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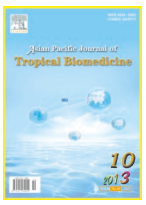

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Seroprevalence of avian influenza (H9N2) in broiler chickens in Northwest of Iran

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PEER REVIEW

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Comments

This is valuable work in broiler chickens of Northwest Iran which demonstrated avian influenza antibodies in serum of chickens at slaughterhouse. HI test was used as serological assay to evaluate sero prevalence of AI infection. High HI titer of AI highlighted role of this infection in the infections of broiler chickens in this area and probably throughout Iran. Details on Page 824

ABSTRACT

Objective: To demonstrate seroprevalence of avian invluenza (H9N2) subtybe in broiler chickens in Northwest of Iran.
Materials: A total of 310 blood samples were collected from 25 broiler flocks in slaughterhouses of West Azarbayjan, Iran. Serum samples were subjected to haemagglutination inhibition test.
Results: The test showed 40.6% of positive serums. Mean antibody titer of avian influenza virus differed between geographical locations in this survey.
Conclusions: High prevalence of avian influenza virus antibodies in serum of birds emphasize that avian influenza has an important role in respiratory complexes in broiler chickens in this region, and probably throughout Iran. Biosecurity measures, monitoring and surveillance programs, and to some degree vaccination are effective tools to prevent introduction of H9N2 infection and its economic losses.

KEYWORDS

Avian influenza, Broiler, HI, Iran

1. Introduction

Influenza type A viruses can be classified into high pathogenic and low pathogenic viruses[1]. They are categorized into several subtypes, based on their surface antigens, haemagglutinin (HA) and neuraminidase (NA). 16 HA (H1-H16) and 9 NA (N1-N9) subtypes are known, and their combinations make different subtypes[1]. Influenza viruses have been isolated from different host species, including mammals and birds[2]. Influenza viruses can produce mild symptoms to highly lethal infections in affected flocks. Avian influenza (AI) is an important zoonotic infection which causes mortality in human[3]. So, eradication of infection in poultry flocks diminishes public health concerns.

The H9N2 subtype is isolated from domestic fowls, ducks, geese, quails and pigeons[4]. This subtype can produce respiratory signs, losses in egg production, and mortality if accompanied with secondary pathogens in affected birds[5].

Molecular assays, viral isolation and presence of specific antibodies are indicative of exposure to influenza disease virus (AIV)[2]. Among serological assays, haemagglutination inhibition (HI) test is commonly used in routine surveillance programs and detection of infection.

The aim of this study was to detect antibody responses against H9N2 subtype in serum of broiler chickens in Northwest of Iran. The results of such surveys can be useful in designing management programs, with regard to H9N2 infections in broiler chickens of Iran.

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2. Material and methods

Three hundred and ten blood samples were collected from 2 slaughterhouses of West Azarbayjan, Northwest of Iran. 25 broiler flocks were included in this survey. Flocks were in West Azarbayjan and East Azarbayjan provinces (16 flocks in West and 9 flocks in East Azarbayjan). Serum were obtained by centrifugation of samples and subjected to HI test according to protocol[6]. Briefly, two-fold serial dilutions of sera were made and 4HA avian influenza virus subtybe H9N2 with equal volume (25 μL) of diluted sera was used in each well of 96 well microplate. After 45 min incubation at room temperature, 25 μL 1% chicken red blood cell was added and after 30 min incubation at room temperature, the last well which had a complete inhibition, was considered as the antibody titer. Statistical analysis was carried out using SPSS version 16.0 (SPSS, Chicago, IL, USA) where applicable.

3. Results

One hundred and twenty six out of the 310 collected broiler sera were positive for H9N2 antibodies in HI test (Table 1).

Mean antibody titer for avian influenza virus was 3.31. Mean titer of antibody of birds was significantly higher in East Azarbayhan province than West Azarbayjan ($P<0.05$). Samples of West Azarbayjan were divided into north and south, based on their geographic locations. There is a significance difference between mean titer of north and south samples ($P<0.05$). Mean avian influenza virus titer in north of west Azarbayjan was 3.35, whereas 2.39 in south.

Table1

Antibody titer of H9N2 in broiler chickens of Northwest of Iran.

Antibody titer	Number (percentage)
$<2^{-4}$	184 (59.4%)
2^{-4}	49 (15.8%)
2^{-5}	39 (12.6%)
2^{-6}	15 (4.8%)
2^{-7}	11 (3.5%)
2^{-8}	7 (2.3%)
2^{-9}	4 (1.3%)
2^{-10}	1 (0.3%)

Sera with titers $\geq 2^{-4}$ were considered positive.

4. Discussion

H9N2 has been reported from different countries including Iran[7], and this subtype is enzootic throughout Asia[8]. H9N2 viruses are not highly pathogenic for poultry, although opportunistic pathogens and immunosuppressive infections can compromise this infection.

Serological tests are useful for early detection and surveillance of infection. In this regard, four major tests agar gel immunodiffusion, ELISA, HI, neuraminidase inhibition were used[9]. HI is more specific and more commonly used in diagnostic laboratories for detection of infection.

Blood samples of slaughtered birds in 2 abattoirs of west Azarbayjan province from October to November 2012 were submitted to laboratory to detect antibody concentration in serum of birds by HI test. 40.6% of samples were positive for H9N2 antibodies. Some differences in mean HI titer were noted between geographic areas. High prevalence of H9N2 antibodies in birds indicates that avian influenza has an important role in respiratory complexes in Northwest of Iran and probably throughout the country.

Biosecurity, vaccination and monitoring are effective strategies of controlling infection[5]. Vaccination is applied in some broiler farms of Iran using killed vaccines. But, there is debate about usefulness of such vaccination. Vaccination increases bird resistance to field challenge, and decreases shed of virus in the poultry environment[5]. Although, cost of vaccination using killed vaccine is high, and vaccinated birds are not fully protected against field challenges, especially with highly pathogenic viruses.

Nili and Asasi reported H9N2 infections in broiler farms of Iran[10]. In order to determine AIV antibody status in migratory waterfowl of Iran, Fereidouni and coworkers collected 217 serum samples from 25 different species of waterfowl during 2003 and 2004[11]. A total of 77 samples from 14 different species were positive (35.5%). Hadipour and Golchin examined serum samples from 160 broilers with respiratory signs by HI test for specific antibodies against H9N2 subtype[12]. They found that mean HI titer and seroprevalence against H9N2 were 7.3 and 75.95% in commercial chicken flocks of Dezful, respectively. Another survey was conducted on 30 broiler flocks of Fars province, Iran. The overall 300 serum samples were considered for avian influenza antibodies by HI and ELISA tests. H9 subtype was detected in 263 and 274 serum samples of birds by HI and ELISA, respectively[13]. In a study on seroprevalence of AIV in pigeons of Kavar area, 34% serum samples had antibody titers $\geq 2^{-5}$ against the H9N2 AI virus[14]. Al-Barwary *et al.*, collected blood samples from 7 broiler farms in Iraq which were clinically infected with avian influenza[15]. The average mortality was 45%. They stated that 87% of birds were positive for avian influenza antibodies at 29-33 days old. Another study was carried out to determine prevalence of avian influenza in serums of 100 broiler flocks of Pakistan. Only 3 farms were positive[16]. A number of 253 poultry flocks in Korea were examined by HI test and agar gel precipitation test for seroprevalence of AIV. Seroprevalence rates in broiler and layer flocks were 23% and 26%, respectively. However, none of the broiler breeder and domestic duck flocks was seropositive[17]. Two hundred and sixty two samples of backyard poultry collected in Maryland were determined in prevalence of avian influenza and 4.2% of birds were found seropositive[18].

High seroprevalence of H9N2 subtype in broiler flocks observed in this survey revealed that such infection was endemic in Iran. Biosecurity measures decrease challenge with the disease and economic losses due to infection. Furthermore, live bird markets and migratory waterfowls should be monitored regularly. In the case of low pathogenic avian influenza infections, there is no eradication policy in Iran. So, vaccination can be used to diminish losses of AIVs.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Avian influenza is one of the most important respiratory diseases in poultry industry which causes devastating losses. This infection is endemic in many countries, including Iran. Surveillance pragmas are necessary to determine prevalence of infection. Results of such surveys are beneficial in designing effective control strategies against infection.

Research frontiers

In this survey 310 blood samples were collected from broiler chickens of West Azarbayjan province and subjected to HI test. Mean antibody titer of broiler chickens in different areas were compared.

Related reports

Avian influenza is endemic in Iran and many other countries. High HI titers are reported in many reports. Several factors are important in epidemiology of infection.

Innovations and breakthroughs

HI test is a specific and sensitive serological test that commonly used in diagnostic laboratories to detect avian influenza titers. Determination of AI titers at slaughterhouse is vital to determine avian influenza status in flock and effectiveness of control strategies. Birds at slaughterhouse have sufficient time to develop antibodies which reflected in serological assays.

Applications

Results of this survey showed avian influenza status in broiler chickens of Northwest Iran. Such surveys are beneficial in designing control strategies in poultry farms.

Peer review

This is valuable work in broiler chickens of Northwest Iran which demonstrated avian influenza antibodies in serum of chickens at slaughterhouse. HI test was used as serological assay to evaluate sero prevalence of AI infection. High HI titer of AI highlighted role of this infection in the infections of broiler chickens in this area and probably throughout Iran.

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