

Histopathology of the lymphatic system in ascitic broilers

YANG SIJUN, GUO DINGZONG, YAO BAOAN

College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, China

ABSTRACT: Histomorphologic changes of the lymphatic system of the liver and thoracic duct were examined. The diameters of lymphatic segments isolated from the thoracic ducts of ascitic and normal broilers at 32 to 37 days of age were measured using an optical micrometer measurement system. The histopathological picture of the segments of lymphatic tissue showed lymphatic cysts bilaterally along the posterior vena cava. The hepatic capsule manifested edema, thickening, and cellular proliferation. Microscopic changes in lymphatic vessels of the hepatic capsule include lymph embolism, and lymphatic plasma retention in lymphatic cysts. In some cases, distended lymphatic vessels exhibited protuberances, and lymph leaked from the lymphatic cysts into the surrounding swollen and degenerated endothelial cells of the thoracic duct. Sometimes, extensive endothelial cell loss was observed, and their exfoliated fragments were also seen. Marked dilatation of thoracic duct and lymph embolism, leaking of lymph, edema in some fibers and the enlargement of spaces between fibers, swollen intimas, and rupture and bleeding of the thoracic duct were also visible. The thoracic duct's long and short semi-axis, and the cross sectional area of the thoracic duct differed significantly between normal and ascitic broilers.

Keywords: histopathology; lymphatic system; ascite; broilers

The frequent occurrence of ascites has been a difficult problem detrimental to the poultry industry around the world. Approximately 4% of all broilers die from pulmonary hypertension syndrome (PHS), causing a loss estimated at \$1 billion annually (Maxwell and Robertson, 1997). General agreement exists regarding the reasonable etiology of ascites that: systemic hypoxia triggers a series of events including peripheral vasodilatation, increased cardiac output and pulmonary arterial pressure, and right ventricular hypertrophy (Wideman and Bottje, 1993; Bottje and Wideman, 1995). The right ventricular hypertrophy that occurs in response to the increased work-load by the right ventricle as a result of pulmonary hypertension (Julian, 1987). Broilers susceptible to pulmonary hypertension undergo the pathophysiological progression leading sequentially to right-side congestion, pressure-induced cirrhosis of the liver, and transudation of fluid into the abdominal cavity. (Peacock *et al.*, 1989,1990; Julian, 1993) All factors such as the housing environment including cold

temperatures, moderate heat, air quality, high altitude hypoxia, and elevated carbon dioxide levels, can trigger PHS (Hernandez, 1987; Julian, 1989). However, relatively little consideration had been given to histopathological change of the lymphatic system in correlation with the pathophysiological progression leading to ascites. In the present study, histomorphologic changes of the hepatic lymphatic system of a single broiler line were studied in order to define the morphologic changes. Features of affected birds were compared with those in age-matched control penmates. The thoracic duct diameter from day 32 to day 37 was measured using an optical micrometer measurement system. Microscopic pathological changes in the liver of ascites of lymphatic tissues were examined.

MATERIAL AND METHODS

Five hundred broilers were obtained from the local hatchery. The birds were raised in a windowless

house, and ventilation was adjusted as needed to maintain air quality. Temperatures in the chamber were 31°C and 30°C for weeks 1 and 2, lowered to 15°C during week 3 and maintained at 12°C for the rest of the study. Water and feed were provided *ad libitum*. The combination of cool temperatures and free access to feed has been reported to induce a high incidence of PHS. The birds were vaccinated at 3 weeks old against infectious bronchitis and Newcastle diseases.

Birds were randomly selected that exhibited overt PHS symptom: abdominal fluid accumulation and systemic cyanosis of the comb, wattle and skin were evident (Cawthon *et al.*, 1999). Blood samples were collected from the wing vein in heparinized vacu-tainers. Each bird (days 32 to 37) was anesthetized to a surgical plane with an intramuscular injection of allobarbitol (5,5-diallyl-barbituric acids; 15 mg/kg body weight). They were fasted in dorsal recumbence on a heated surgical board that was maintained at a surface temperature 30°C. A cut was made along the cartilage costalis and the cavum thoracic was opened. The heart was obtained and after careful removal of the atria, the right ventricle (RV) and total ventricle (TV) weights were determined to calculate the RV : TV weight ratio, which is a sensitive indicator of prior exposure of the heart to elevated pulmonary arterial pressure. Birds with an RV : TV > 0.30 were classified as having PHS, whereas those with RV : TV ≤ 0.27 that did not have abdominal or precardiac fluid were classified as non-PHS birds.

The position and distribution of the thoracic duct were observed, and the thoracic cavity and viscera organs were fixed in 10% neutral formalin solution. Then the whole liver was dissected from the chest, being careful to keep the thoracic duct intact when separating the viscera organs from the

thoracic cavity. The thoracic duct was obtained by incising the abdominal aorta from the aortic arch to near the arterial inlet to the kidneys. Vessels selected for study were 1cm in length; 6 µm paraffin-embedded tissue sections stained with hematoxylin and eosin were used for histological examination. The thoracic duct diameter from day 32 to day 37 was measured using an optical micrometer measurement system. The cross section area was calculated using the equation $A = \pi ab$ (a = denoted the long semi-axis, b = denoted the short semi-axis). Microscopic pathological changes in the liver and lymphatic tissues were examined.

Statistics

Significant differences in RV : TV, hematocrit, and the cross section area of thoracic ducts were determined by Student's *t*-test. Probability values ≤ 0.05 were considered significant. Data are presented as mean ± SD for individual experiments.

RESULTS

Frequently, the liver capsule was 2 to 7 times thicker than normal due to fibrosis, oedema and fibrin clots. In some cases there were severe congestion and an obvious dilation of the sinusoids that caused atrophy of hepatocytes and fibrosis of the parenchyma. Accumulation of inflammatory and immune cells consisting of lymphocytes, macrophages and a few heterophils were associated with the portal triads, especially perivascular areas. Occasionally, foci of degenerating hepatocytes around these areas were seen. In addition, centrilobular fatty changes together with other lesions



Figure 1. Lymphatic cysts, lymphatic plasma in lymphatic cysts

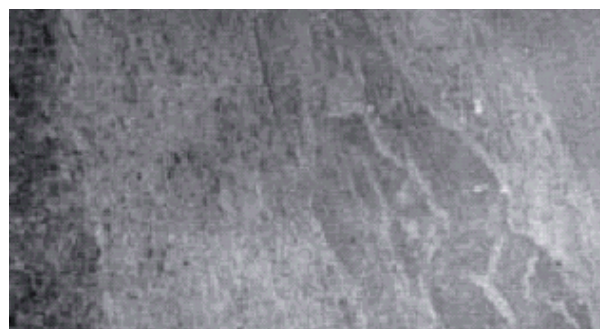


Figure 2. Hepatic capsule manifested edema and thickened, hepatic capsule manifested cell proliferation.

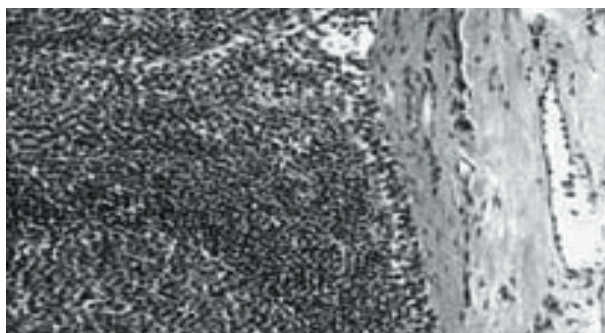


Figure 3. Lymphatic embolisms in lymphatic vessels of hepatic capsule

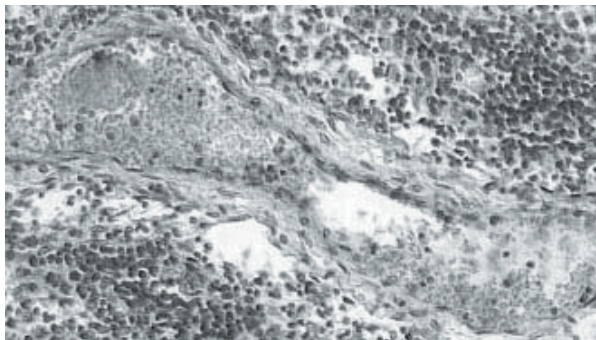


Figure 4. Swollen lymphatic vessels exhibited protuberance

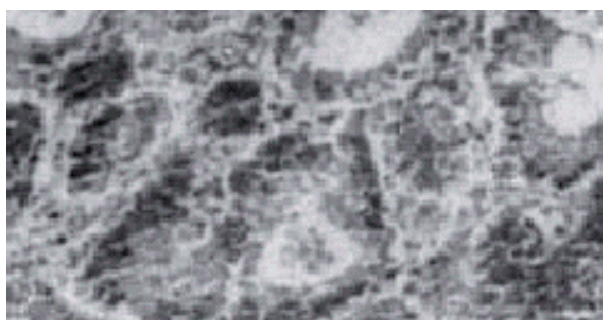


Figure 5. Lymph leaking from lymphatic cyst

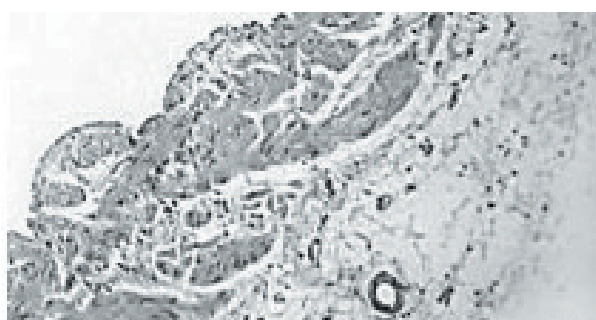


Figure 6. Thoracic duct of normal broilers chickens

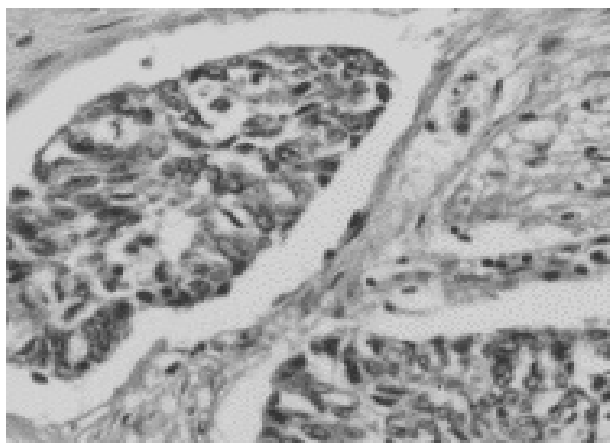


Figure 7. Endothelial cells of thoracic duct swollen and degenerated

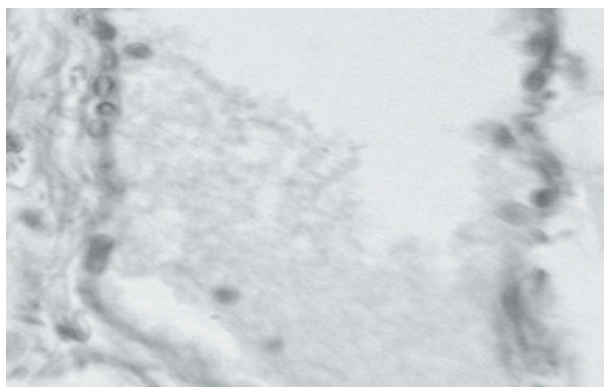


Figure 8. Thoracic duct dilation and embolism of lymph, the swollen intimas of thoracic duct broke

were sometimes observed (unpublished observation).

The histopathological picture of the segments of lymphatic tissue showed: lymphocysts bilateral to the posterior vena cava (Figure 1); hepatic capsule manifested edema, thickening, and cell proliferation (Figure 2); lymph embolism induced by ob-

struction of lymphatic vessels in the hepatic capsule (Figure 3); extended protuberances from lymphatic vessels retained lymphatic plasma (Figure 4); lymph leaking from lymphatic cyst into the surrounding interstitial space (Figure 5); endothelial cells of the thoracic duct became swollen and degenerated, sometimes, extensive epithelial cell loss occurred

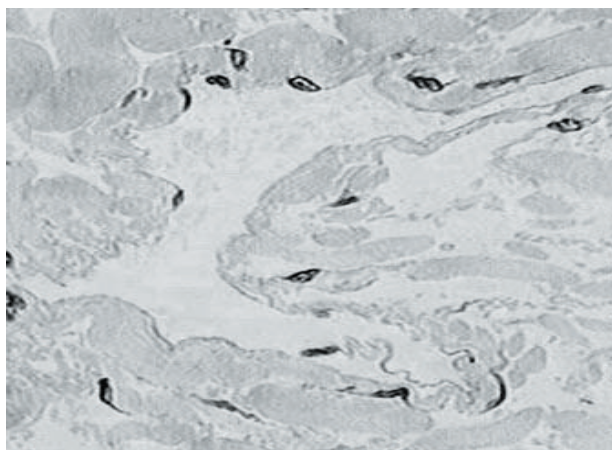


Figure 9. Lymph leaking from the vessels

and the swollen intimas of thoracic duct broke and bled (Figures 6 and 7); histological examination revealed marked thoracic duct dilatation and embolism of lymph suggesting lymph retention and elevation of venous pressure (Figure 8); lymph leaking from the vessels of thoracic duct was observed (Figure 9).

Physiological analyses of broilers from normal and ascites are presented in Table 1, Lower body weight, RV/TV and hematocrit were observed in broilers with PHS that were typical indications of this metabolic diseases. The long and the short semi-axis of the thoracic duct, the cross section area

Table 1. Physiological analyses of broilers in controls and those with ascite

Variables	<i>n</i>	Non-ascite	Ascite
BW (kg)	6	1.736 ± 87.1	1.227 ± 139.3
RV/TV	6	0.219 ± 0.02	0.397 ± 0.13
Hemocrit	6	32.39 ± 1.62	52 ± 3.21

of thoracic duct observed between normal and ascites differed significantly. ($P < 0.05$) (Table 2), suggesting dynamics change of lymphatic circulation in the thoracic duct.

DISCUSSION

Ascites is a significant cause of mortality in many flocks of growing broiler chickens and the incidence appears to be increasing, This syndrome may result from vascular damage, increased vascular hydraulic pressure, or blockage of lymph drainage (Julian, 1983). The main causes of ascites in broiler chickens are usually chronic passive congestion caused by right ventricular failure (Julian and Wilson, 1986) and hepatic fibrosis secondary to hepatitis (Julian, 1988; Calnek *et al.*, 1991). Genetic selection experiments have confirmed that susceptibility to PHS depends substantially on an inherent

Table 2. The values of thoracic duct' long semi-axis and short semi-axis, areas of cross section in normal broilers chickens and those with ascites, the areas was calculated with the equation similar to $A = \pi ab$ (a = denoted long semi-axis, b = denoted short semi-axis)

Category	<i>n</i>	Age	Location	Long semi-axis	Short semi-axis	Areas
Normal	6	37	A	0.5346 ± 0.005 ^a	0.1399 ± 0.0102 ^a	0.2379 ± 0.0134 ^a
			B	0.5284 ± 0.0047 ^a	0.1462 ± 0.0051 ^a	0.2351 ± 0.0177 ^a
Ascites	6	37	A	1.3554 ± 0.1838 ^b	0.3474 ± 0.0934 ^b	1.4799 ± 0.5368 ^b
			B	1.4228 ± 0.2516 ^b	0.3400 ± 0.0805 ^b	1.6053 ± 0.6901 ^b

Notes:

A = denotes the thoracic duct closed to the arterial arch

B = denotes the thoracic duct near the *vena cava*

values = mean ± SEM, values in column with different superscript ($P < 0.05$)

inability of the pulmonary vasculature to accommodate the requisite cardiac output (Wideman and French 1999, 2000). All factors contributing to an increase in cardiac output or an overall reduction in the pulmonary vascular capacity theoretically can accelerate the pathogenesis of PHS by forcing the right ventricle of the heart to increase the pulmonary arterial pressure to propel blood flow through the lungs (Wideman, 2000).

The results of present study demonstrate pathological changes of the liver that correspond closely with those reported earlier at high and low altitudes (Lohr, 1975; Sanger *et al.*, 1985; Maxwell *et al.*, 1986; Witzel *et al.*, 1990). The study further showed the histomorphologic changes of the lymphatic system of the liver and thoracic duct in response to PHS. The lymphatic circulation is acknowledged as a factor involved in the functional regulation of the circulatory system, with appreciable evidence concerning its role in the blood circulation system. Lymphatic vessels are important in reducing edema formation by removing excess fluid and transporting the fluid to veins in the presence of an elevated central venous pressure (Drake *et al.*, 1998). Thoracic duct lymph flow (TDF) and its driving pressure (DP) are positively correlated. The DP is the main factor determining TDF when venous pressure (VP) rises in conjunction with increased lymph production (Inagaki *et al.*, 2000). Hepatic lymph drains into the thoracic duct and then into the systemic venous circulation. Since systemic venous pressure (SVP) must be overcome before liver lymph can flow, variations in SVP may affect lymph flow rate and therefore the rate of fluid accumulation within the liver. Edema develops when lymph does not return to the venous circulation at a rate equal to the rate of capillary filtration (Wilson *et al.*, 1988). Ascitic broilers develop edema as well as an increased central venous pressure while undergoing PHS (Bezuidenhout, 1988; Sakumi *et al.*, 1996). A further study should be made of the relationship between the histomorphologic changes of lymphatic system, lymphatic flow and pressure in response to central venous pressure to assess the severity of broilers during the pathophysiology precession leading to ascites.

Although the specific role of lymphatic system could not be determined in this study, this study nevertheless permits the following conclusions. First, this lymph hypothesis would provide new information on the pathophysiology of ascites. Second, it would help explain the discordant re-

sults between the PHS and heart failure in the development of a systemic ascites syndrome. Finally, understanding the mechanisms underlying the pulmonary hypertensive responsiveness to velocity of lymphatic flow change will likely contribute to our understanding of the multifactorial pathogenesis of PHS in broilers.

REFERENCES

- Bezuidenhout A.J. (1988): Ascites and the anatomy of the peritoneal sacs of broilers. *Onderstepoort J. Vet. Res.*, 55, 23–25.
- Bottje W.G., Wideman R.F. Jr. (1995): Potential role of free radicals in the etiology of pulmonary hypertension syndrome. *Poul. Avian. Biol. Rev.*, 6, 211–231.
- Calnek B.W., Barnes H.J., Beard C.W., Reid W.M., Yoder H.W. (1991): *Diseases of Poultry*. Wolfe Publishing LTD, London.
- Cawthon D.C., McNew R., Beers K.W., Bottje W.G. (1999): Evidence of mitochondrial dysfunction in broilers with pulmonary hypertension syndrome (ascites). Effect of t-butyl hydroperoxide on hepatic mitochondrial function, glutathione, and related thiols. *Poultry Sci.*, 78, 114–124.
- Drake R.E., Teague R.A., Gabel J.C. (1998): Lymphatic drainage reduces intestinal edema and fluid loss. *Lymphology*, 31, 68–73.
- Hernandez A. (1987): Hypoxic ascites on broilers: A review of several studies done of Columbia. *Avian. Dis.*, 31, 358–361.
- Inagaki M., Onizuka M., Ishikawa S., Yamamoto T., Mitsui T. (2000): Thoracic duct lymph flow and its driving pressure in anesthetized sheep. *Lymphology*, 33, 4–11.
- Julian R.J. (1983): Foci of cartilage in the lung of broiler chickens. *Avian Dis.*, 27, 292–295.
- Julian R.J. (1987): The effect of increased sodium in the drinking water on right ventricular hypertrophy, right ventricular failure and ascites on broiler chickens. *Avian Pathol.*, 16, 61–71.
- Julian R.J. (1988): Ascites in meat-type ducklings. *Avian Pathol.*, 17, 11–21.
- Julian R.J. (1989): Lung volume of meat-type chickens. *Avian Dis.*, 33, 174–176.
- Julian T.J. (1993): Ascites on poultry. *Avian Pathol.*, 22, 419–454.
- Julian R.J., Wilson J.B. (1986): Right ventricular failure as a cause of ascites in broiler and roaster chickens. In: *Proceeding 4th International Symposium Veterinary Laboratory Diagnostic*, 608–611.

- Lohr J.E. (1975): Congestive heart failure in broilers, resembling toxic heart degeneration and chick edemas disease. *NZ Vet. J.*, 23, 200–206.
- Maxwell M.H., Robertson G.W. (1997): World broiler ascites survey 1996. *Poultry, Int.* (April), 16–30.
- Maxwell M.H., Robertson G.W., Spence S. (1986): Studies on an ascites syndrome in young broilers. 1. Haematology and pathology. *Avian Pathol.*, 15, 511–524.
- Peacock A.J., Pickett C., Morris K., Reeves J.T. (1989): The relationship between rapid growth and pulmonary hemodynamics of the fast-growing broiler chicken. *Am. Rev. Respir. Dis.*, 139, 1524–1530.
- Peacock A.J., Pickett C., Morris K., Reeves J.T. (1990): Spontaneous hypoxaemia and right ventricular hypertrophy on fast-growing broiler chickens reared at sea level. *Comp. Biochem. Physiol.*, 97A, 537–541.
- Sanger V.L., Scott L., Hamdy C., Gale W.D., Pouden (1985): Alimentary toxemia in chickens. *J. Am. Vet. Med. Assoc.*, 133, 172–176.
- Sakumi (1996): Liver capsule thickening characterized by mesothelial cell proliferation with vascularization in broilers ascites syndrome. In: *Avian Pathology*, 25–153.
- Wideman R.F. (2000): Cardio-pulmonary hemodynamics and ascites in broiler chickens. *Poult. Avian Biol. Tech.*, 11, 1–23.
- Wideman R.F., Bottje W.G. (1993): Current understanding of the ascites syndrome and future research directions. In: *Nutrition and Technical Symposium Proceedings*, Novus International, Inc., St. Louis, MO, 1–20.
- Wideman R.F., French H. (1999): Broiler breeder survivors of chronic unilateral pulmonary artery occlusion produce progeny resistant to pulmonary hypertension syndrome induced by cool temperatures. *Poultry Sci.*, 78, 404–411.
- Wideman R.F., French H. (2000): Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. *Poultry Sci.*, 79, 396–401.
- Wilson J.B., Julian R.J., Barker I.K. (1988): Lesions of right heart failure and ascites in broiler chickens. *Avian Dis.*, 32, 246–261.
- Witzel D.A., Huff W.E., Kubena L.F., Harvey R.B., Elissalde M.H. (1990): Ascites in growing broilers; a research model. *Poultry Sci.*, 69, 741–745.

Received: 02–08–16

Accepted after corrections: 02–09–24

Corresponding Author

Yang Sijun, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
E-mail: sijunjang@21cn.com
