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

M. Haghighat-Jahromi1,2, K. Asasi1, H. Nili1 and H. Dadras1
1Poultry Research Center, School of Veterinary Medicine, Shiraz University, P.O. Box 1731, Shiraz 71345, Iran
2Group 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 synergetic 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<sub>50</sub>/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

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Groups</th>
<th>21</th>
<th>24</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (negative control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>IBLV</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>H9N2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>IBLV + H9N2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>IBLV</td>
<td>H9N2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>H9N2</td>
<td>-</td>
<td>IBLV</td>
<td>-</td>
</tr>
</tbody>
</table>

AIV: Avian influenza virus (H9N2 subtype, $10^6$ EID$_{50}$/bird), IBLV: Infectious bronchitis live vaccine (H120, one vaccine dose)

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 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...
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.

### References


