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Pathomorphological alterations due to pulmonary hypertension syndrome in broiler chicken reared under temperate climatic conditions of Northern Himalayas

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Abstract
Present study was aimed to investigate path anatomical alterations in commercial broiler chickens with spontaneous Pulmonary Hypertension Syndrome (Ascites).Gross lesions included ascites, cyanotic musculature, right ventricular hypertrophy/ cardiomegaly, and congestion, haemorrhages, and oedema of different organs. Histopathologically marked changes were observed in heart, lungs, liver, kidneys, brain, GIT thyroid, adrenal gland and lymphoid organs. the lesions, in general, included varying degrees of congestion, haemorrhages and perivascular leakage of lymph / oedema; thickening of vascular walls with medial hypertrophy and cystic dilatation of lymphatics; inflammatory changes with predominantly perivascular mononuclear cell and also heterophil infiltration in; neuronal and purkinje cell degeneration and lymphoid depletion. It was concluded that PHS caused marked patho-anatomical changes in all organs. The inflammatory changes observed suggest it as a model for PAH in humans.

Keywords: Broiler chicken, pathology, pulmonary hypertension syndrome

Introduction
Ascites/ pulmonary hypertension syndrome (PHS) syndrome in fast growing broilers is the terminal consequence of progressive pulmonary arterial hypertension leading to cardiac decompensation. (Wideman *et al.*, 2007) [27]. Cardiomegaly associated with right ventricular hypertrophy and dilationhas been reported consistent feature in PHS (Moayyedian *et al.*, 2011; Davis *et al.*, 2012) [26, 5]. Infact anatomical changes in heart can be detected before a bird exhibits gross ascites syndrome. It is generally accepted that RV/TV index greater than 0.30 is indicative of right ventricular hypertrophy, pulmonary hypertension and, ultimately, ascites syndrome (Wideman *et al.*, 1998) [45]. The syndrome is multifactorial involving complete epidemiological triad including nutritional, management, environmental, and genetic influences (Baghbanzadeh and Decuypere, 2008; Wideman *et al.*, 2013) [1, 44]. Cold exposure and reduced oxygen tension are two important factors favouring development and progression of the disease condition (Julain, 2005; Hassanzadeh *et al.*, 2008) [6, 1]. Owing to high altitude and temperate climate in Kashmir valley, the birds in closed houses frequently encounter the conditions like cold and reduced oxygen tension, and hence are prone to develop ascites. However, many intrinsic and extrinsic factors have been incriminated, warranting detailed path biological studies in given environmental conditions (Hassanzadeh, 2009) [13]. Present study was aimed to investigate detailed pathology of Pulmonary Hypertension Syndrome in broiler chicken.

Materials and Methods
Samples comprised of mortalities from various poultry farms operating in Srinagar, Ganderbal, and Budgam, Districts along with their adjoining areas brought to Division of Veterinary Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, for post-mortem examination during April 2014 to June 2015. Pulmonary hypertension syndrome was diagnosed on the basis of presence of ascites or right ventricular hypertrophy. The carcasses were subjected to a thorough systematic necropsy examining and gross lesions were

recorded. Also, total ventricular weight and right ventricular weights were taken for 14 cases each PHS/ascitic chicken and normal healthy chicken. Representative samples of liver, heart, caeca, lung, kidney, intestines, bursa, spleen, thymus, thyroid, brain, proventriculus, pancreas, ceaca and intestines were collected from ascetic birds, subsequently preserved and fixed in 10% buffered formalin for histopathological examination and processed by routine paraffin embedding technique employing alcohol and acetone as dehydrating agent, choloroform as clearing agent and paraffin wax of congelling temperature 60 ℃. The sections of 5µm thickness were cut and stained with Harris haematoxylin and eosin technique for routine examination (Luna, 1968) [23].

Results

The carcasses revealed varying degree of abdominal distension due to ascites. In high grade ascites abdominal musculature was thin and transparent. The colour of breast musculature varied from pale to dark/cyanotic. Haemorrhages ranging from ecchymosis to suffusions were occasionally observed in thigh musculature. Transudation into coelomic cavity varied from slight quantity of the fluid in caudal coelomic cavity to large quantities occupying all chambers of coelomic cavity. In most of the cases, fluid was coagulated forming gelatinous masses, loose or adhering to viscera (Fig. 1).

Cardiovascular System

Grossly normal to increased quantities of pericardial fluid was observed. Heart invariably contained blood clot in both ventricles and atria (Fig 2a). Other changes included right ventricular hypertrophy with firm consistency of myocardium (Fig 2b) which was frequently associated with dilation of right ventricle, right atrium, sinus venosus, and cardiac vena cava, leading to cardiomegaly (Fig 2c). The mean total ventricular weight (Tvw), right ventricular weight (Rvw), and Rvw: Tvw ratio in normal and ascitic birds were 5.4423 ± 0.30419 vs 5.7229 ± 0.40899, 1.5146 ± 0.09676 vs 1.9562 ± 0.12555 and 0.2775 ± 0.00515 vs 0.3440 ± 0.00456 respectively. In general these weights were significantly higher in ascitic birds (Table 1). Occasionally haemorrhages ranging from petechiae to suffusions were noted on pericardium. In cases complicated with colibacillosis fibrinous pericarditis was noted (Fig 2d).In general blood vessels including cutaneous vessels, major blood vessels in the coelome, and mesenteric vessels were distended with blood (Fig 3).

Table 1: Total Ventricular Weight (Tvw), Right Ventricular Weight (Rvw) and Rvw: Tvw ratio of healthy and PHS affected broiler chicken (Mean ± SE)

	N	Tvw (gm)	Rvw (gm)	Rvw: Tvw
Healthy Chicken	14	5.4423 ± 0.30419 (4.10 - 8.78)	1.5146 ± 0.09676 (1.02 - 2.57)	0.2775 ± 0.00515 (0.24 - 0.30)
Ascitic Chicken	14	5.7229 ± 0.40899 (4.21 - 8.78)	1.9562 ± 0.12555 (1.49 - 2.87)	0.3440 ± 0.00456 (0.32 - 0.38)

Values in braces are range

Histopathologically, heart revealed mild to severe vascular congestion, focal to diffuse areas of haemorrhage in epicardium and myocardium, and varying degrees of myocardial degeneration. Focal to diffuse mononuclear and heterophil infiltration was observed in myocardium, epicardium and pericardium. Heterophils predominated in birds dead during 3rd week whereas mononuclear were predominant cell type in birds dying during later stage (Fig 4a-c). Occasionally, oedema was observed in birds with mild ascites. In birds with moderate to severe ascites vascular wall was frequently thickened with lateral cystic dilatation of lymphatics and leakage of lymph into the surrounding interstitial space (Fig 4d).

Lungs

Grossly lungs revealed varying degrees of congestion, and haemorrhages. In severely affected cases lungs were oedematous and voluminous. Sanguineous fluid oozed from the cut surface (Fig 5a).Occasionally pneumonic consolidation was observed. Trachea and bronchi were full of dirty frothy exudate. In some cases, caseous plug was present in the trachea. In general, air sacs were clear.

Histopathologically, lungs showed moderate to severe congestion of interstitial vessels and blood capillaries, varying degrees of haemorrhages and interstitial oedema. Pulmonary arterioles appeared thickened with medial cystic dilatations. Frequently perivascular leukocytic infiltration, consisting of heterophils admixed with mononuclear cells, was observed. Occasionally plexiform lesions were observed (Fig 5b). In some cases par bronchi, air spaces and air capillaries appeared normal, but frequently broncho interstitial pneumonia and haemorrhagic bronchopneumonia were seen. Broncho interstitial pneumonia was characterized by thickening of interstitium with heterophilic infiltration admixed with mononuclear cells and presence of inflammatory exudates in parabronchi, (Fig 5c). Haemorrhagic bronchopneumonia was associated with presence of necrotic material along with haemorrhagic inflammatory exudates including heterophils and mononuclear cells in the lumen of parabronchi, mesobronchi, and metabronchi, proliferation of fibrous connective tissue in the interstitum, and thickening of the air space wall due to hypertrophy of muscle trabiculae were evident (Fig 5d).Occasionally, osseous metaplasia was noted.

Liver

Grossly, liver was swollen with varying degrees of congestion, oedema and haemorrhages. Frequently the liver surface was smooth but occasionally liver was irregular giving lobulated appearance. In two cases rupture of liver with large blood clot adhering to ruptured site was seen. Occasionally, fibrinous clots of ascitic fluid covered the liver lobes completely (Fig 6).

Histopathologically, liver revealed mild vascular congestion, hepatocellular degeneration with increased eosinophilia and vacuolar changes. Occasionally rounding and individualization of hepatocytes and focal necrosis with infiltration of heterophils and mononuclear cells were observed. Necrosis was also seen in the perivascular region. Glisson's capsule was variedly thickened, revealing cystic dilatation of lymphatics and leukocytic infiltration. Focal

necrosis and focal haemorrhages were noted. Kuffer cell proliferation was noted which was more prominent in sub-capsular region. Occasionally hepatocytes revealed eosinophilic granular change. Diffuse mononuclear cell infiltration frequently admixed with heterophilsand leakage of lymph from cystic lymphatics were seen (Fig 7).In one outbreak basophilic, intracytoplasmic inclusions were observed.

Kidneys

Grossly, kidneys were swollen and oedematous distending out from the bony sockets. Frequently they were congested with dark discolouration. However, occasionally pale to greyish white mottling was noted (Fig 8a, b). Varying degrees of haemorrhages were also seen.

Histopathologically, kidneys showed mild to severe vascular congestion, focal to wide spread haemorrhages in cortex and medulla, nephrosis, and occasional capsular thickening. Nephritic changes ranged from swelling of tubular epithelium to denudation of cortical tubular epithelium with formation of hyaline and granular casts in lumen. Occasionally swollen glomeruli with vacuolation podocytes and focal nephritis were seen. Inflammatory reaction was characterized by mononuclear and heterophilic infiltration (Fig 8c, d).

Brain

Grossly, brain generally appeared normal. Occasionally mild to severe congestion of meningeal vessels was noted. Histopathologically brain revealed varying degree of vascular congestion in meninges, choroid plexus, cerebrum and cerebellum. In cerebral cortex the space around the microvasculature was dilated. Varying degree of degeneration and necrosis of both granule as well as large and small pyramidal cells was seen. The necrotic cells were shrunken with increased cytoplasmic basophilia, and nuclear pyknosis, karyorhexis and karyolysis associated with dialatation of neuropil, mild satellitosis and neuronophagia (Fig 9a). Neuronal degeneration and necrosis, denoted by nuclear condensation and neuropil dilatation associated with satellitosis and neuronophagia, were also observed in thalamus (Fig 9b). Occasionally marked gliosis was noted (Fig 9c). Medullary tracts revealed mild demyelination (Plate 27B). Cerebellum revealed congestion and marked purkinje cell degeneration (Fig 9d).

Gastrointestinal tract

Grossly, proventriculus occasionally revealed oedema and thickening of mucosa. Marked congestion of intestines was a consistent feature. Lumen was filled with semisolid or fluid contents (Fig 10a). Often diffuse haemorrahges were seen in duodenum and jejunum. Frequently mucosa presented a velvety appearance.

Microscopically, proventriculus revealed mild to severe vascular congestion and oedema in submucosa and mucosa. Occasionally mononuclear cell and heterophil infiltration was evident in mucosa (Fig 10b). Intestines showed mild to severe congestion and oedema of mucosa which frequently extended to submucosa. Frequently, mucous degeneration of goblet cells and varying degrees of heterophils and mononuclear cellinfiltrationsin lamina propria was seen (Fig 10c,d).

Thyroid

Grossly, thyroid was oedematous, congested and swollen. Frequently dark discolouration was observed. Microscopically, thyroid revealed mild to severe vascular congestionin the interstitium and capsule. Thyroid follicles of varying sizes lined with essentially squamous type epithelium were filled with colloid. Occasionally active follicles with cuboidal to columnar epithelium and peripheral vacuolation of colloid were seen (Fig 11a). Occasionally mononuclear cell infiltration was noted especially in the perivascular region.

Parathyroid Gland

No gross changes were observed in Parathyroid. Microscopically, parathyroid revealed normal densely packed cords of basophilic cells. Occasionally severe vascular congestion was observed (Fig 11b).

Adrenal Gland

No gross or histomorphological alterations were observed in adrenal gland, except for marked vascular congestion, focal haemorrhages in medulla and cortex, and occasional cortical necrosis.

Pancreas

No gross changes or histomorphological alterations in either exocrine glands or islets of Langerhans were seen, except occasional vascular congestion (Fig 11c, d).

Spleen

Grossly, spleen in most cases was enlarged, congested with varying degrees of haemorrhages. Occasionally severe oedema with mottling was seen (Fig 12a). Microscopically spleen revealed mild to severe congestion, areas of haemorrhage and mild to severe lymphoid depletion. In some cases multifocal necrosis and thickening of vascular wall with lateral cystic dilatations with leakage of lymph into the peripheral interstitum was evident (Fig 12b). Spleenic capsule was thickened. Frequently mononuclear and heterophil infiltration was seen in necrotic areas and capsule.

Thymus

Grossly, thymus lobes generally revealed congestion. Frequently oedema and swelling was also seen. Microscopically, thymus revealed varying degrees of vascular congestion and lymphoid depletion. Also, varying degree of oedema and infiltration of heterophils in Hessel's corpuscles were seen.

Bursa

Grossly, bursa of Fabricious was oedematous, swollen with varying degrees of congestion and haemorrhages. Lumen often contained serous exudates.

Microscopically, bursa revealed mild vascular congestion, focal haemorrhages, interstitial oedema, varying degrees of lymphoid depletion, and infiltration of heterophils admixed with mononuclear cells was seen in mucosa and submucosa. Degeneration of mucosal epithelium and presence of cystic spaces in the mucosa were consistently seen (Fig 12c,d).

Discussion

Dark/ cyanotic discolouration of muscles may be attributed to PHS associated hypoxia (Maxwell *et al.*, 1986) [25]. Ascites has been considered as the most prominent lesion in clinical cases of PHS (Dimple *et al.*, 2005) [6]. It has been attributed to systemic hypotension caused by dilation of major veins stimulating rennin-angiotensin-aldosterone cascade leading to sodium and water retension by kidneys, and hence venous

congestion, increasing hydrostatic pressure and ascitic transudation (Wideman *et al*., 2013) [44].

Cardiomegaly associated with right ventricular hypertrophy and dilation has been consistently reported (Olkowski *et al*., 2003; Davis *et al.*, 2012) [28, 5]. Moayyedian *et al.* (2011) [26] reported cardiac enlargement due to dilatation of the right atrium, sinus venosus, cardiac vena cava and right ventricle. In cases of early mortality, transudate in coelomewas not marked and right ventricular hypertrophy was not associated with dilatation/ cardiomegaly. However, increase in RV/TV ratio was consistently noted in subclinical as well as clinical cases. This is consistent with the reports by other workers suggesting RV/TV index greater than 0.30 as indicative of right ventricular hypertrophy, pulmonary hypertension and, ultimately, ascites syndrome (Reza *et al*., 2008; Tekeli, 2014) [30, 36]. Theright ventricular hypertrophy may be attributed to increased pulmonary arterial resistance (Lorenzoni *et al.*, 2008; Lorenzoni and Wideman, 2008; Wideman *et al.*, 2010; West *et al.*, 2010) [17, 19, 41, 39]. The cardiomegaly due to right ventricular dilatation may be attributed to cardiac decompensation associated with weakening of ventricular muscle (Olkowski, 2007; Lorenzoni *et al.*, 2008; Wideman *et al*., 2013) [27, 17, 19, 44]. Histopathological alterations observed in heart are in congruence with earlier reports (Sijun *et al.*, 2002; Dimple *et al*., 2005) [34, 6]. However, Habib-ur-Rehman *et al*. (1999) [10] reported that heart from ascitic birds did not show any significant lesion except mild congestion. The differences may be attributed to stage and duration of ailment as well as concurrent clinical or subclinical affections. The degenerative changes may be attributed to the consistent high cardiac pressure load on account of increased pulmonary resistance due to limited pulmonary reserves (Wideman *et al.*, 2010; West *et al.*, 2010) [41, 39], pulmonary arterial-constriction (Zhou *et al*., 2008; Hassan pour *et al*., 2010) [12, 46], increased flow of blood to lungs under metabolic demand (Lorenzoni *et al.*, 2008) [17, 19] and increased blood viscosity due to elevated haematocrit (Wideman *et al*., 2013) [44], as well as increased volume load on account of systemic hypotension leading to increased venous return(Wideman *et al*., 2007, 2013) [42, 44], besides the direct effect of hypoxaemia.

Congestion, haemorrhage and oedema of lungs observed grossly and in histopathological sections, are consistent with earlier reports (Maxwell *et al.*, 1986; Biswas *et al.*, 1995; Habib-ur-Rehman *et al.*, 1999) [25, 4, 10]. Lungs besides being the primary target of PHS, have reduced proportionate weight in fast growing chicken when compared with slow growing lines which acts as a predisposing factor for development of pulmonary arterial hypertension (Malan *et al.*, 2003) [24]. Higher pulmonary arterial pressures and pulmonary vascular resistances with or without differences in cardiac output has been observed to be a consistent feature in PHS susceptible broiler chicken (Lorenzoni *et al.*, 2008; Kluess *et al.*, 2012) [17, 19, 16]. Histopathological lesions in lungs are consistent with earlier reports (Biswas *et al.*, 1995; Habib-ur-Rehman *et al*., 1999; Dimple *et al.*, 2005) [4, 10, 6]. Medial hypertrophy and intimal proliferation of pulmonary arterioles has been consistently reported in broilers with PHS/PAH but is unevenly distributed (Wideman and Hamal, 2011; Bautista-Ortega and Ruiz-Feria, 2012; Weidner *et al*., 2012) [43, 2, 38]. Plexiform lesions have been observed in PAH-susceptible lines as early as first week post hatch. The lesions develops from an early compact lesion having relatively homogeneous endothelial matrix and sparse vascular channels into larger mature plexiform lesions exhibiting numerous vascular channels and multiple cell types including connective tissue, inflammatory cells, and proliferating intimal cells (Wideman *et al*., 2011; Wideman and Hamal, 2011; Kluess *et al*., 2012) [43, 16]. Immunohistochemical studies have confirmed that the plexiform lesions of broilers contain immune/inflammatory cells viz. monocytes/macrophages, cytotoxic lymphocytes, B cells, and MHC class II cells; and express antiproliferative factors von Willebrand factor, a smooth muscle actin, vascular endothelial growth factor and its type 2 receptor, hypoxia inducible factor-1a, survivin, and tenascin (Hamal *et al*., 2012) [11]. Predominance of heterophils in some cases may be attributed to concurrent subclinical infections.

Varying degrees of liver lesions have been reported in chicken affected with PHS depending on degree of ascites and associated complications (Habib-ur-Rehman *et al.*, 1999; Dimple *et al.*, 2005; Davis *et al.*, 2012) [10, 6, 5]. Histopathological alterations in liver were similar to the changes reported by other workers (Dimple *et al.*, 2005; Davis *et al.*, 2012) [6, 5]. Degenerative and necrotic changes may be attributed to hypoxaemia and venous congestion associated with progressive development of right sided congestive heart failure (Wideman *et al*., 2013) [44]. Sijun *et al.* (2002) [34] reported that histomorphologic changes of the lymphatic system of the liver in ascitic broilers were edema, thickening, and cellular proliferation of hepatic capsule; lymph embolism, and lymphatic plasma retention in lymphatic cysts of hepatic capsule. Leukocytic infiltration particularly heterophils and mononuclear cells have been frequently reported (Maxwell *et al.*, 1986; Habib-ur-Rehman *et al*., 1999) [25, 10]. Dimple *et al.* (2005) [6] reported that ascitic birds showed acute to chronic perihepatitis along with fibrosis and pseudolobulation in liver. The inflammatory response may be attributed to oxidative stress associated with decreased antioxidants leading to elevated free radicals (Wang *et al.*, 2012) [37].

Grossly kidneys were congested, swollen, oedematous and showed haemorrhages, dark discolouration and occasionally greyish white mottling. Histopathologically, kidneys showed vascular congestion, haemorrhages, nephrosis, occasional swollen glomeruli with vacuolation podocytes, focal nephritis, and occasional capsular thickening. Inflammatory reaction was characterized by mononuclear and heterophilic infiltration. The lesions are consistent with earlier reports (Habib-ur-Rehman *et al.*, 1999; Dimple *et al.*, 2005; Davis *et al.*, 2012) [10, 6, 5]. The renal morphological implications of PHS may be attributed to hypoxaemia leading to increased systemic blood flow, oxidative stress, and metabolic acidosis (Wideman *et al*., 2013) [44]. Kidneys being the vital organs playing central role in homeostasis and maintenance of blood pressure, the progressive right side congestive heart failure has severe implications (Lorenzoni *et al.*, 2008; Wideman *et al*., 2013) [17, 19, 44]. The systemic hypotension caused by dilation of major veins stimulates rennin-angiotensin-aldosterone cascade causing sodium and water retention by kidneys leading to vascular congestion further aggravating hypoxaemia (Wideman *et al*., 2013) [44]. Inflammatory changes may be attributed partially to oxidative stress and concurrent infections.

The changes observed in brain may be attributed to consistently aggravating hypoxaemic condition during the pathogenesis of ascites. Ascites in broiler chicken has been invariably associated with increase in blood PCO_2 and decrease in blood PO_2, pH and saturated PO_2 (Malan *et al.*, 2003; Moayyedian *et al.*, 2011) [24, 26]. Scheele *et al.* (2003)

[33] reported that pCO_2 tensions at day 11 affected the incidence of ascitic signs up to week 5 and suggested that high pCO_2 tension in venous blood measured at day 11 was a reliable predictor for ascites susceptibility. Tekeli (2014) [36] reported that ascites in chicks reared at high altitude (1727m) was characterized by significant ($P<0.05$) decrease in blood oximetry parameters viz. SO_2 and FO_2Hb which can be used as indicators of ascites susceptibility in broilers grown at high altitudes.

Gross and histopathological changes in proventriculus and intestines including congestion, haemorrhages and oedema have been consistently reported (Habib-ur-Rehman *et al.*, 1999) [10]. The inflammatory changes, with mononuclear cell and heterophil infiltration, have been reported by Dimple *et al*., 2005 [6].

The histopathological picture of thyroid was indicative of low activity. Malan *et al.* (2003) [24] reported that the fast-growing chickens had low heat production per kg metabolic body weight (H/W = 0.75) values, lower plasma thyroid hormone compared with the slower-growing lines. The ascites incidence was associated with lower heat production per metabolic body weight and therefore a lower oxygen requirement per metabolic weight. Supplementation with exogenous thyroxin in broilers under experimental conditions has been found to cause significant reduction in percentage of ascites (21.5% / 23% vs 7%), whereas propylthiouracil induce hypothyroidism caused significant increase in appearance of ascites (35%) (Luger *et al.*, 2002) [20]. Ascites has been associated with decrease in thyroxine (T4) and increase in triiodothyronine (T3) concentration (Luger *et al.*, 2001; Guo *et al.*, 2007; Moayyedian *et al.*, 2011) [21, 9, 26].

Histopathological changes in adrenal may be attributed to stress response which plays a critical role in development and progression of PHS (Lorenzoni and Ruiz-Feria, 2006; Ruiz-Feria, 2009; Bautista-Ortega and Ruiz-Feria, 2010; Kluess *et al.*, 2012; Wang *et al.*, 2012) [18, 32, 3, 16, 37]. Luger *et al*. (2003) [22] reported that ascitic chickens revealed a uniquely continuous stress response: expressing an increase ($P\leq0.05$) in plasma corticosterone concentration 2 to 3 week before death.

No gross or histopathological changes were observed in parathyroid and pancreas. The presence of normal islets of Langerhans suggest that the observed increase in blood glucose levels of ascetic birds is mediated through stress hormones.

Degenerative and inflammatory changes have been reported in spleen, bursa, and thymus of ascitic birds (Dimple *et al.*, 2005) [6]. Depletion of lymphoid elements observed in present study may be attributed to stress associated mobilization causing lymphocytic leucocytosis (Ruiz-Feria, 2009; Bautista-Ortega and Ruiz-Feria, 2010; Hassanpour *et al*., 2010; Kluess *et al.*, 2012) [32, 3, 12, 16]. The compromised immunity in ascetic birds is buttressed by the observation of high incidence of concurrent infections.

In general, the vascular changes including congestion, haemorrhages, and perivascular leakage of lymph / oedema in different organs were consistently associated with PHS. This may be attributed generalized vascular response to a cascade of changes initiated by cardio-pulmonary imbalance leading to development of PAH and ascites (Lorenzoni and Wideman, 2008) [17, 19]. Hypoxaemia due to compromised pulmonary blood oxygenation incites compensatory responses including increased haematopoiesis and systemic arteriolar dilation which in turn leads to systemic hypotension, and increased blood flow (Malan *et al.*, 2003; Moayyedian *et al.*, 2011; Wideman *et al*., 2013) [24, 26, 44]. This in turn stimulates rennin-angiotensin-aldosterone cascade causing sodium and water retension by kidneys leading to venous congestion, increasing hydrostatic pressure and ascitic transudation (Lorenzoni *et al.*, 2008; Wideman *et al*., 2013) [17, 19, 44]. Stress hormone response also plays a critical role in haemodynamic changes (Lorenzoni and Ruiz-Feria, 2006; Gomez *et al*., 2007; Zhou *et al.*, 2008; Ruiz-Feria, 2009; Bautista-Ortega and Ruiz-Feria, 2010; Hassanpour *et al*., 2010; Kluess *et al.*, 2012) [18, 8, 46, 32, 3, 12, 16].

Frequently thickening of vascular walls with medial hypertrophy and cystic dilatation of lymphatics was observed in different organs. *et al.* (2002) [34] also reported presence of lymphatic cysts bilaterally along the posterior vena cava; protuberances of distended lymphatic vessels; leakage of lymph from the lymphatic cysts into the surrounding; swelling and degeneration of endothelial cells of the thoracic duct; occasionally extensive endothelial cell loss and their exfoliation; and marked dilatation of thoracic duct and lymph embolism, leaking of lymph, edema in some fibers and the enlargement of spaces between fibers, swollen intima, and rupture and bleeding of the thoracic duct. The authors reported significant difference in long and short semi-axis, and the cross sectional area of the thoracic duct, between normal and ascitic broilers. They also reported lymphatic plasma retention in lymphatic cysts of hepatic capsule.

During present study inflammatory changes characterized by predominantly perivascular mononuclear cell infiltrations and also heterophil infiltration, were seen in all organs. Immune system and Inflammation especially perivascular mononuclear cell infiltration including macrophages, dendritic cells, T and B lymphocytes, and mast cells, has been reported to play an important role in pathogenesis of PAH in humans. Expression of inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, chemokines (RANTES/CCL5, CXC3L1/Fractalkine CCl2), T helper cell-type 1 cytokines (i.e., interferon-γ; IFN-γ), and T helper cell-type 1-associated activities down-stream of IFN-γ, such as IL-18 and chemokine CXCL10 has been implicated especially IL-18 and CXCL10 (Kherbeck *et al.*, 2011; Price *et al.*, 2012; Ross *et al.*, 2012; Stacher *et al.*, 2012) [15, 29, 31, 35]. Elevated circulating levels of inflammatory cytokines (IL-1, IL-6) and chemokines (IL-8) as well as C-reactive protein have been associated with local pulmonary inflammatory activity and implicated for the worse clinical outcome in human (Dorfmű*ller et al.*, 2003; Kherbeck *et al.*, 2011) [15]. Thus aberrant innate and adaptive immune response including autoimmunity, besides other factors, seems to play a critical role in pathogenesis of PAH in humans (Wideman *et al.*, 2013) [44]. However, characterization of such responses in chicken is warranted.

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