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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Role of Infectious Bronchitis Live Vaccine on Pathogenicity of H9N2 Avian Influenza Virus

M. Haghighat-Jahromi[1,2], K. Asasi[1] H. Nili[1] and H. Dadras[1]
[1]Poultry Research Center, School of Veterinary Medicine,
Shiraz University, P.O. Box 1731, Shiraz 71345, Iran
[2]Group of Veterinary Medicine, Darab Branch, Islamic Azad University (IAU), Darab, Iran

Abstract: Based on experimental inoculation of chickens and sequence of amino acids at cleavage site, H9N2 AIV is pathotyped as low pathogenic avian influenza virus. But our extensive field experiences during last decade show serious disease problems and high mortality associated with this subtype in some Asian countries. One of the possible explanations for such a high mortality and great economic losses could be circulation of the virus and mixed infection with other respiratory pathogens. Infectious Bronchitis Live Vaccine (IBLV) is being used broadly in chicken farms of these countries. So it was decided to experimentally study the effect of infectious bronchitis live vaccine (H120) on enhancing of pathogenicity of H9N2 in broiler chicks. Clinical signs, gross lesions, viral shedding and mortality rate were compared between groups. Results of the present study showed that co-infection of IBLV with H9N2 AI virus not only increased the severity of H9N2 AIV clinical sings and gross lesions; but also increased the mortality rate and extended viral shedding period of H9N2 avian influenza virus.

Key words: H9N2 avian influenza virus, Infectious bronchitis virus, co-infection

Introduction

Influenza viruses are segmented, negative-sense, single-strand RNA viruses of the family Orthomyxoviridae and are divided into types A, B and C on the basis of the antigenic character of their internal nucleoprotein and matrix proteins. Only type A influenza viruses have been known to cause natural infections in birds. Type A influenza viruses are further divided into subtypes based on the antigenic relationships in the surface glycoproteins haemagglutinin (H) and neuraminidase (N) (Wood *et al*., 1993). To date, 16 H subtypes (H1 to H16) and 9 N subtypes (N1 to N9) have been recognized (Fouchier *et al*., 2005). Each virus has one H and one N antigen, apparently in any combination. Viruses of all subtypes and the majority of possible combinations have been isolated from avian species (Wood *et al*., 1993). Avian influenza viruses can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause Highly Pathogenic Avian Influenza (HPAI), which may result in flock mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. All other viruses cause a much milder disease consisting primarily of mild respiratory disease, depression and egg production problems in laying birds (low pathogenicity avian influenza [LPAI]) (Capua and Marangon, 2000). Replication of field situation in experimental study is almost impossible. Although laboratory examination in SPF chicken show that H9N2 avian influenza virus is non-highly pathogenic, it is almost one decade that Middle East and Asian countries are facing frequent outbreaks of H9N2 infection with high mortality (Naeem *et al*., 1999; Alexander, 2000; Guo *et al*., 2000; Bano *et al*., 2002; Nili and Asasi, 2002; Nili and Asasi, 2003; Naeem *et al*., 2003). Co-infection study is one approach in defining possible synergist effects of different organism on each other. Field and vaccine strains of infectious bronchitis virus are circulating in broiler farms in Iran and some other Asian countries (Haqshenas *et al*., 2005; Nouri *et al*., 2003). Personal experiences showed that some broiler flocks which had been vaccinated with live infectious bronchitis vaccine, showed extraordinary high mortality due to H9N2 infection. Therefore in this study it was decided to experimentally study the effect of infectious bronchitis live vaccine (H120) on enhancing of pathogenicity of H9N2 in broiler chicks.

Materials and Methods

One hundred and eighty one-day-old broiler chicks (*Ross 308*) were randomly divided into six equal groups. The chicks raised for 42 days in Animal Research Unit of Shiraz University Veterinary School in isolated groups. The birds were inoculated with 10^6 EID_{50}/bird H9N2 AIV [A/chicken/Iran/SH-110/99(H9N2)] via nasal route and/or one dose of IBLV vaccine [Freeze-dried Live attenuated vaccine, Mass type, H120 strain, Merial Company] via spray route (Table 1). No other vaccines were used in birds in control or treatment groups. The chickens were daily monitored for general condition, clinical signs, gross lesions, mortality and viral shedding. In order to

Table 1: Groups of broiler chicks inoculated with H9N2 AIV and/or infectious bronchitis live vaccine

Age (days) Groups	21	24	26
1(negative control)	-	-	-
2	IBLV	-	-
3	H9N2	-	-
4	IBLV+H9N2	-	-
5	IBLV	H9N2	-
6	H9N2	-	IBLV

AIV: Avian influenza virus (H9N2 subtype, 10^8 EID_{50}/bird), IBLV: Infectious bronchitis live vaccine (H120, one vaccine dose)

Fig. 1: A four weeks old broiler chick from group 4 (inoculated with AIV+IBLV) showing conjunctivitis, ocular discharge and swelling of the periorbital tissues and sinuses

investigate viral shedding; fecal samples were collected every day starting on day 1 post-infection. AIV Ag test kit (Felco) was used and samples, which were negative with the kit, were inoculated into 10-days-old embryonated chicken eggs via the chorioallantoic cavity route (0.2 mL per egg, five eggs per specimen). The eggs were incubated in a stationary incubator at 37°C, 55% relative humidity for 48 hours and chilled at 4°C for a minimum of 4 hr. Chorioallantoic fluids were harvested from dead (> 24 hr post inoculation) and live embryonated chicken eggs and tested for the presence of AIV by hemagglutination (HA) and Hemagglutination inhibition (HI) tests with known H9N2 positive antiserum (Johnson, 1990; Lennette, 1995).

Results and Discussion

Prior to inoculation, all chicks were normal and they did not show any clinical signs. In groups 1 (negative control) and 2 (IBLV) clinical signs were not observed in any of the chickens during their entire trial periods. Only

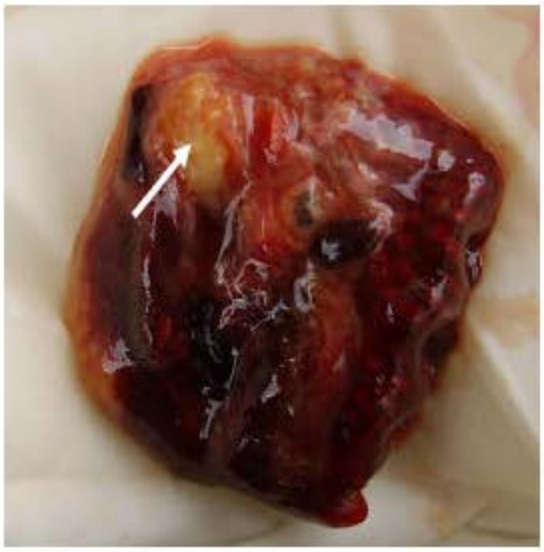

Fig. 2: Severe edema and congestion of the lung with accumulation of yellowish fibrinous (arrow) exudates on the pleura of a dead bird in group 4 (inoculated with AIV+IBLV)

Fig. 3: Formaldehyde fixed tracheal bifurcation showing bilateral cast formation in the lumen. Arrows showing extended tips of casts from bronchial lumen, from a dead bird in group 4 (inoculated with AIV+IBLV)

few birds in group 3 (AIV inoculated) showed mild clinical signs such as depression, ruffled feathers, nasal and ocular discharge and conjunctivitis.

The majority of birds in groups 4, 5 and 6 showed depression, ruffled feathers, respiratory distress (coughing, sneezing and dyspnea), swelling of the periorbital tissues and sinuses, conjunctivitis and discharge from the eyes, nose and mouth from day 2 to

Table 2: Virus isolation results for different groups after AIV inoculation

Days (PI) Groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 (negative control)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-
4	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
6	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-

6 post-infection (Fig. 1). In some cases, dyspnea was very severe and affected birds gasped for air with the mouth open, the head raised and the neck extended. A squeaking sound was often audible as the affected birds struggled for breath.

During acute stage of infection, the birds in groups 4, 5 and 6 died and post-mortem lesions were identified primarily in the respiratory tract (Fig. 2 and 3). The most severe cases had tubular casts in the tracheal bifurcation, which extended into the secondary and lower bronchi. Complete or partial blockage of the tracheal bifurcation resulted in asphyxiation (Fig. 2).

No mortality was observed in negative control birds (group 1) and the birds that inoculated either with IBLV (group 2) or AIV (group 3), while mortality rate in groups 4, 5 and 6 was 3%, 3% and 6.6% respectively.

As indicated in Table 2, except groups 1 and 2, AIV was isolated from the fecal samples of other groups and viral shedding period extended in groups 4, 5 and 6 as compared with group 3.

Results of the present study showed that co-infection of IBLV with H9N2 AI virus not only enhanced the virulence of H9N2 avian influenza virus and extended the viral shedding period but also increased the rate of mortality in infected birds. Gross lesions such as tracheal and lung hyperemia and exudation of the trachea with tubular cast formation in the tracheal bifurcation, which have been reported previously (Nili and Asasi, 2002), were observed in the necropsy examination (Fig. 2). Cast formation in the tracheal bifurcation abruptly reduced airflow, causing respiratory distress, dyspnea, cyanosis and asphyxiation. Kishida *et al*., 2004 showed that co-infection of *Staphyloccocus aureus* or *Haemophilus paragallinarum* exacerbates H9N2 influenza A virus infection in chickens (Kishida *et al*., 2004). Also Bano *et al*., 2002 showed that *Escherichia coli* has a significant role in viral shedding and virulence of H9N2 pathogenicity (Bano *et al*., 2002). Similarly, these lesions were commonly reported in turkeys during the 1999 outbreak of MP H7N1 AI in Italy in association with secondary bacterial pathogens such as *E. coli*, *Riemerella anatipestifer* and *Pasteurella multicida* (Capua *et al*., 2000). These suggests a common pathogenic mechanism with multiple subtypes of MP AI causing extensive damage to respiratory airways, fallowed by additional damage by secondary pathogens. In the present study gross lesions and viral shedding pattern are very typical and influenced by IBLV. Inoculation of IBLV 3 days before AIV has caused viral shedding started earlier and synchronous or delayed inoculation of IBLV has extended shedding period.

In this study we used vaccine strains of infectious bronchitis virus; therefore in field situation it is most likely that wild and field strains of infectious bronchitis virus could increase the severity of clinical manifestation, although the individual role of infectious bronchitis virus needs to be determined in the future.

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