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HISTOPATHOLOGICAL STUDY OF A/CHICKEN/IRAN/772/99 (H9N2) INFLUENZA VIRUS IN COMMERCIAL BROILER CHICKENS

M. M. HADIPOUR

Department of Clinical Sciences, School of Veterinary Medicine, Islamic Azad University, Kazeroun Branch, Kazeroun, Iran

Summary

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Avian influenza outbreaks in 1998 due to H9N2 subtype of avian influenza virus (AIV) that occurred in poultry industry in Iran caused serious economic losses. The aim of this study was to investigate the pathogenesis, clinical signs, gross and histopathological findings of the chickens experimentally inoculated with A/Chicken/Iran/772/99(H9N2) influenza virus, isolated from the commercial broiler chickens of Iran with 60% mortality. Eighteen 3-week-old commercial broiler chickens were inoculated with 10^6 EID_{50} per bird with A/chicken/Iran/772/99(H9N2) avian influenza virus. On days 1, 2, 4, 6, 8 and 11 post-inoculation (PI) samples of the trachea, lungs, liver, pancreas, spleen, thymus, duodenum, kidneys, brain and bursa of Fabricius were collected for histopathological study. Clinically, depression, crouching, huddling, puffing, oedema of face and head, conjunctivitis and ruffled feathers were observed. In necropsy, turbidity of the thoracic and abdominal air sacs and mild congestion of the trachea and lung and mild accumulation of fibrinous exudate on the tracheal mucosa were seen. Tracheitis, pneumonia and tubulointerstitial nephritis were the most frequent histological lesions. The results indicated that the A/Chicken/Iran/772/99 (H9N2) avian influenza virus has pathogenicity for the trachea, lungs (pneumotropic) and kidneys (nephrotropic).

Key words: avian influenza, broiler chickens, H9N2 subtype, histopathology

INTRODUCTION

Avian influenza viruses (AIVs) are a diverse group of viruses in the family Orthomyxoviridae, genus Influenzavirus A and can be categorized into subtypes based on two surface glycoproteins, the haemagglutinin (H) and the neuraminidase (N). There are 16 different haemagglutinin (H1–16) and 9 different neuraminidase (N1–9) subtypes, which make 144 possible combinations of H and N subtypes. AIVs can be further classified into two different pathotypes (low and high pathogenicity), based on the ability to produce disease and death in the major domestic poultry species, the chicken (*Gallus domesticus*) (Alexander, 2000; Fouchier Ron *et al*., 2005; Swayne, 2007). Avian influenza disease due to H9N2 subtype in poultry during the second half of the 1990s has been noticeably increased worldwide. The H9N2 subtype outbreaks have occurred in domestic ducks, chickens and turkeys in Germany (1995 and 1998), in chickens in Italy (1994 and 1996), in pheasants in Ireland (1997), ostriches in South Africa (1995), turkeys in the USA (1995 and 1996) and in chickens in Korea (1996) (Bano *et al*., 2003;

Capua & Alexander, 2004; Naeem *et al*., 1999). More recently, H9N2 viruses have been reported in Middle Eastern countries and have been responsible for widespread and serious disease in commercial chickens in Iran, Pakistan, Saudi Arabia and United Arab Emirates (Naeem *et al*., 1999; 2007; Banks *et al*., 2000; Nili & Asasi, 2002; 2003; Alexander, 2003; Capua & Alexander, 2004; Aamir *et al*., 2007). In 1998 an outbreak caused by the low-pathogenicity AIV H9N2 subtype has occurred in Iranian poultry industry (Nili & Asasi, 2002; 2003). Earlier pathogenetic studies revealed that low-pathogenicity AIVs (LPAIV) are pneumotropic following intranasal inoculation (Swayne & Slemons, 1994). Data collected from recent avian influenza outbreaks indicate that LPAIV may mutate and become highly pathogenic, probably after introduction to poultry (Garcia *et al*., 1996), and therefore to cause extremely complex situations with dramatic effects on the poultry industry.

The aim of this study was to investigate the pathogenesis, clinical signs, gross and histopathological findings of the chickens after experimental intranasal inoculation with A/Chicken/Iran/772/99 (H9N2) influenza virus, isolated from the commercial broiler chickens of Iran with 60% mortality.

MATERIALS AND METHODS

Thirty six 3-week-old commercial broiler chickens (Ross, UK) were randomly divided in two groups (experimental with 18 birds and control with 18 birds) and were housed under the same conditions in two separate rooms. Chickens were monitored on a daily basis for general condition and the presence of clinical signs. All birds were bled for detection of specific antibodies against H9N2 subtype of AIV. Subsequently the experimental group was inoculated intranasally with 10^6 EID_{50} per bird of A/chicken/Iran/772/99(H9N2) avian influenza virus at 20 days of age. Three birds from each group (test and control) were randomly selected on days 1, 2, 4, 6, 8 and 11 post-inoculation (PI). Then they were humanly sacrificed and were subjected to thorough necropsy. The clinical signs and gross lesions were recorded. Samples of different organs including the trachea, lungs, liver, pancreas, spleen, thymus, duodenum, kidneys, brain and bursa of Fabricius were collected for histopathological study from all six chickens and fixed in 10% neutral buffered formalin solution. Tissue samples were routinely processed to paraffin wax blocks and 5 μm sections were prepared and stained with haematoxylin-eosin (H & E) stain for light microscopic examination.

RESULTS

Daily monitoring did not show any changes in the clinical behaviour of the birds from the control group.

Infected chickens exhibited clinical signs such as depression, crouching, huddling, puffing, oedema of face and head, conjunctivitis and ruffled feathers.

The most frequent gross lesions in infected birds were turbidity of the thoracic and abdominal air sacs, mild congestion of the trachea and lungs and mild accumulation of fibrinous exudate on the tracheal mucosa. Control chickens did not show any gross lesions.

In inoculated chickens, acute tracheitis associated with hyperaemia, oedema, deciliation, degeneration of mucous glands, infiltration of heterophils were seen on day 2 PI. However, on days 4, 6, 8 and 11 PI, lymphocytic tracheitis, associated with

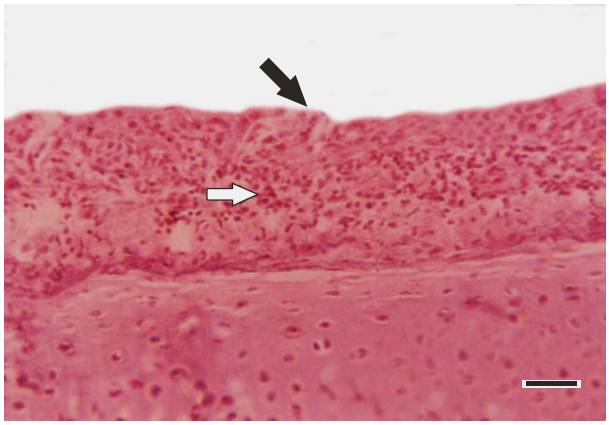

Fig. 1. Lymphocytic tracheitis on day 4 PI. Deciliation (black arrow) and infiltration of lymphocytes (white arrow) are seen; some regenerative processes especially between tracheal epithelial cells could be also seen. H&E, bar = 200 μm.

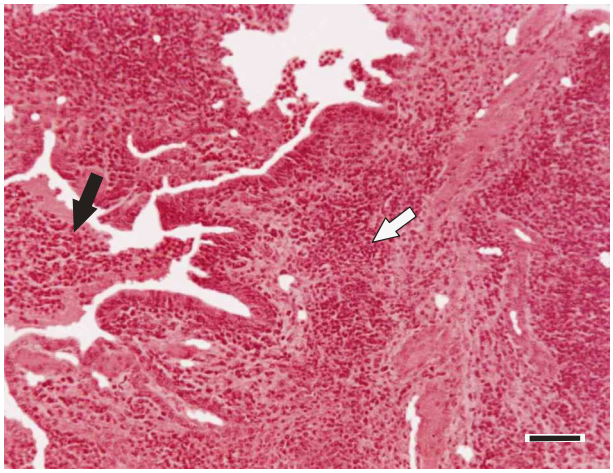

Fig. 2. Lung section (5 μm) from a chicken on day 6 PI showing accumulation of exudate in lumen (black arrow) and associated pneumonia (white arrow), as well as some consolidation of air capillaries. H&E, bar = 100 μm.

infiltration of lymphocytes, deciliation, and hyperplasia of epithelium were observed (Fig. 1). On day 11 PI the number and the severity of lesions had been considerably reduced. Lymphocyte infiltration in the lamina propria of the secondary bronchi and associated pneumonia were the most prominent histological changes in the lung, on days 4–11 PI (Fig. 2).

Lymphocytic tubulointerstitial nephritis was observed in kidneys on days 6, 8 and 11 PI (Fig. 3). Spleen exhibited the highest frequency of histological changes among lymphoid tissues. Mild to modera-

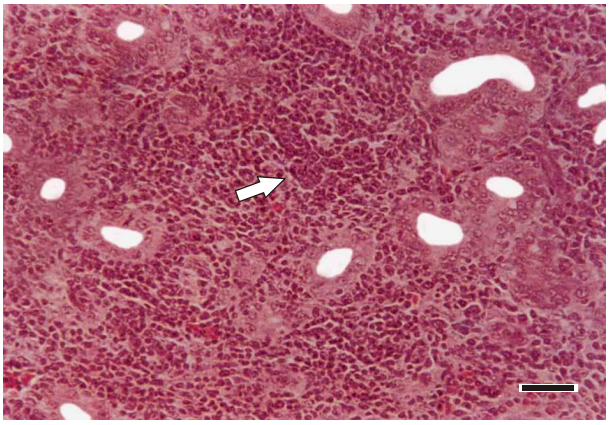

Fig. 3. Kidney section (5 μm) from a chicken on day 8 PI showing large aggregate of lymphocytes in the interstitial tissues of the kidney. The lesion was characterized as tubulointerstitial nephritis (white arrow). H&E, bar = 200 μm.

te reticuloendothelial cell hyperplasia and increased number of lymphoid follicles on days 6, 8 and 11 PI were found, but necrosis and inflammation were not seen. Thymus of some samples showed depletion of lymphocytes in the cortical region on days 6, 8 and 11 PI. In bursa of Fabricius on days 8 and 11 PI there were three cases of lymphoid atrophy and cystic follicles. A mild vacuolation of pancreatic cells was present in a chicken on day 8 PI, and lymphoid hyperplasia was observed in the duodenum of two chickens on day 6 PI. There were no changes in the liver and brain of chickens.

In control chickens, all examined organs were histologically normal with no detectable lesions.

DISCUSSION

Although experimental study of low-pathogenicity AIVs in specific pathogen-free chickens was related to absence of or low mortality, high mortality rates have been frequently reported in field cases (Vasfi Marandi & Bozorgmehri Fard, 2002; Naeem *et al.,* 1999; 2003; Nili & Asasi, 2002; 2003; Bano *et al.,* 2003). This experiment was conducted to investigate the histopathology of the A/chicken/Iran/772/99(H9N2) infection in commercial broiler chickens.

In this study the clinical signs and lesions found at *post mortem* examination were almost similar and milder than lesions reported in naturally infected chickens during H9N2 AIV outbreak in Iran and Pakistan (Naeem *et al.,* 1999; Nili & Asasi 2002, 2003; Bano *et al.,* 2003).

In some researches (Slemons *et al.,* 1990; Slemons & Swayne, 1990, 1995; Swayne & Slemons, 1990, 1992, 1994; Swayne *et al.,* 1994; Swayne & Pantin-Jackwood, 2006), inoculation of chickens by the intranasal (IN) and the intratracheal (IT) route with low virulence chicken- or duck-originating AIV isolates produced mortality and kidney lesions in 1-day-old chickens and adult hens. However, in other studies no mortality has been reported (Shalaby *et al.,* 1994; Swayne *et al.,* 1994).

In this experiment, tracheitis, pneumonia and tubulointerstitial nephritis were the most frequent histological lesions. The lesions were obvious on days 2–8 PI but on day 11 PI the severity of lesions was reduced. Clinically, this was associated with improvement in the general condition of inoculated birds.

Mo *et al*. (1997) inoculated chickens intratracheally with AIV isolates of either low- or high-pathogenicity and concluded that most frequently, the low-pathogenicity isolates produced either histological lesions in the trachea and lungs or no histological lesions. In another study after intravenous (IV) inoculation of avirulent H4N4, H6N2 and H3N8 viruses into chickens, specific lesions were noted in the kidneys only (Hooper *et al.,* 1995). Swayne & Beck (2005) inoculated two low-pathogenicity and two high-pathogenicity AIVs into chickens by intranasal route. The LPAIV caused localized viral infections in the respiratory and gastrointestinal tracts. Swayne & Slemons (1994) inoculated three AIV strains with chicken/duck origins to 5-week-old chickens by IV, IN and IT routes. Chickens inoculated by IT and IN routes had mild to severe tracheitis, bronchitis and pneumonia associated with secondary bronchi but lacked renal tubular necrosis and nephritis. As abdominal air sacs are next to kidneys, presumably, presence of nephritis foci in the kidneys during 6–11 days PI could be a result from respiratory tract infection (air sacs) (Shalaby *et al*., 1994). Regarding kidney lesions, the results obtained from the present study are in agreement with the findings of Slemons & Swayne (1990) and Slemons *et al*. (1990) indicating that renal failure resulting from kidney lesions could be encountered in H9N2 AIV infection in chickens. According to Swayne & Slemons (1995), lymphoid and reticuloendothelial hyperplasia of the duodenum and the spleen that were seen in the present study could be an immune response of B- and T-lymphocytes to foreign antigens. Lymphoid atrophy of the thymus and the bursa of Fabricius was most compatible with non-specific endogenous glucocorticoid response. The mild infiltration of lymphocytes into the pancreas was similar to mild non-specific immunological reaction. Absence of lymphocytic necrosis and viral antigens in primary and secondary lymphatic organs showed that lymphoid tissues were not targets of AIV H9N2.

The most frequent sites of histological lesions observed in this study – the trachea, lungs and kidneys of chickens, indicated that the A/Chicken/Iran/772/99 (H9N2) isolate had a pathogenicity for the trachea, lungs (pneumotropic) and kidneys (nephrotropic).

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Correspondence:
Dr. Mohammad Mehdi Hadipour
Department of Clinical Sciences,
School of Veterinary Medicine,
Islamic Azad University, Kazeroun Branch,
P.O.Box: 73135-168
Kazeroun, Iran
Tel: 00989177189086; fax: 00987118317975
E-mail: hadipourmm@yahoo.com