Journal of Animal and Veterinary Advances 7 (11): 1358-1363, 2008
ISSN: 1680-5593

Pathological and Microbiological Investigations on Lung Lesions of Slaughtered Broilers in the Slaughterhouse

[1]Necati Timurkaan, [2]Fethi Yılmaz, [1]Ayşe Kılıç and [3]Gökben Özbey
[1]Sivrice Vocational School, [2]Elazig School of Health Science,
[3]Vocational School of Health, University of Fırat, 23119 Elazığ, Turkey

Abstract: The purpose of this study was to identify the pathologic and microbiologic findings and their ratios, which were detected in the lungs of broilers slaughtered in slaughterhouse. For this purpose, lung tissues of 21,600 animals, which were slaughtered in a commercial broiler slaughterhouse were examined and the tissues in which lesions were observed, underwent microbiologic and histopathological examination. In 275 (1.27%) of the examined broilers, pathologic lesions were detected and in 244 of these samples, different bacterial agents were identified. All of the histopathological findings detected in the lungs had an inflammatory nature and when they were categorized according to predominant features in the inflammation area, exudative inflammation in 241 broilers (87.63%), proliferative inflammation in 24 (8.72%) and exudative-proliferative (bronchointersititiel) inflammation in 10 (3.63%) were seen. In all of the cases, peribronchial and perivascular lymphoid hyperplasia and osseous and cartilaginous nodules in parenchyma of the lungs appeared as common findings. It was found that among the microbiologically identified agents, *E.coli* ranked first. No clear relation between the type of lesions and the infectious agents identified was established. In conclusion, these results indicated that the ratio of lung lesions were 1.27% in slaughtered broiler chickens.

Key words: Broiler chicken, slaughterhouse, lungs, pathology, microbiology

INTRODUCTION

Diseases of the respiratory tract have an important role in poultry. In survey studies, which were carried out on diseased or dead chickens it is reported that, among the causes of the diseases, respiratory system diseases generally ranked first (Kusama *et al*., 1985; Rathore *et al*., 1985; Reece *et al*., 1986). These diseases may emerge as the reflection of a primary or a multisystemic disease (Glisson, 1998). In the etiology of respiratory system diseases, bacteria, virus, parasite, fungi, nutrition and environmental factors are effective (Brugere-Picoux, 1984). In the etiology of respiratory system diseases, bacterial agents have an important role. However, bacterial agents generally colonize in respiratory system after a primary or viral infection or after a nutritional or an environmental stress and then cause infection (Glisson, 1998). The data on poultry diseases are generally based on the data, which are obtained from experimental studies (Garside, 1965; Gauffaux *et al*., 1977) or from the samples taken to diagnosis laboratories (Fletcher, 1980). There are no pathologic studies, which analyze lung lesions based on slaughterhouse material. The purpose of this study is to determine the pathologic and microbiologic findings and their ratios, which were detected in the lungs of broilers slaughtered in the slaughterhouse in our region, where broiler production is common.

MATERIALS AND METHODS

The material of the study consisted of lung tissues of 21,600 broilers, which were examined with inspection and palpation in a commercial broiler slaughterhouse. The broilers studied were from the flocks with prior history of respiratory distress. During the examination, the lesioned or lesion-suspected lungs were collected and after the determination of the distribution of the lesions, they were taken to the laboratory. Lung tissues of all of the samples underwent microbiologic and histopathologic analyses. Microbiologic cultivations from the lung with lesions were made to blood agar. Identification or confirmation of bacterial species was assessed by observation of the colonial morphology and Gram staining results and biochemical methods. These biochemical methods were briefly as follows; catalase, nitrate reduction, H_2S production in Triple Sugar Iron (TSI), growth on

Corresponding Author: Necati Timurkaan, Sivrice Vocational School, University of Fırat, 23119 Elazığ, Turkey

MacConkey, Indole, Urease, Metil Red, Voges Proskauer, oxidase reaction, motility and carbonhydrate fermentation (Sneath *et al*., 1986).

Tissue samples, which were collected for histopathological examination were fixed in 10% buffered neutral formalin solution. The cross-sections, which were taken to paraffin blocks after routine processes were stained with heamatoxylineosin (H and E) and then were examined by light microscope.

RESULTS

Gross findings: Gross lesions were observed in 275 (1.27%) of the examined broilers in the lungs. It was found that 152 broilers the lesions were colonized in the right lobe, in 81 broilers the lesions were colonized in the left lobe and the lesion had bilateral colonization in 42 broilers. It was found that, the size of the lesions, which were macroscopically detected ranged from the size of lentil to chick pea, had dark brown or blackish color and had liver consistency. And in some of the broilers, the lesions were extended in a way to cover the entire lung, had dark red color, were thick and were, in some places, covered with a grey-colored mass.

Histopathological findings: All of the findings detected in the lungs were of inflammatory nature and the detected lesions were categorized as exudative, proliferative and exudative-proliferative (bronchointerstitial) bronchopneumonia according to the predominant feature in inflammatory area and then were given in Table 1 with the microbiological cultivation results of the samples. In all 3 types of bronchopneumonia, peribronchial and perivascular lymphoid hyperplasia and osseous and cartilaginous nodules (Fig. 1) in parenchyma of the lungs were observed as common findings. It was observed that among all samples, exudative bronchopneumonia constituted the majority. In the samples with exudative type bronchopneumonia, especially hemorrhage in inflammatory area was observed to be a common finding.

Histopathologic findings characterized with catarrhal, hemorrhagic, necrotic and fibrinous inflammation according to the predominant feature were detected in the exudative type bronchopneumonia. In the catarrhal bronchopneumonia, degeneration, necrosis or hyperplasia were seen in the epithelium of the secondary and tertiary bronchi and air vesicles. There was exudate containing degenerating and necrotic epithelial cells, heterophils, red blood cells and mucus in the lumen of the air ways (Fig. 2). It was seen edema, congestion, heterophil and mononuclear cell infiltrations in the interstitial area. In the hemorrhagic bronchopneumonia, severe hemorrhage and necrosis were prominent feature and acute inflammatory cells infiltration and tissue debris were often present (Fig. 3). Severe multifocal to coalescing necrosis with vasculitis and vascular thrombosis were the main microscopic lesions of necrotic bronchopneumonia. In the fibrinous bronchopneumonia, tertiary bronchus and air vesicles were filled with fibrinous exudate admixed with various amount of hemorrhage, small numbers of heterophils and macrophages (Fig. 4). The fibrinous mass in the air ways appeared as pink, fibrillar or more

Table 1: Histopathological and microbiological results observed in the lungs with lesions slaughtered broiler chickens

	Histopathological findings									
	Exudative BP*						Exudative-proliferative BP	Proliferative BP		
Infectious agents	Catarrhal BP	Catarrhal-hemorrhagic BP	Hemorrhagic BP	Hemorrhagic-necrotic BP	Necrotic BP	Fibrino-necrotic BP	Broncho-interstitial BP	Non granulo-matous BP	Granul-omatous BP	Total
Staphylococcus (*Staph*) sp.	19	12	3	2	-	-	4	-	-	40
E. coli	23	19	-	3	6	3	-	1	8	63
E. coli +Fungi	-	3	2	-	-	-	-	-	-	5
P. multocida	5	6	2	-	-	-	-	-	-	13
Streptococcus sp	5	2	-	-	-	-	-	-	-	7
Staph sp. + *E. coli*	14	8	5	2	-	-	-	3	2	34
Corynebacterium sp.	2	-	2	-	-	-	-	-	-	4
Proteus sp.	21	19	3	2	2	-	2	8	-	57
Proteus sp. + *Staph.* sp.	3	-	--	-	-	-	-	-	-	3
Pseudomonas sp.	3	3	2	2	-	-	-	-	-	10
Fungi	-	-	2	-	-	-	2	-	-	4
Bordatella sp.	2	-	-	-	-	-	-	-	-	2
Bacillus sp.	-	-	-	2	-	-	-	-	-	2
No agent grew	12	7	6	2	-	-	2	-	2	31
Total	109	79	27	15	8	3	10	12	12	275

*BP: Bronchopneumonia

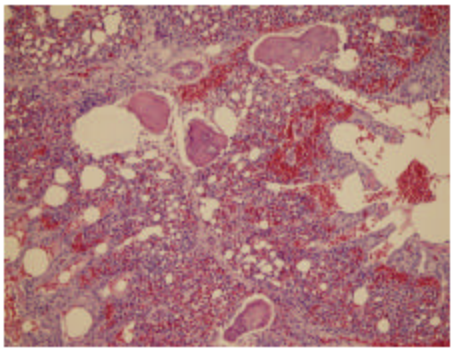

Fig. 1: Osseous nodules around tertiary bronchi in the lung in a broiler chicken H and EX320

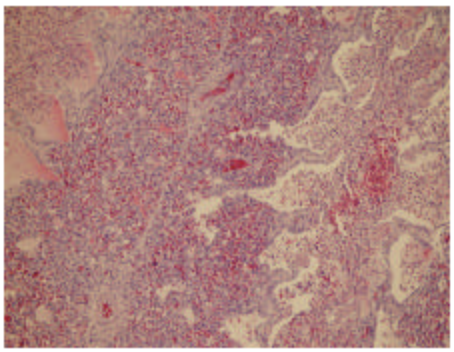

Fig. 2: Catarrhal bronchopneumonia. Edema in tertiary bronchi and lumen of air vesicles in the lung of a broiler, desquamated epithelial cells, heterophyl and mononuclear leucocytes and erythrocytes H and EX320

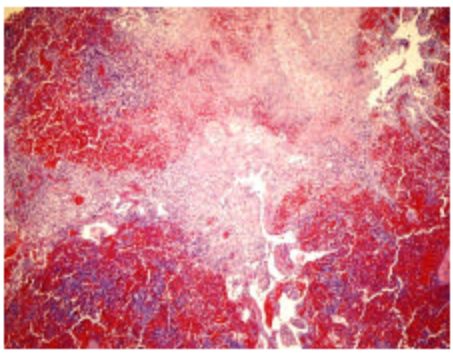

Fig. 3: Hemorrhagic-necrotic bronchopneumonia. Severe hemorrhage, necrosis, acute inflammatory cells infiltration and tissue debris in a lung of a broiler chicken H and EX160

Dhomogeneous. The capillaries were compressed by fibrinouse exudate and became occluded by thrombi in some areas.

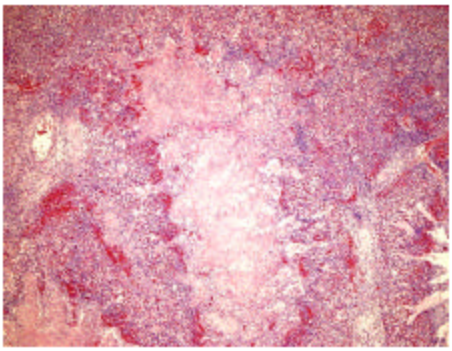

Fig. 4: Fibrinous bronchopneumonia. Air ways were filled with fibrinous exudate admixed with various amount of hemorrhage, small numbers of heterophils and macrophages in the lung of a broiler chicken H and EX160

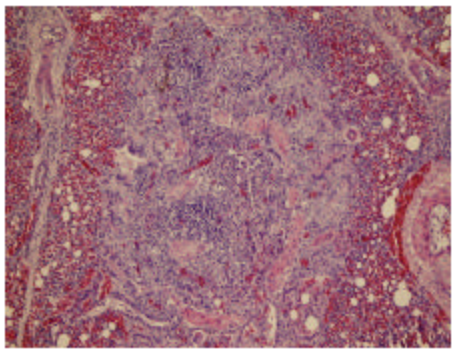

Fig. 5: Proliferative bronchopneumonia. Macrophages, lymphocytes, plasma cells, a few heterophils, type II cell and connective tissue proliferation in the interstitial area in the lung of a broiler chicken H and EX320

The characteristic findings of proliferative lesions were focal epithelial hyperplasie of secondary and tertiary bronchi and air vesicles, muscle trabeculae hypertrophy in the walls of the tertiary bronchi. Macrophages, lymphocytes, plasma cells, a few heterophils, type II cell and connective tissue proliferation caused thickening of interstitial area (Fig. 5). Emphysematous findings, which are characterized by collapse and dilatation in some of the air vesicles or some ruptures in the walls were detected. Some of the proliferative lesions were of granulomatous nature. The granulomas consisted of a caseous mass with necrotic heterophils in the center and were surrounded by epithelioid macrophages, plasma cells, lymphocytes, giant cells and connective tissue cells (Fig. 6). Bacterial aggregates were present in the some necrotic areas.

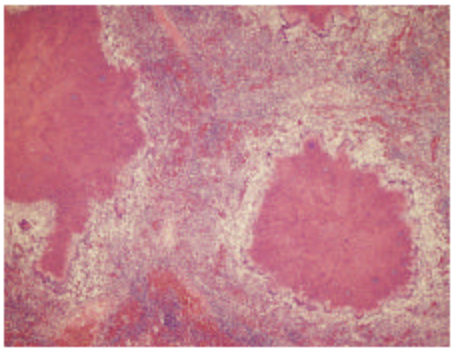

Fig. 6: Granulomatous bronchopneumonia. The granulomas consisted of necrotic heterophils in the center and were surrounded by in the lung of a broiler chicken H and EX160

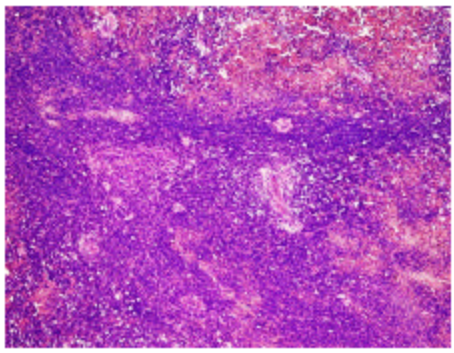

Fig. 7: Bronchointerstitial pneumonia. Severe lymphocyte infiltrations in the lung of a broiler chicken H and EX320

The cases in which exudative findings and proliferative lesions occurred together were considered to be bronchointerstitial pneumonia. In these cases, along with a catarrhal or catarrhal-hemorrhagic bronchopneumonia, findings composed of macrophage, lymphocyte, plasma cell, hypertrophy of muscle trabeculae and proliferation of connective tissue cells were observed. In 5 cases with bronchointerstitial pneumonia, diffuse lymphoid cells infiltrations were observed (Fig. 7). In addition, in 3 of these cases, syncytial cells were also observed in the desquamated cells in secondary and tertiary bronchi lumens.

Microbiological findings: In 244 (88.72%) of the 275 lung tissues with macroscopic lesions, different bacteria were isolated and in 31 (11.28%) samples, no bacterial agent was produced. It was found that among the agents which were identified from pneumatic lungs, mostly *Echerichia coli* (*E. coli*), *Proteus* sp., *Staphylococcus* sp. (*Staph.* sp) and *Staph.* sp. + *E. coli* were observed. While in the majority of lungs with bronchopneumonia single agent was cultivated, in some cases more than one bacterial species were cultivated.

DISCUSSION

Pathological changes were detected in the lungs of 275 (1.27%) of 21,600 broilers, which were examined in the study. There are a limited number of studies based on slaughterhouse material in poultry (Ansari-Lari and Rezagholi, 2007; Herenda and Jakel, 1994), but there are no pathologic studies, which evaluated lung lesions. The reason of this is probably the fact that in poultry production places, when a disease appears in a poultry house, as the individual economic value of the poultry are low, necropsies can be applied on animals. In addition, the fact that slaughter process in poultry slaughterhouse are quite fast and the fact that making lung examinations without interrupting slaughter process is difficult can also be listed among these reasons. In addition, it was also reported that slaughterhouse data is a potential information source in the epidemiology and prevention of the diseases (Ansong-Danquah, 1987; Herenda and Jakel, 1994; Gracey *et al*., 1999).

In this study, in which cultivations were made only on bloody agar, in 244 of 275 samples, bacterial agents were isolated and identified and in 30 samples, no agent was isolated or identified. No isolation or identification study was made for the agents and viruses, which reproduced in other cultures. It was reported that *E. coli* ranked first among the causes of bacterial diseases or death of both broiler and egg-laying hens (Sharma and Kaushik, 1986; Samberg, 1984). In this study, it was found that among the agents, which were produced in lungs with lesion of broilers, *E. coli* ranked first and *Proteus* sp., *Staph.* sp. and *Staph.* sp. + *E. coli* followed it. As it can be deduced from Table 1, generally no relationship was established between the type of the lesions in the lungs and identified agents. However, relationship was found between granulomatous bronchopneumonia and *E coli*. It is reported that bacterial agents mostly cause infections in the lungs based on a viral infection or nutritional or environmental factors (Glisson, 1998). In this study, in some of the samples, which were detected with bronchointerstitial pneumonia, the fact that dense lymphocyte infiltrations occurred in interstitial area and the fact that in addition to desquamated cells, syncytial cells were observed in secondary and tertiary bronchi lumens make us think that in addition to the bacterial agents detected in these samples, a viral etiology also existed.

In this study, it was found that all of the lesions, which were detected by histopathologic evaluations of the lungs were of inflammatory character and when they were categorized according to the type of the exudate in inflammatory area or the nature of the inflammation, catarrhal bronchopneumonia was observed the most. Different assessment can be made in the classification of pulmonary inflammatory response. The inflammation of the lung can be classified on temporal basis as acute, subacute and chronic, on an etiologic basis of causative agent, or according to the type of the inflammation as exudative, proliferative and exudative-proliferative bronchopneumonia. One approach is to classify according to initial site of involvement and the pattern of spread of the lesion as bronchopneumonia, lobar pneumonia and interstitial pneumonia (Jubb *et al*., 1985; Thomson, 1984). In survey studies carried out in poultry, the classification of lung lesions was generally made according to their etiologies (Garside, 1965; Riddell, 1987). However, since this study only indicates the agents, which reproduce only in blood agar in aerobic media, an etiologic assessment could not be made and as a result of this, the lesions detected in the lungs were assessed by categorizing them according to the nature of the inflammation. As it can be understood from Table 1, different agents cause the same inflammation type or the same agent causes different inflammation types. It is thought that like in our study, the fact that no relationship was made between the agent and inflammation type can be due to course of the disease, acuteness of the disease, the pathogenity of the agent and environmental factors and to the immune status of the animal. In addition, the fact that more than one agent was identified in some samples in this study can also be given as a probable reason.

The avian heterophil has been a counterpart to the neutrophil in mammals. Although, there are many similarities between these 2 granulocytes, there are also important differences. So, the pathogenesis of lesions as a result of heterophil infiltration in avian differs markedly from that created by neutrophils in mammals (Harmon, 1998). The heterophils accumulation commonly involves inspissations of the necrotic heterophils into a caseous mass in the lesion area, rather than the liquefaction and abscess formation, which is observed in neutrophyls in mammals (Thomson, 1984; Harmon, 1998). As seen in this study, these necrotic heterophils are surrounded by epithelioid macrophages, lymphocyte, fibrous connective tissue and giant cells to form heterophil granulomas.

In this study, osseous and cartilaginous nodules in lungs with pneumonia were common. The existence of osseous and cartilaginous nodules in the lungs of poultry were reported by many researchers and different views were suggested concerning the existence of these nodules. Some researchers suggest that osseous and cartilaginous nodules are a normal histological findings of poultry lung (Riddell, 1987), while some other researchers report that these nodules appear as a result of the reproduction of abnormal embrional cartilage and bone cells in the lungs after reaching there through various routes (Wight and Duff, 1985; Sarango and Riddell, 1985). Maxwell (1988) and Tafti and Karima (2000) reported that these nodules were common in chicken with ascites syndrome and they probably occurred as a result of hypoxia and they pointed out that there was a relationship between these nodules and the lesions in the lungs.

CONCLUSION

To our knowledge, this study is the first report investigating the lung lesions pathologically in slaughtered broiler chickens. The ratio of the lesions were found 1.27%. In this study, among respiratory organs of the broilers, only lungs were examined and it is thought that a slaughterhouse study including the entire respiratory system would be more beneficial.

REFERENCES

Ansari-Lari, M. and M. Rezagholi, 2007. Poultry abattoir survey of carcass condemnations in Fars province, southern Iran. Prev. Vet. Med., 79: 287-293. DOI: 10.1016/j.prevetmed.2006.12.004. PMID: 17254653.

Ansong-Danquah, J.A., 1987. Survey of carcass condemnation at a poultry abattoir and its application to disease management. Can. Vet. J., 28 (1-2): 53-55. PMID: 17422886. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1680381&blobtype=pdf.

Brugere-Picoux, J., 1984. Differential diagnosis of respiratory diseases of poultry. Rec. Med. Vet., 160 (11): 1069-1078.

Fletcher, C.J., 1980. Pathology of the avaian respiratory system. Poult. Sci., 59: 2666-2679. PMID: 7267515.

Garside, J.S., 1965. Histopathological diagnosis of avian respiratoy infections. Vet. Rec., 13 (77): 354-366. PMID: 14320639.

Glisson, J.R., 1998. Bacterial respiratory diseases of poultry. Poult. Sci., 77: 1139-1142. PMID: 9706078. http: //ps.fass.org/cgi/reprint/77/8/1139.

Gouffaux, M., H. Vindevogel, G. Meulemans, A. Dewaele and P. Halen, 1977. Elements du diagnostic histopathologique differentiel des principales affections respiratoires de la poule. Avian. Pathol., 6: 61-76.

Gracey, J.F., D.S. Collins and R.J. Huey, 1999. Poultry Production, Slaughter and Inspection. 10th Edn. In: Meat Hygiene. W.B. Saunders Company LTD, pp: 261-287. ISBN: 0702022586.

Harmon, B.G., 1998. Avian heterophils in inflammation and disease resistance. Poult. Sci., 77: 972-977. PMID: 9657606. http://ps.fass.org/cgi/reprint/77/7/972.

Herenda, D. and O. Jakel, 1994. Poultry abattoir survey of carcass condemnation for standard, vegetarian and free range chickens. Can. Vet. J., 35: 293-296. PMID: 8050075. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1686657&blobtype=pdf.

Jubb, K.V.F., P.C. Kennedy and N. Palmer, 1985. Pathology of Domestic Animals. 2nd and 3rd Edn. pp: 414-541. Academic Pres, New York, USA. ISBN: 0-12-391602-X.

Kusama, Y., M. Inoue, M. Nakamura and T. Masegi, 1985. Pathological investigations of current diseases of broiler chickens in Gifu prefecture. Res. Bull. Fac. Agric. Gifu Uni., 50: 241-250. http://www.affrc.go.jp/.

Maxwell, M.H., 1988. The histology and ultrastructure of ectopic cartilaginous and osseous nodules in the lungs of young broiler with an ascitic syndrome. Avian Pathol., 17: 201-219. PMID: 18679911.

Rathore, B.S., S. Rajendra and S.S. Khera, 1985. Survey on causes of poultry mortality in India-based on postmortem examinations conducted at 10 diagnostic centres. Ind. J. Poult. Sci., 2 (20): 135-139.

Reece, R.L., V.D. Beddome and D.A. Barr, 1986. Diseases diagnosed in replacement layer and breeder chicken flocks in Victoria, Australia, 1977-1985. Vet. Rec., 19 (119): 471-475. PMID: 3788011.

Riddell, C., 1987. Avian Histopathology Allen Press Inc. 1th Edn. pp: 37-45. Lawrence, Kansas. ISBN: 0-915538-03-2.

Samberg, Y., 1984. The control of poultry diseases in Isreal, 1972-1981. Ref. Vet., 3 (41): 91-103.

Sarango, J.A. and C. Riddell, 1985. Study of cartilaginous nodules in lungs of domestic poultry. Avian Dis., 29: 116-127. PMID: 2580509.

Sharma, N.K. and R.K. Kaushik, 1986. Surveillance of diseases in broilers in Hayrana State. Ind. J. Anim. Sci., 7 (56): 762-764.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt, 1986. Bergey's Manual of Systematic Bacteriology. 1th Edn. 2: 965-995. Willims and Wilkins. ISBN: 0-683-07893-3.

Tafti, A.K. and M.R. Karima, 2000. Morphological studies on natural ascites syndrome in broiler chickens. Vet. Arhc., 5 (70): 239-250. ISSN: 0372-5480. http://www.vef.hr/vetarhiv/papers/70-5/tafti.pdf.

Thomson, R.G., 1984. General Veterinary Pathology. 2th Edn. W.B. Saunders Company, London, pp: 163-272. ISBN: 0-7216-8851-9.

Wight, P.A.L. and S.R.I. Duff, 1985. Ectopic pulmonary cartilage and bone in domestic fowl. Res. Vet. Sci., 39: 188-195. PMID: 4070786.