SEROSURVEY OF H9N2 AVIAN INFLUENZA VIRUS DURING RESPIRATORY DISEASE OUTBREAKS IN BROILER FLOCKS IN DEZFUL, SOUTHERN IRAN

M. M. HADIPOUR & P. GOLCHIN
Department of Clinical Sciences, School of Veterinary Medicine, Islamic Azad University, Kazerun Branch, Kazerun, Iran

Summary


Since 1998, an epidemic of avian influenza occurred in the Iranian poultry industry. The identified agent was a low-pathogenicity H9N2 avian influenza virus that caused frequent episodes of high mortality in broiler chicken farms in Iran and some other Asian countries. This study was designed to investigate the prevalence of AIV H9N2 subtype in commercial chicken flocks in Dezful, southern Iran. Serum samples from 160 broilers (8 broiler flocks) with respiratory symptoms, were examined by hemagglutination inhibition (HI) test for specific antibodies against AIV H9N2 subtype. Overall HI titre and seroprevalence against H9N2 were 7.3 and 75.95%, respectively.

Key words: Avian influenza, broiler chickens, Dezful, H9N2 subtype, prevalence

Avian influenza virus (AIV) has been recognized as one of the most important pathogens in poultry. The H9N2 avian influenza virus (AIV) was reported to be of low pathogenicity in chickens (Alexander, 2003; Bano et al., 2003). The H9N2 subtype was first reported in 1966 in the United States (Homme & Easterday, 1970). Since then, the virus has been isolated in various countries (Alexander, 2003; Senne, 2003). In Middle Eastern countries during 1998–2000, H9N2 viruses were responsible for widespread and serious disease in commercial chickens in Iran (Nili & Asasi, 2002; 2003) Pakistan (Naeem et al., 1999; 2003; 2007), the United Arab Emirates (Mannell et al., 2000; Aamir et al., 2007) and Saudi Arabia (Banks et al., 2000). In Iran, the incidence and severity of respiratory disease in commercial chicken flocks have been recently increased due to poultry industry intensification. AIV is believed to be one of the main causes of chicken respiratory diseases in the country as indicated by many field reports (Nili & Asasi, 2002; 2003). Phylogenetic analysis of H9N2 isolates in Pakistan, Iran and Saudi Arabia showed very close relationships, suggesting a common source (Banks et al., 2000).

Numerous infections of poultry and other birds with the subtype H9 during the 1990s originated from separate intro-
DUCTIONS FROM FERAL BIRDS (BANKS ET AL., 2000; AL-NATOUR & ABO-SHEHADA, 2005).

DEZFUL IS SITUATED IN SOUTHERN IRAN, NEAR TO KAROUN LAKE AND PERSIAN GULF AND IS ON THE ROUTE OF MIGRATORY WILD BIRDS. LOCAL WILD AND FERAL BIRDS REMAIN IN THE AREA FOR SEVERAL MONTHS EACH YEAR. IN THE AREA OF THIS STUDY, THERE ARE ALSO SEVERAL BACKYARD CHICKEN FLOCKS. THE PRESENCE OF DIFFERENT BIRD SPECIES IN THIS REGION AND AROUND THE BROILER FARMS MAY RESULT IN THE RISK OF TRANSMISSION OF INFECTIOUS AGENTS, SUCH AS AVIAN INFLUENZA VIRUS (HADIPOUR, 2010).

THIS STUDY WAS DESIGNED TO INVESTIGATE THE PREVALENCE OF AIV H9 SUBTYPE IN BROILER FLOCKS IN THE DEZFUL REGION, SOUTHERN IRAN, USING A SEROLOGICAL TECHNIQUE.

DURING THE PERIOD FROM FEBRUARY 2010 TO SEPTEMBER 2010, 160 COMMERCIAL BROILERS FROM 8 BROILER FARMS LOCATED IN DEZFUL, SOUTH OF IRAN WITH RESPIRATORY DISEASE OUTBREAKS WERE EXAMINED. EACH FARM OWNED ONE FLOCK, AND THE CHICKENS WERE OF VARIOUS AGES RANGING FROM 20 TO 43 DAYS. THE BIRDS ARE USUALLY KEPT FOR A PRODUCTION LIFE OF 41–45 DAYS, AND THEN SOLD FOR SLAUGHTER. NONE OF FLOCKS RECEIVED ANY OF AIV VACCINES. THE STOCKING DENSITY WAS 10 BIRDS/M². BEFORE BIRDS WERE PLACED, THE HOUSES WERE CLEANED, WASHED, DISINFECTED AND PROVIDED WITH NEW WOOD SHAVINGS. INFORMED CONSENT WAS OBTAINED FROM FARMS’ OWNERS TO PARTICIPATE IN THE STUDY. BLOOD SAMPLES WERE COLLECTED BY VENIPUNCTURE OF THE BRACHIAL VEIN FROM 20 BIRDS FROM EACH FARM IN AN ACUTE PHASE OF RESPIRATORY SYMPTOMS. SERA WERE SEPARATED AND STORED AT –20 °C UNTIL TESTED FOR AIV H9 SUBTYPE ANTIBODIES.


IN THE MAJORITY OF ALL BROILER FLOCKS, CHICKENS SUFFERED FROM GASPING, COUGHING, CONJUNCTIVITIS, NASAL AND OCULAR DISCHARGE, DEPRESSION, INAPPETENCE, WEAKNESS AND WERE RELUCTANT TO MOVE. THE MORTALITY RATE WAS 15–25%. ALL FARMS HAD CHICKENS POSITIVE FOR ANTIBODIES AGAINST H9N2 AVIAN INFLUENZA VIRUS. MEAN ANTIBODY TITRES RANGED BETWEEN 8.3 AND 6.4 LOG₂, AND THE SEROPREVALENCES BETWEEN 84.3% AND 68.2% (TABLE 1). THE OVERALL HI TITRE AND SEROPREVALENCE OF AIV H9N2 SUBTYPE ANTIBODIES IN THIS STUDY WERE 7.3 AND 75.95%, RESPECTIVELY. NO SIGNIFICANT STATISTICALLY VARIATION IN H9N2 AIV ANTIBODY TITRE OR SEROPREVALENCE WERE FOUND AMONG THE EIGHT FLOCKS.

IN THE PRESENT STUDY, H9N2 AIV ANTIBODY TITRES BETWEEN 0 TO 10 LOG₂ WERE FOUND IN ALL FLOCKS. THIS MAY BE EXPLAINED BY THE INTENSIFICATION OF BROILER FARMS WHICH MAY RESULT IN DIFFERENT STAGES OF INFECTION IN THESE CHICKENS. THE PRESENCE OF CLINICAL SIGNS OF INFLUENZA IN BROILER FARMS ASSOCIATED WITH HIGH ANTIBODY TITRES IN SOME OF NON-VACCINATED BIRDS, COULD BE DUE TO PERSISTENT EXPOSURE OF BROILER FARMS TO AIV. ALL COMMERCIAL BROILER FARMS EXAMINED WERE POSITIVE FOR H9 AIV.
subtype. In the serological and molecular assays for detection of avian influenza in domestic pigeons in Kavar area of Iran, 34% of samples had antibody titres $\geq 2^3$ against the H9N2 AI virus (Mohammadi et al., 2010). In another survey of H9N2 avian influenza virus in backyard chickens around the Caspian Sea in Iran, the overall HI titre and seroprevalence against H9N2 were 6.52 and 72.98%, respectively (Hadipour, 2010).

Table 1. Mean HI antibody titres and seroprevalence in eight broiler flocks. Data are presented as mean ± SEM (n=20)

<table>
<thead>
<tr>
<th>Flock</th>
<th>Mean HI antibody titres</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.8 ± 0.37</td>
<td>84.30</td>
</tr>
<tr>
<td>2</td>
<td>7.3 ± 0.24</td>
<td>72.25</td>
</tr>
<tr>
<td>3</td>
<td>8.3 ± 0.40</td>
<td>78.40</td>
</tr>
<tr>
<td>4</td>
<td>6.5 ± 0.15</td>
<td>70.13</td>
</tr>
<tr>
<td>5</td>
<td>7.7 ± 0.26</td>
<td>79.50</td>
</tr>
<tr>
<td>6</td>
<td>6.4 ± 0.13</td>
<td>68.20</td>
</tr>
<tr>
<td>7</td>
<td>7.5 ± 0.31</td>
<td>74.23</td>
</tr>
<tr>
<td>8</td>
<td>6.9 ± 0.18</td>
<td>80.60</td>
</tr>
</tbody>
</table>

In virological, molecular and serological studies carried out to determine the status of infections with avian influenza viruses in different wild waterfowl species in Iran during 2003–2007, 48.5% of serum samples were positive by using a nucleoprotein-specific competitive ELISA (NP-C-ELISA). Duck species including mallards, common teals, common pochards, Northern shovelers and Eurasian wigeons revealed the highest antibody prevalence from 44 to 75% (Fereidouni et al., 2010). An investigation was undertaken by Nacem et al. (2003) in selected broiler-breeder, broiler and layer flocks, from which nine H9N2 AIV isolates were recovered. Serological data from this investigation indicated that both chickens in flocks with a previous history of respiratory tract infection and some without overt clinical respiratory signs had seroconverted. Al-Natour et al. (2005) reported that the seroprevalence of avian influenza was 71% among broiler-breeder flocks in Jordan. The number of positive sera correlated with flock size and to farms located within the migratory route of migratory wild fowl. In another study, 54.2% of broiler flocks and 78.3% of layer flocks in Jordan were positive for AIV H9 subtype antibodies (Roussan et al., 2009). Woo et al. (2008) reported that the 26% of layers and 23% of broilers in Korea were seropositive against H9N2 AIV. The high seroprevalence of AIV H9 subtype antibodies observed in the current investigation and previously reported by Nili & Asasi (2002, 2003) and Hadipour (2010) suggests the endemic nature of the disease in Iran. The H9N2 virus has been isolated from various avian species in other countries in the region (Banks et al., 2000; Manvell et al., 2000; Aamir et al., 2007; Naeem et al., 2003; Kwon et al., 2006).

In conclusion, it is essential that the biosecurity on poultry farms should be improved to prevent the introduction and dissemination of influenza and other viruses. Furthermore, farmers need to be educated about the signs, lesions, and the importance of this viral infection.

REFERENCES


Paper received 27.10.2010; accepted for publication 03.12.2010

**Correspondence**

Dr. Mohammad Mehdi Hadipour  
Department of Clinical Sciences,  
School of Veterinary Medicine,  
Islamic Azad University, Kazerun Branch,  
Kazerun, Iran  
P.O.Box 73135-168; phone: 00989177189086  
e-mail: hadipourmm@yahoo.com