Journal of Animal and Veterinary Advances 10 (13): 1706-1710, 2011
ISSN: 1680-5593

Nephropathogenicity of H9N2 Avian Influenza Virus in Commercial Broiler Chickens Following Intratracheal Inoculation

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Abstract: H9N2 avian influenza virus is responsible for the majority of death in Iranian poultry farms due to renal damage. For understanding the nephropathogenicity of H9N2 avian influenza virus in commercial broiler chickens, thirty 20 days old chickens were intratracheally inoculated with 10^6 EID_{50} per bird with A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus. Then on days 1, 2, 4, 6, 8 and 10 Post-Inoculation (PI) samples of the kidney were collected for histopathologic studies. In inoculated chickens tubulointerstitial nephritis in the kidney were observed on days 6, 8 and 10 PI. The results indicated that the A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus after IT inoculation has pathogenicity for kidney (nephrotropic).

Key words: H9N2, influenza, nephropathogenicity, commercial broiler chickens, avain, virus, Iran

INTRODUCTION

Avian influenza viruses are caused a viral, highly contagious disease of domestic and wild birds and can be 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; 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 (Bano *et al*., 2003; Naeem *et al*., 1999; Nili and Asasi, 2002, 2003).

Influenza A viruses of the H9N2 subtype have become highly prevalent in poultry in many countries and although, these viruses generally cause only mild to moderate disease, they have been associated with severe morbidity and mortality in poultry as a result of co-infection with other pathogens (Brown *et al*., 2006; Nili and Asasi, 2002, 2003).

Antigenic and genetic analyses of H9N2 viruses isolated during the last two decades indicate that these viruses are extensively evolving and have reassorted with other avian influenza viruses to generate multiple novel genotypes (Li *et al*., 2003, 2005; Xu *et al*., 2004, 2007a, b). Prior to 1990, H9N2 viruses were mainly detected in avian species in North America and healthy ducks during surveillance in Southeast China (Brown *et al*., 2006). In 1988, the isolation of an H9N2 virus from Japanese quail in Southern China was the 1st recorded land-based poultry case of H9N2 in Asia (Perez *et al*., 2003a, b; Fedorko and Nelson, 2006; Liu *et al*., 2003). Since, 1998, an outbreak of low pathogenic avian influenza virus (H9N2 subtype) has occurred in Iranian poultry industry (Nili and Asasi, 2002, 2003). The H9N2 subtype outbreaks have occurred in domestic ducks, chickens and turkeys in different parts of the world (Capua *et al*., 2000; Bano *et al*., 2003; Capua and Alexander, 2004; Naeem *et al*., 1999; Alexander, 2000; Nili and Asasi, 2002, 2003). 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; Aamir *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 majority of death caused by A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus in commercial broiler farms of Iran might be due to renal

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damage, so the aim of this study was to investigate the nephropathogenicity of A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus following Intratracheal (IT) inoculation of this isolate in commercial broiler chickens.

MATERIALS AND METHODS

Experimental design: Sixty 20 days old broiler chickens were randomly divided in 2 equal groups (test 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 test group was inoculated intratracheally with 10^6 EID_{50} per bird of A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus at 20 days of age.

Five 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 kidney were collected for histopathologic studies.

Light microscopy: Tissue samples were taken from each of five 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 and E) stain for light microscopic examination.

RESULTS AND DISCUSSION

Clinical findings: 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 days 2-4 PI.

Gross necropsy findings: 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, decrease size of bursa of Fabricius and abnormal kidney. Thymus did not show obvious gross lesions.

Histopathology: In the control chickens (30 birds) all of the examined organs were histologically normal and there was no detectable lesion. The results of histopathology in kidney of inoculated group (30 birds) are as follow: Lymphocytic tubulointerstitial nephritis was predominant histologic change observed in the kidney on days 6, 8 and 10 PI (Fig. 1 , 2). The frequency of histologic changes in kidney was 26.6%. 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 field cases (Nili and Asasi, 2002, 2003; Naeem *et al*., 1999; Bano *et al*., 2003).

This experiment was conducted to study the nephropathogenicity of H9N2 AIV in the commercial broiler chickens. Histopathologic study of experimental intratracheal infection of chickens with Iranian AIV H9N2 isolate revealed that the virus is epitheliotropic and produced tubulointerstitial nephritis in commercial broiler chickens. 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;

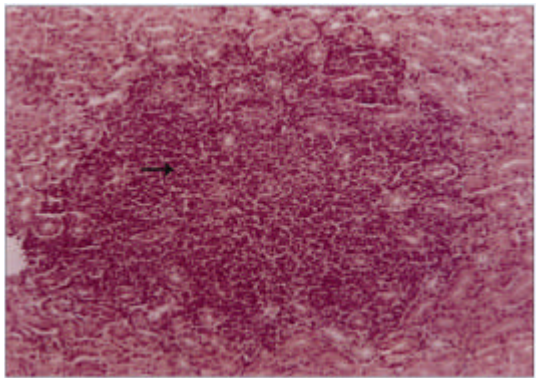

Fig. 1: Large aggregate of lymphocytes in the interstitial tissues of the kidney (black arrow) 8 days PI (H and E ×100)

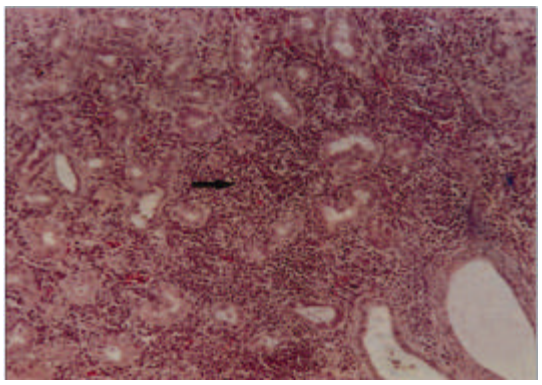

Fig. 2: Lymphocytic tubulointerstitial nephritis (black arrow) 10 days PI (H and E ×200)

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).

Recent studies indicate that co-infection of even infectious bronchitis live vaccine with H9N2 AI virus not only increased the severity of H9N2 AIV clinical sings and gross lesions but also increased the mortality rate due to the synergistic effects of both viruses on kidney and subsequent severe renal failure (Haghighat-Jahromi *et al*., 2007, 2008). In some researches (Slemons and Swayne, 1990, 1995; Slemons *et al*., 1990; Swayne and Slemons, 1990, 1992, 1994; Swayne *et al*., 1994; Swayne and Pantin-Jackwood, 2006), inoculation of chickens by Intranasal (IN) and Intratracheal (IT) route with low virulence chicken or duck-origin influenza virus isolates produced mortality and kidney lesions in 1 day old chickens and adult hens. However in other studies absence of mortality has been reported (Swayne *et al*., 1994; Shalaby *et al*., 1994).

In the current study, presence of inflammation and infiltration of lymphocytes in the renal tissues indicate that the A/Chicken/Iran/SH-110/99 (H9N2) avian influenza virus has pathogenicity for kidney of commercial broiler chickens. Hablolvarid *et al*. (2004) inoculated 5 weeks old chickens with an isolate of H9N2 avian influenza virus by intratracheal route. Tubulointerstitial nephritis and pancreatitis were the most frequent histologic changes. Influenza nucleoprotein was demonstrated in the kidney and pancreas of inoculated chickens. 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 antigen was detected in the trachea, lung, kidney and cloacal bursa among infected birds. Mo *et al*. (1997) inoculated chickens inoculated 4 weeks old SPF chickens intratracheally with A/Chicken/ Pennsylvania/ 21525/83 (H5N2) and histologic lesions were seen in lymphoid organs and kidney. In another study after intravenous inoculation of avirulent H4N4, H6N2 and H3N8 viruses into chickens, specific lesions and immunoperoxidase staining were noted in the kidney only (Hooper *et al*., 1995). As abdominal air sacs are next to kidneys, presumably, presence of nephritis foci in the kidneys during 6-10 days PI could be resulted from infection of the respiratory tract (air sacs). Regarding kidney lesions, the result obtained from the present study is in agreement with finding by Slemons *et al*. (1990). This finding indicate that renal failure resulted from kidney lesion could be encountered in H9N2 AIV infection in chicken.

CONCLUSION

The presence of abnormal kidney in gross necropsy findings, concurrent with histologic lesions were seen in the renal tissue in this study indicate that the A/Chicken/Iran/SH-110/99 (H9N2) isolate is epitheliotropic which in IT route of inoculation has pathogenicity for the kidney (nephrotropic).

ACKNOWLEDGEMENTS

The researchers thanks the Pathology Department Staff of Shiraz University of Medical Sciences and Histopathology Laboratory Staff of Chamran Hospital for technical assistance in preparing histopathologic sections.

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