

Histologic Lesions of Thymus and Bursa of Fabricius in Commercial Broiler Chickens Inoculated with H9N2 Avian Influenza Virus

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Abstract: Low pathogenic avian influenza (H9N2) is of major concern for the poultry industry especially in Iran, as the virus can spread rapidly in and between flocks, causing high mortality and severe economic losses. The aim of this study was to determine the pathogenicity of H9N2 avian influenza virus in thymus and bursa of Fabricius of commercial broiler chickens, so we studied the histologic lesions of this isolate in these organs following intranasal (IN) inoculation. Twenty-four 3-week-old chickens were inoculated with 10^6 EID₅₀ per bird of H9N2 avian influenza virus. Then on days 1, 2, 4, 6, 8 and 10 post-inoculation (PI), samples of the thymus and bursa of Fabricius were collected for histopathological studies. In inoculated chickens, lymphocyte depletion in the thymus, follicular atrophy and cystic follicles in the bursa of Fabricius were seen. The results indicated that the H9N2 influenza virus has some immunosuppressive effects on chicken lymphoid organs.

Key words: H9N2 influenza, histopathology, thymus, bursa of Fabricius, broiler chickens

INTRODUCTION

Avian influenza (AI) is a viral, highly contagious disease of domestic and wild birds (Alexander, 2000). Avian influenza viruses are a diverse group of viruses in the family Orthomyxoviridae, genus influenza virus A and can be categorized into subtypes based on the two surface glycoproteins, the haemagglutinin (H) and 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. Avian influenza viruses 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 (Alexander, 2000; Fouchier Ron *et al.*, 2005; Swayne, 2007). Low Pathogenic Avian Influenza (LPAI) viruses are capable of replicating only in few organs, mainly the respiratory and GI tracts, and do not invade the rest of the body. However frequent incidences of high mortality have been reported in field situation in outbreaks of low pathogenic avian influenza viruses such as H9N2 subtypes. In 1998 an outbreak of low pathogenic avian influenza virus (H9N2 subtype) has occurred in Iranian poultry industry (Nili and Asasi, 2002, 2003). 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 different parts of the world (Naeem *et al.*, 1999;

Alexander, 2000; Nili and Asasi, 2002, 2003; Bano *et al.*, 2003; Capua and Alexander, 2004). 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; Banks *et al.*, 2000; Nili and Asasi, 2002, 2003; Alexander, 2003; Capua and Alexander, 2004; Amir *et al.*, 2007). Earlier pathogenesis studies revealed that LPAI viruses are pneumotropic following intranasal inoculation (Swayne and Slemons, 1994). Data collected from recent avian influenza outbreaks indicate that LPAI virus may mutate and become HPAI (Garcia *et al.*, 1996; Perdue *et al.*, 1997), and therefore to cause extremely complex situations with dramatic effects on the poultry industry. The aim of this study was to investigate histopathological findings of the thymus and bursa of Fabricius of commercial broiler chickens after intranasal inoculation with H9N2 avian influenza virus.

MATERIALS AND METHODS

This study was conducted in veterinary research laboratory from May 2010 to August 2010. Forty-eight 3-week-old commercial broiler chickens were randomly divided in two equal groups (Treatment and Control) and were housed in the same condition in two separate rooms. Chickens were monitored on a daily basis for general condition and the presence of clinical signs. Subsequently

the treatment group (twenty-four birds) was inoculated intranasally with 10^6 EID₅₀ per bird of H9N2 avian influenza virus at 20 days of age. Four birds from each group were randomly selected on days 1, 2, 4, 6, 8 and 10 post-inoculation (PI). Then they were humanly sacrificed and were subjected to throughout necropsy. Gross lesions were recorded and samples of thymus and bursa of Fabricius were collected for histopathological studies. Tissue samples were taken from each of four inoculated and uninoculated chickens and then fixed in 10% neutral buffered formalin solution. Tissue samples were routinely processed to paraffin wax blocks and five micrometer sections were prepared and stained with haematoxylin-eosin (H & E) stain for light microscopic examination.

RESULTS AND DISCUSSION

Daily monitoring did not show any changes in clinical behaviour of the birds in control group. Infected chickens showed clinical signs such as depression, puffing, oedema of face and head, conjunctivitis and ruffled feathers on day 4 PI. Control chickens did not show any gross lesions. However the most frequent gross lesions in infected birds were turbidity of the thoracic and abdominal air sacs, mild congestion of the trachea and lung, mild accumulation of fibrinous exudate on the tracheal mucosa and decrease size of thymus and bursa of Fabricius. In the control chickens, all of the examined organs were histologically normal and there was no detectable lesion. The chickens in the treatment group showed necrosis and lymphocyte depletion in medulla of thymus on days 8 and 10 PI (Fig. 1). In bursa of Fabricius on day 10 PI, lymphoid atrophy and cystic follicles were observed (Fig. 2). The frequency of histologic changes in thymus was 16.6% and in bursa of Fabricius was 12.5%. Although experimental study of low pathogenic AI viruses in SPF chicken produce no or low mortality, frequent high mortality rates have been reported in the commercial broiler chicken farms of Iran, this possibly due to the effects of H9N2 avian influenza viruses on chicken lymphoid organs and different degrees of immunosuppression (Hooper *et al.*, 1995, Mo *et al.*, 1997; Naeem *et al.*, 1999; Nili and Asasi 2002, 2003; Bano *et al.*, 2003). This experiment was conducted to study the histopathological changes of the H9N2 avian influenza virus in thymus and bursa of Fabricius of commercial broiler chickens. In this study, the predominantly histologic changes were mild lymphocyte necrosis, then lymphocyte depletion in the medulla of thymus, atrophy and cystic formation in the lymphoid follicles of bursa of Fabricius. Presence of mentioned histologic changes in the thymus and bursa of Fabricius in this study possibly secondary to stress or only transitory infection of virus. Our results were similar to lesions in chickens intratracheally inoculated with

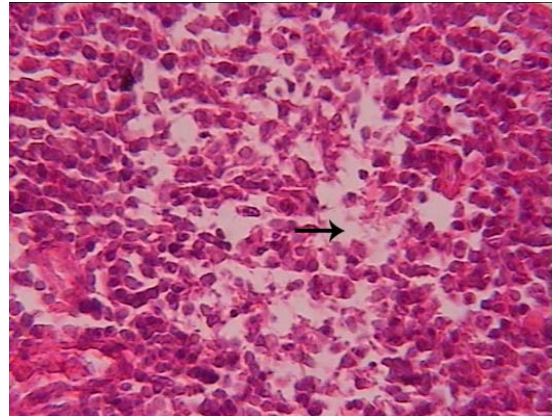


Fig. 1: Lymphocyte depletion in the medulla of thymus (black arrow) on day 8 PI. (H&E $\times 400$)

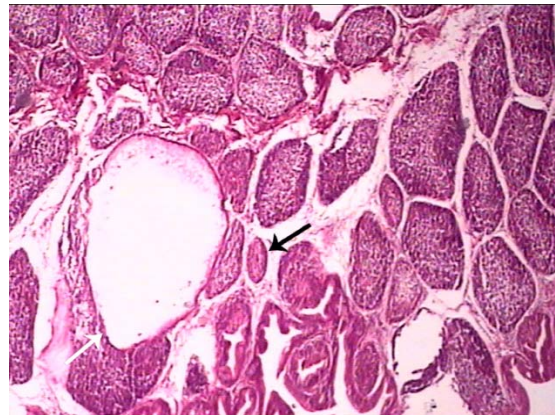


Fig. 2: Follicular atrophy (black arrow) and cystic follicle (white arrow) in bursa of Fabricius on day 10 PI. (H&E $\times 100$)

A/Chicken/Pennsylvania/21525/83 (H5N2) that was low pathogenic and lesions were limited to lymphoid organs and kidney (Mo *et al.*, 1997).

In another study after intravenous inoculation of avirulent H4N4, H6N2 and H3N8 viruses and intranasal/ocular inoculation of virulent H7N7 and H7N3 viruses into chickens, necrosis of the bursa of Fabricius was a common finding in all the birds. The degree of necrosis was usually very severe, with most lymphocyte being destroyed. There was no evidence of direct virus action on the lymphocytes as detected by the immunoperoxidase test, and it is possible that the necrosis in the bursa and some other lymphoid organs was secondary to stress (Hooper *et al.*, 1995). Other workers using similar immunohistochemical methods demonstrated specific action by another virulent avian influenza virus against lymphoid tissues (Van Campen *et al.*, 1989). Bano *et al.* (2003) inoculated an isolate of H9N2 avian influenza virus to chickens using different routes and subsequently challenged with other

infectious agents. The AIV histologic changes were detected in the trachea, lung, kidney and cloacal bursa among infected birds. In another study, 4 days after intranasal inoculation of 6-week-old chickens by H7N7 avian influenza virus, pyknosis and loss of lymphocytes in both the cortex and medulla of bursa of Fabricius were seen (Hooper, 1989). In this study the clinical sign and lesions found at postmortem examination were almost similar and milder than lesions produced in naturally infected chickens during H9N2 AIV outbreak in Iran and in Pakistan (Naeem *et al.*, 1999; Nili and Asasi, 2002, 2003; Bano *et al.*, 2003). However frequent cast formation in the tracheal bifurcation which has been reported in field cases of H9N2 avian influenza outbreaks, were not observed in this experiment. This finding shed some light on the H9N2 problem in Iran and some other Middle East countries, which has been reported to be associated with cast formation in the tracheal bifurcation. This could be due to mixed infection with other respiratory pathogens such as infectious bronchitis virus in field situation (Nili and Asasi, 2002, 2003).

CONCLUSION

Presence of histologic lesions in thymus and bursa of Fabricius suggests injury and death of lymphocytes are not the result of direct virus replication in lymphocytes but are probably secondary to stress, possibly from endogenous glucocorticoid secretion or from production of specific cytokines. The new results of the current study showed that the atrophy and lymphoid depletion of thymus and bursa of Fabricius and probably some other lymphoid organs, resulted in immunosuppression, predisposing to secondary bacterial infections and subsequent high mortality in field situations.

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