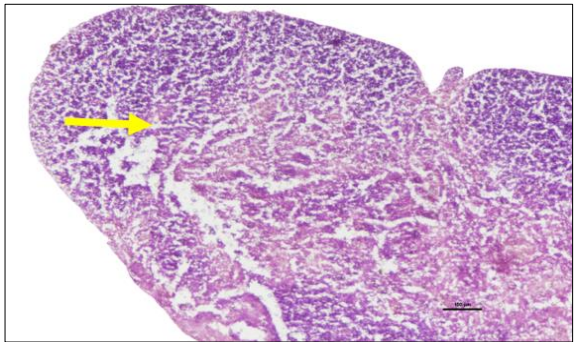


Fig 3a: Photomicrograph of Thymus revealing necrosis within the thymic follicles

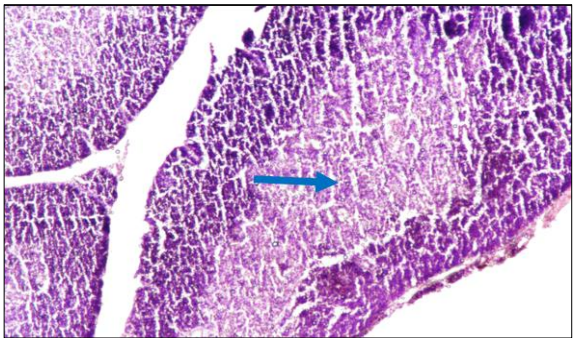


Fig 3b: Photomicrograph of Thymus revealing lymphoid depletion from the lymphoid follicles

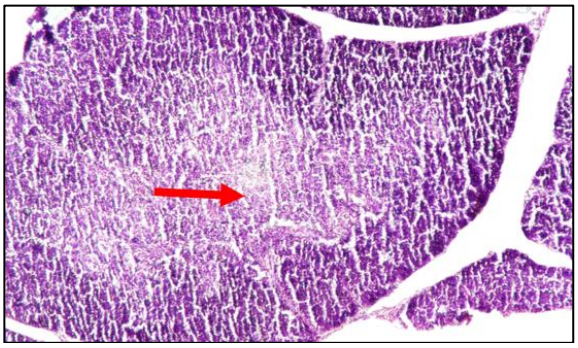


Fig 3c: Photomicrograph revealing hypocellularity of the lymphoid follicles of Thymus

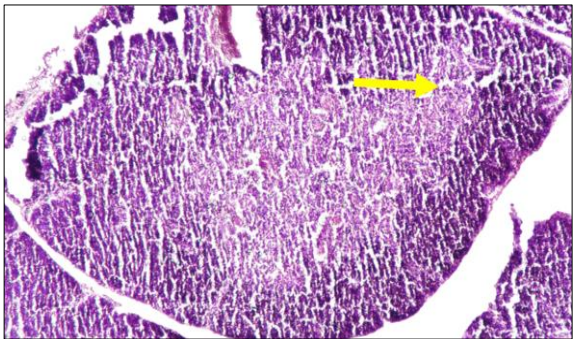


Fig 3d: Photomicrograph of Thymus revealing degeneration with severe lymphoid depletion from thymic follicles

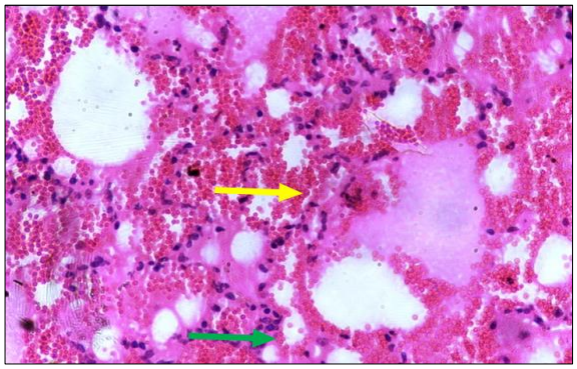


Fig 3e: Photomicrograph of the lung indicating the presence of erythrocytes (yellow arrow) within the hemorrhagic area and cellular infiltration in the affected tissue with (green arrow)

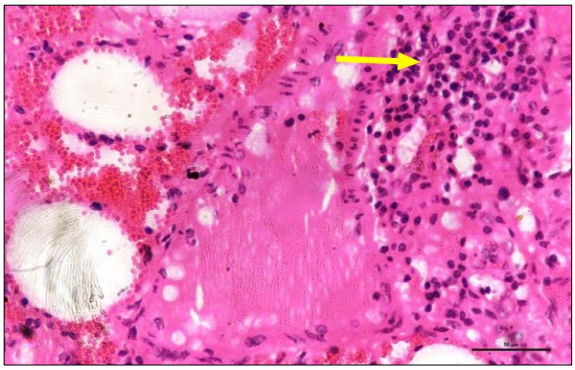


Fig 3f: Photomicrograph of the lung revealing Pneumonia as it is marked by the infiltration of inflammatory cells into the alveoli and interstitial spaces of the lung tissue

Plate 4: Histopathology of Kidney

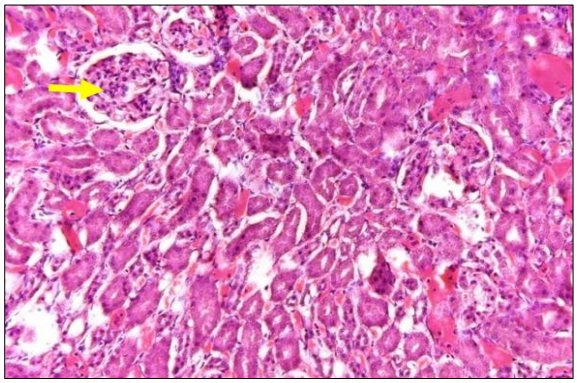


Fig 4a: Photomicrograph of Kidney revealing glomerular congestion with degeneration

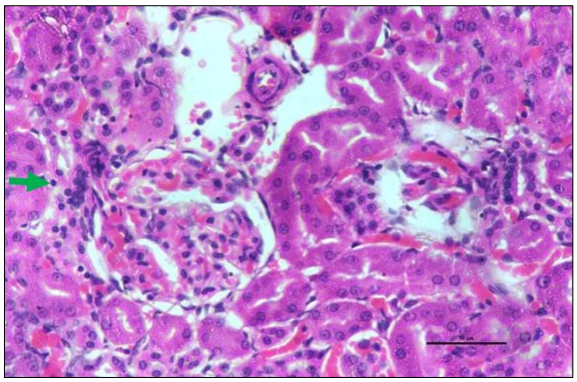


Fig 4b: Photomicrograph of Kidney revealing Interstitial nephritis

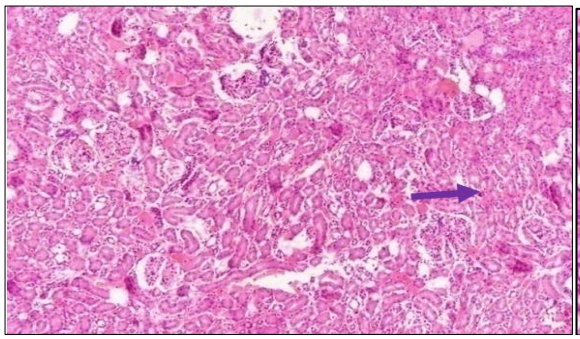


Fig 4c: Photomicrograph of Kidney revealing renal tubular necrosis

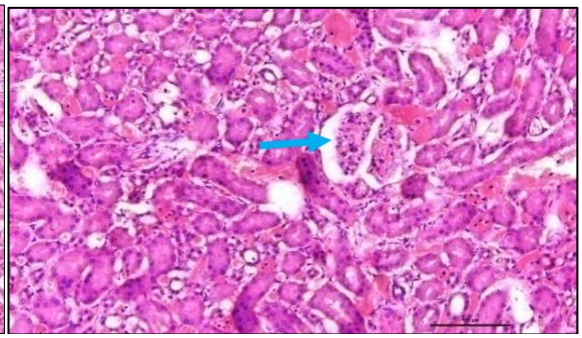


Fig 4d: Photomicrograph of Kidney revealing atrophy of the glomerular

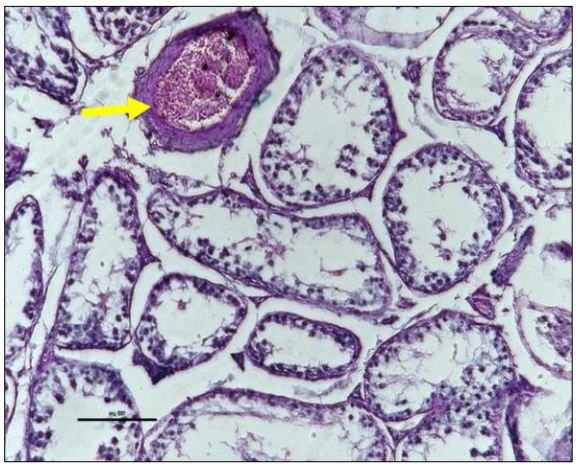


Fig 4e: Photomicrograph of Kidney revealing congested blood vessel with thickening of the wall of blood vessel

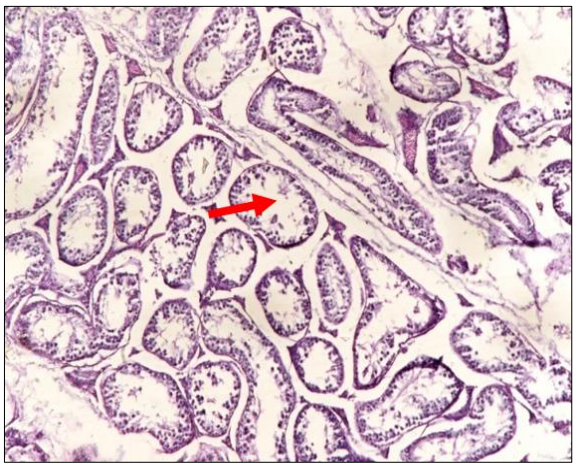


Fig 4f': Photomicrograph of Kidney revealing dilatation of the renal tubules

Plate 5: Histopathology of Liver

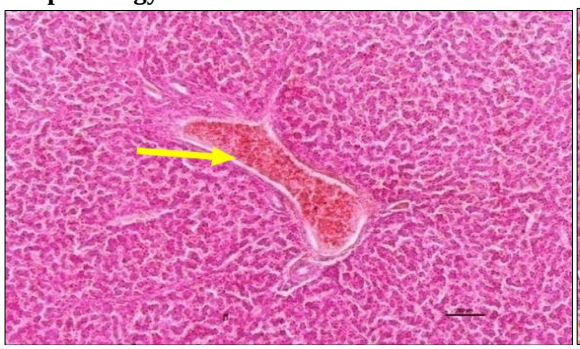


Fig 5a: Photomicrograph of Liver revealing congestion in the blood vessel

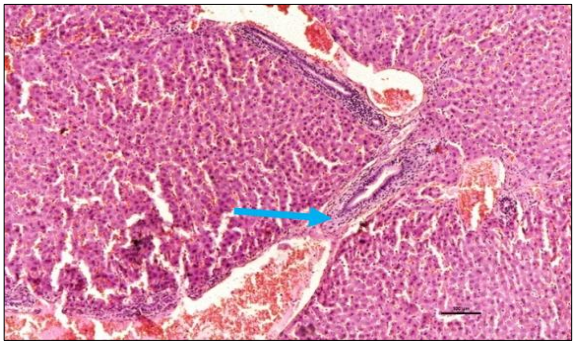


Fig 5b: Photomicrograph of Liver revealing hepatocellular degeneration with thickening of the wall of blood vessel

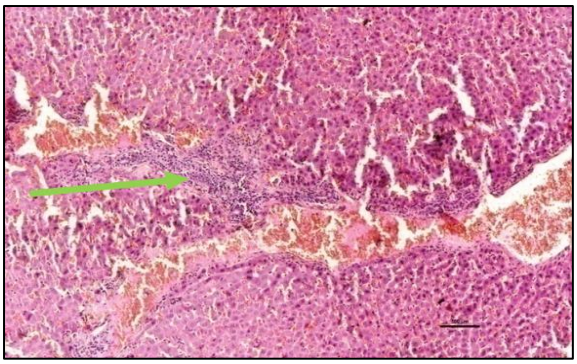


Fig 5c: Photomicrograph of Liver revealing cellular infiltration with congestion

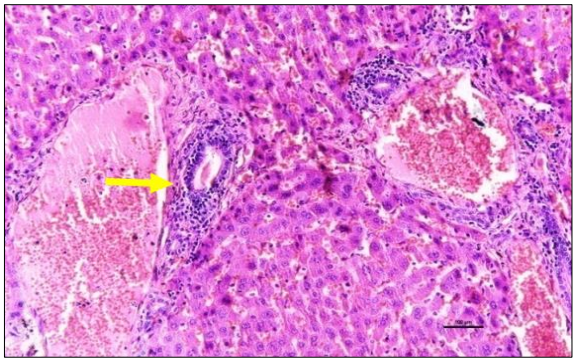


Fig 5d: Photomicrograph of Liver revealing congestion with pervascular infiltration of heterophils

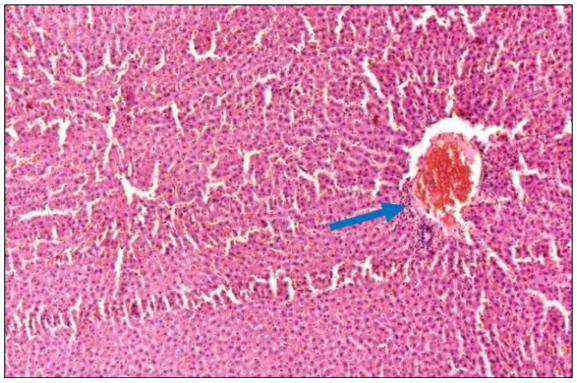


Fig 5e: Photomicrograph of Liver revealing degeneration of the hepatocytes around the congested central vein

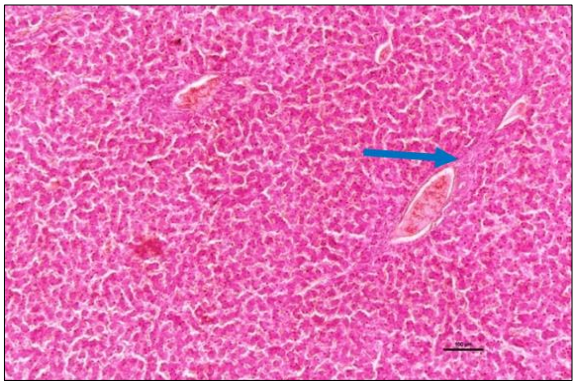


Fig 5f: Photomicrograph of Liver revealing necrosis around the wall of the congested blood vessel

Conclusion

Infectious bursal disease is one of the highly prevalent diseases in commercial broiler farms of Kashmir Valley. This study was designed to assess the different clinical signs, hematological parameters and pathomorphology of Infectious bursal disease of affected broiler chickens. In this study, marked haematological alterations were observed in the infected chickens. Among the different histological changes the higher frequencies of changes were found as destruction of lymphoid follicles in lymphoid organs of infected chickens which were suggestive of immunosuppressive and immunomodulatory effects of birna virus in broiler chickens.

Acknowledgments: The authors are highly thankful to the Dean of F.V.Sc & AH for providing the necessary facilities

Conflict of Interest

All authors declare no conflict of interest

References

1. Bancroft D, Layton C. Theory and Practical of Histological Techniques. Churchill Livingstone; c2014.
2. Balamurugan V, Kataria M, Bhattacharya K. Antigenic evolution and Pathomorphology of infectious bursal disease virus in India. Virology. 2014;159(11):2869-2876.
3. Cortes L, Montassier H, Sakamoto M. Molecular characterization of infectious bursal disease virus from Brazil. Brazilian Journal of Microbiology. 2005;36(1):13-20.
4. Jackwood D, Sommer E. Immunohistochemical detection of infectious bursal disease virus antigen in paraffin-embedded tissues. Avian Diseases. 2017;51(3):635-639.
5. Jung A, Rautenschlein S, Kraemer C. Pathogenicity of three recent infectious bursal disease virus field isolates. Avian Pathology. 2014;41(5):461-469.
6. Mardani K, Assarehzadegan A, Sharifzadeh A. Pathogenicity of infectious bursal disease virus in lymphoid organs of experimentally infected chickens. Veterinary Research Forum. 2013;4:85-86.
7. Mahmood S, Siddique M, Yaqub T, Abbas Z. Pathology and hematological alterations in infectious bursal disease virus-infected broiler chicken. Tropical Animal Health and Production. 2018;50:341-348.
8. Muller H, Mund E. Elicitation of protective immune responses against infectious bursal disease virus after DNA immunization with the whole inactivated virus. Virology. 2012;86(8):3842-3851.
9. Panigrahy B, Rowe D, Corrier E. Haematological values and changes in blood chemistry in chickens with Infectious Bursal Disease. Veterinary Science Research. 1996;40:86-88.
10. Rautenschlein S, Suhr K, Kraemer C. Pathogenicity of infectious bursal disease viruses in White Leghorn chickens is influenced by their genomic sequences and their compatibility with host immune competence. Avian Pathology. 2016;45(3):366-378.
11. Singh S, Taneja K. Competitiveness of Indian Poultry Industry in Global Context. Indian Journal of Animal Sciences. 2018;88:587-593.
12. Sanjit K, Sanjiv R. A Study on Mortality Pattern of Poultry in and around Ranchi. International Journal of Current Microbiology and Applied Sciences. 2018;7:713-716.

13. Sohini D, Dinesh C, Pathak N, Mohan C. Prevalence and impact of Infectious bursal disease virus in chickens. Veterinary Medicine. 2019;10:85–97.
14. Zekarias B, Fitzgerald D, Riddell C. Pathogenesis of Infectious Bursal Disease in chickens experimentally infected with very virulent infectious bursal disease virus. Veterinary Pathology. 2018;45:535-546.