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Pathological Findings in Quails Infected with Avian Influenza A Virus H7 N1 Subtype

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Abstract: The aim of this study was to identify the clinical signs, gross and histopathological findings of the quails experimentally inoculated with Avian Influenza A virus H7 N1 subtype. Two groups of 6-day-old, ninety quails (45 quails were inoculated, 45 quails were kept as control) (Coturnix coturnix japonica) were used. Avian Influenza A virus H7 N1 subtype was inoculated intra nasally into the experimental group of the quails. Decrease in feed and water consumption, calmness, reluctance, and diarrhea were observed as clinical symptoms after the 15 days after of inoculation. Pathologically, mild inflammation with congestion and haemorrhage in the respiratory tract and intestines, degenerative and necrotic changes in the liver and heart, and non suppurative meningoencephalitis were observed. In conclusion, H7N1 of AIV subtype caused a mild form of the disease in quails. The clinical signs and pathological findings were not specific for the diagnosis of the disease. The results of the present study suggest that the cases with the symptoms written above should be taken into consideration of possibility of AI in quails.

Key words: Avian influenza, H7 N1, quail, pathology

Introduction

Avian Influenza (AI), which is also known as fowl plaque, is a zoonotic viral disease characterized with respiratory, gastrointestinal and nervous system findings at high morbidity and mortality in the avian species (Jordan, 1996). Many species of birds, domesticated or wild, can be infected with the virus. It is observed in various wild winged animals and water birds such as hens, turkeys, ducks, geese, and pheasants (Astorga *et al*., 1994; Jordan, 1996; Swayne, 1997).

The causative agent of the AI is Influenza A virus from Orthomyxoviridae family. It is reported that there are numerous subtypes of AI virus according to haemagglutinin (H) and neuroaminidas (N) (Hooper *et al*., 1995; Jordan, 1996). Fifteen different H and 9 N types of the virus have been reported, and H5 and H7 are referred as the highly pathogenic serotypes (Hooper *et al*., 1995; Jordan, 1996; Alexander and Gough, 1997). However, most AI viruses of all H subtypes, including H5 or H7, have been mildly pathogenic based on the information produced from experimental inoculation of chickens (Alexander and Gough, 1997; Swayne, 1997).

Avian influenza epidemics in Asia and Europe and zoonotic features of the disease prompted the studies on AI due to its great significance for human health. Although the quails, like many avian species, are said to be sensitive to the disease, the studies on the pathological changes of the quails caused by the virus are indeed limited (Marangon *et al*., 2002, Tashiro *et al*., 1987, Zhao *et al*., 1998). Besides, it has been stated that the virus replication developed more rapidly in the quails compared to the other species, and that the wild quails might have played role in the spread of the disease (Perez *et al*., 2003).

The objective of this study was to describe the clinical, gross and histopathological findings observed in the quails experimentally infected by H7 and N1 of the AI virus.

Materials and Methods

Quails: We obtained 90 of one-day old quails from the Directorate of Bingol Trade College of Firat University, and were fed *ad libitum* by standard grower diet throughout the experiment.

Virus: In the study, the AI-VX73-67 virus strain with H7 N1 subtype isolated from a parrot in North Ireland in 1973 was obtained from Manisa Poultry Diseases Research and Vaccine Production Institute.

Trial: A total of 90 quails at 6 days age (Coturnix coturnix japonica) were divided into two groups: 45 quails in the trial group and 45 in the control group. Each group was placed in separate pens under the same care and feeding conditions with separate ventilation systems. The quails of the trial group were given 0.1 ml intranasal VX73-67 (H7 N1) (HA 256) of the AI virus diluted in 1/64 phosphate buffered saline. On the other hand, the quails of the control group were given phosphate buffered saline at the same dose and in the same way.

Totally 90 quails were applied necropsies on 5 randomly selected quails from each of the trial group and control group in the 3, 7, 11, 15, 19, 23, 27, 31 and 35 days after the inoculation. Tissue samples were taken from the

nares, trachea, lungs, air sacs, liver, spleen, pancreas, stomach, intestine, kidneys, brain, adren and skin, and fixed in 10% neutral formalin solution. Tissue samples were embedded in paraffin, and then cross sections were made and stained with Haematoxylin-Eosin (H&E). In addition, the fixed liver tissues were cut in the frozen microtome, stained with the Oil Red O staining procedure and examined under the light microscope.

Findings: Similar clinical findings were observed between 15 and 27th days of the trial. These findings were observed in the form of decrease in feed and water consumption; calmness, reluctance, and diarrhea in some birds.

Different macroscopic and microscopic findings were observed between days 11 and 31 after inoculation. These findings were not appreciable different. No macroscopic finding was determined in the quails slaughtered in the 35th day of the trial.

Macroscopic findings were mild in severity, and were observed mostly in the respiratory system, liver, heart and intestines. The trachea and upper respiratory tissues were congested and had accumulation of mucus or exudates. It was seen that bilateral ventral parts of the lungs were covered with slightly dark focus and had edematous appearance. The liver was congested and yellowish-gray focuses at needle head size were observed in some cases. The content of the intestines was dilute, and congestion in the mucous and sometimes petechial haemorrhagia was observed generally in the first 1/3 of the duodenum and jejunum in the small intestine. In some cases, there was a yellowish exudate at a lesser amount in the abdominal cavity. There were sub-epicardial and in some cases sub-endocardial hemorrhages in the heart, and approximately 1 ml liquid was observed in the pericardium.

Microscopically, the tracheal mucosa was inflamed as characterized by congestion, edema, and deciliation, necrosis and detachment of the epithelial cells. Severe infiltration of lymphocytes, macrophages, and infrequently heterophils were observed in the lamina propria, which increased the thickness of the tracheal mucosa. Mild to moderate interstitial pneumonia was a common lesion in quails. Infiltration of macrophages, lymphocytes and few heterophils were seen in addition to hyperemia, edema and hemorrhages in the lungs (Fig. 1). In the epithelia of the primer bronchia, degeneration, desquamation and focal hyperplasia as well as peri-bronchial edema and congestion were notable.

The virus produced focal lymphohistiocytic myocarditis with myocyte degeneration and necrosis. Muscle fibers were somewhat homogenous pink color, the citriation disappeared. Besides, myocardial hyperemia and hemorrhages were found in the sub-epicardial region.

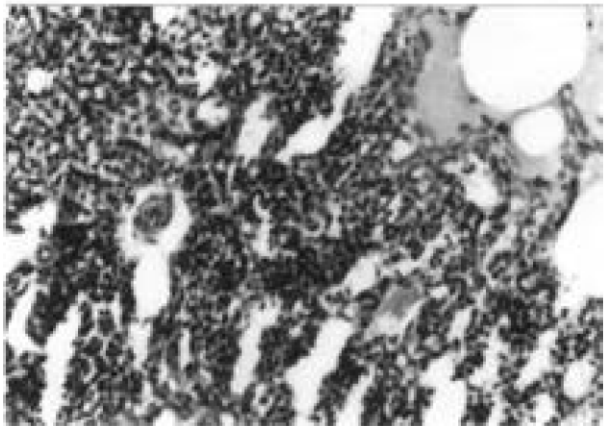

Fig. 1: Lung, inflammatory cells infiltration in the interstitial area with hemorrhage and edema of a quail infected with H7 N1 of AIV subtype on day 21 pi, H&EX360.

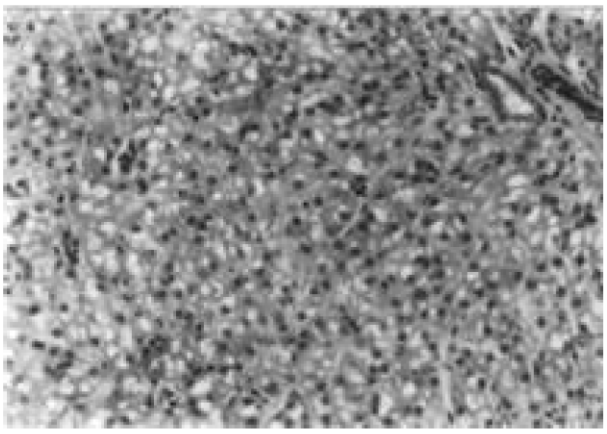

Fig. 2: Liver, hydropic degeneration of a quail infected with H7 N1 of AIV subtype on day 24 pi, H&EX360.

In the liver, usually in the centrilobular area, hydropic degeneration, and tiny droplets with a foamy appearance were seen (Fig. 2). In the staining with Oil Red O, such vacuoles were determined to be fat globules. Besides, apoptosis, focal necrosis and Kupffer cell activation were found. In the portal area mononuclear and heterophil cell infiltration were also observed.

There were non-suppurative encephalitis and meningitis in the brain. In all regions there was perivascular cuffing with lymphocytes and macrophages. In addition, in medulla, midbrain and cerebellar molecular layer there were micro gliosis and vascular endothelial swelling. Neuronal degeneration and necrosis (Fig. 3) were determined in the brain, especially in the region of substantia grisea.

Degeneration in cells of lamina epithelia, desquamation and rare hyperplasia were observed in the duodenum

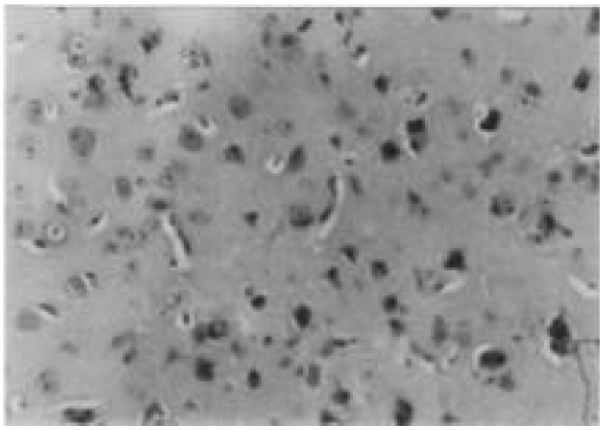

Fig. 3: Brain, neuronal degeneration and necrosis in the substantia grisea of a quail infected with H7 N1 of AIV subtype on day 19 pi H&EX400.

and jejunum. Hyperemia, edema and mononuclear cell and heterophil infiltration were observed in the lamina propria. Degenerative changes were also determined in the crypt epithelial cells in the mucosa.

No substantial macroscopic and microscopic findings were determined in the spleen, pancreas, gland stomach, air sacs, kidney, adren and skin. No pathological finding was observed in the quails of the control group.

Results and Discussion

Avian influenza infection in domestic poultry come out within different clinical syndromes. The occurrence of the disease syndrome or the degree of severity of the disease depends on multiple factors, including the pathogenicity of the virus, care and feeding conditions, the host species, age of the host, route of infection, and existence of secondary bacterial infections (Swayne and Slemons, 1994; Brugh and Beard, 1986). Besides, it was reported that the severity of the disease depended whether the infection was natural or experimental. Although some AIV stains caused sever systemic infections and high mortality in natural conditions, (Brugh and Purdue, 1991; Naem and Hussain, 1995; Nili and Asasi, 2002), the virus produced in mild disease in the experimental infections (Alexander *et al.*, 1986; Halvorsan *et al.*, 1980; Johnson and Maxfield., 1976; Skeeles *et al.*, 1981). In an outbreak of H9 N2 in Iran, mortality rates increased up to 65% in chickens (Vasfi Marandi and Bozorgmehrifard, 1999). However, experimental studies with virus alone have shown all H9 N2 AI viruses from the Middle East to be mild (causing low or no mortality) infection (Banks *et al.*, 2000). In the present experimental study, while H7 N1 of AIV strain caused no mortality and mild infection in quails, virus having same antigenic structure with the virus used in this study was reported to cause serious epidemics in turkey and quail farms in Italy in 2000-2001 (Marangon *et al.*, 2002). It was stated that low virulence AIV strains obtained high virulence by means of the passages and genetic mutations experienced in the field (Tashiro *et al.*, 1987). This situation reminds the probability that mild AIV infections might turn into epidemics that cause serious losses.

It has been reported that in AI infections in avian species, congestion and hemorrhage occurred in the internal organs such as lungs, liver, spleen, kidneys, brain, adren, cardiac, and pancreas, and the skin. However, these lesions were not specific for diagnosis of the disease (Jordan, 1996). In this study, the findings such as congestion, hemorrhage, degeneration and necrosis were determined in the respiratory system, liver, cardiac, brain and small intestines of the quails. However, no finding was observed in the spleen, kidney, adren, pancreas and skin. It is known that there is a relation between the severity of the AIV infections and the tissue and organ tropism in chickens. Swayne *et al.* (1997) reported that in chickens the respiratory system, spleen, kidney and pancreas were affected in the low severity AIV infections; however, the skin, brain, cardiac and the adren were affected in the severe cases. It seems it is hard to draw such a difference as far as the findings of this study are concerned. Besides, it is reported that the way of giving the virus is significant for its effects on the organs (Swayne and Slemons, 1994; Swayne *et al.*, 1994).

It is known that AI epidemics are seen in many parts of the world in the last years, and that it caused significant economical losses. Besides, the zoonotic structure of the virus not only affects the health of the animals but also have a threat on the people working in this field of production. It is stated that AIV is infected via oral or aerosol ways in the normal conditions, and the water birds play the most important role in the spread of the disease (Astorga *et al.*, 1994; Jordan, 1996). Accordingly, it is recorded that the prevalence of the disease is higher in the farms of the avian species that are located on the way of migration of the water birds (Jordan, 1996; Astorga *et al.*, 1994). In this respect, the farms located on the way of migration of the birds carrying the virus are under the risk of the infection.

In conclusion, in this study, it was determined that the H7 N1 sample of AIV resulted in a mild disease in the quails, and that the clinical and pathological findings were not specific for the diagnosis. It was considered that the AI probability should be noted in such non-specific findings as well as the regions under the risk of AI infection from the epidemiological view, and that in addition to clinical and pathological findings, other laboratory techniques would be useful for the diagnosis of the disease.

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