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Technical Note

Seroprevalence Survey of H_9N_2 Avian Influenza Virus in Backyard Chickens around the Caspian Sea in Iran

■ Author(s)

Hadipour MM

Department of Clinical Sciences
School of Veterinary Medicine
Islamic Azad University
Kazerun Branch
Kazerun, Iran

■ Mail Address

Dr. Mohammad Mehdi Hadipour
School of Veterinary Medicine
Islamic Azad University
Kazerun Branch
Kazerun, Iran
P.O.Box: 73135-168

Tel: 091 7718 9086
Fax: 072 1223 0508

■ Keywords

Avian Influenza virus, backyard chickens, Caspian Sea, H_9N_2,seroprevalence, Iran, HI

ABSTRACT

Since 1998, an epidemic of avian influenza occurred in the Iranian poultry industry. The identified agent presented low pathogenicity, and was subtyped as an H_9N_2 avian influenza virus. Backyard chickens can play an important role in the epidemiology of H_9N_2 avian influenza virus infection. Close contact of backyard chickens with migratory birds, especially with aquatic birds, as well as neighboring poultry farms, may pose the risk of transmitting avian influenza virus, but little is known about the disease status of backyard poultry. A H_9N_2 avian influenza virus seroprevalence survey was carried out in 700 backyard chickens from villages around the Caspian Sea, Northern Iran, using the hemagglutination-inhibition (HI) test. The studied backyard chickens had not been previously vaccinated and showed no clinical signs of disease. The mean antibody titers found were 6.8, 7.5, 5.9, 7.2, 5.7, 6.4, 6.2 and the seroprevalence was 76.2%, 79.5%, 68.18%, 78.27%, 65%, 72.31% and 71.4% as found in seven villages. Overall HI titer and seroprevalence against H9N2 were 6.52 and 72.98%, respectively.

INTRODUCTION

Avian influenza due to H9N2 subtype in poultry during the later part of the 1990s has noticeably increased worldwide. H_9N_2 subtype outbreaks occurred in domestic ducks, chickens and turkeys in Germany during 1995 and 1998, in chickens in Italy in 1994 and 1996, in pheasants in Ireland in 1997, ostriches in South Africa in 1995, turkeys in the USA in 1995 and 1996, and in chickens in Korea in 1996 (Bano *et al*., 2003; Capua & Alexander, 2004; Naeem *et al*., 1999). More recently, H9N2 viruses have been reported in Middle Eastern countries and shown to be responsible for widespread and serious disease problems in commercial chickens in Iran, Pakistan, Saudi Arabia, and United Arab Emirates (Aamir *et al*., 2007; Alexander, 2003; Banks *et al*., 2000; Capua & Alexander, 2004; Naeem *et al*., 1999, 2007; Nili & Asasi, 2002, 2003). In 1998 an outbreak caused by a low pathogenicity avian influenza virus (LPAIV, H9N2 subtype) occurred in the Iranian poultry industry (Nili & Asasi, 2002, 2003). Backyard (village) chickens throughout the world, especially in Middle Eastern countries, play an important role in people nutrition due to meat and egg production. Caspian Sea is the largest body of inland water all over the world, and it is situated at the north of Iran. Numerous different species of birds, particularly aquatic birds, migrate to the Caspian Sea, where they remain for several months each year. Close contact of these birds with backyard chickens may result in the risk of transmission of infectious agents. such as avian influenza virus, but little is known about the disease status of backyard poultry. The aim of this study was to evaluate LPAIV H_9N_2 exposure of village chickens around the Caspian Sea using the hemagglutination inhibition test.

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MATERIALS AND METHODS

Serum samples and HI assay. A total of 700 blood samples were randomly collected from the wing vein of backyard chickens (unvaccinated, mature, and healthy chickens) belonging to seven villages around the Caspian Sea. Samples were maintained at room temperature and transported to the laboratory within 24 h. If a delay in sample transportation was expected, samples were centrifuged and frozen at -20 °C before being submitted to the laboratory. Antibodies against H_9N_2 avian influenza virus (A/chicken/Iran/SH-110/99(H_9N_2)) present in the serum were evaluated using the hemagglutination inhibition (HI) assay. The HI assay was performed using 96 'U'-well microtiter plates, doubling dilution in PBS, 1 % v/v red blood cells (RBC), and 4 HA units of AIV antigen (Alexander *et al*., 1983).

RESULTS

Samples were considered negative if titers were ≤8. Positive flocks had at least one serum sample with titer > 8 or at least 3/15 with titer = 8 (Nooruddin *et al*., 2006). Results revealed that all seven villages had chickens that were positive for antibodies against H_9N_2 avian influenza virus. Mean antibody titers were 6.8, 7.5, 5.9, 7.2, 5.7, 6.4, 6.2 $\log_2$, and the seroprevalences were found to be 76.2%, 79.5%, 68.18%, 78.27%, 65%, 72.31% and 71.4%, respectively, in the seven villages.

Overall antibody titer and seroprevalence of H_9N_2 avian influenza virus recorded were 6.52 and 72.98%, respectively. The geometric mean titers in each group were 49.1, 69.1, 28.5, 52.3, 23.7, 41.4 and 37.6, respectively. The results were statistically analyzed by one-way analysis of variance (Miller, 1997). No significant variation ($p>0.05$) in H_9N_2 avian influenza virus antibody titer or seroprevalence of H9N2 AIV were found among the seven villages, although within each village, significant variation ($p<0.05$) was observed among individuals. In spite of presence of high antibody titers among chickens in each group, no clinical symptoms were observed.

DISCUSSION

In the present study, H_9N_2 AIV antibody titers between zero to 10 $\log_2$ HI ($p<0.05$) were found in all villages. This may be explained to the extensive rearing system in village chicken,s which may result in different stages of infection in these chickens. The absence of clinical signs of influenza in backyard chickens, in spite of high antibody titers in some birds, could be due to persistent exposure and acquired resistance of these birds to influenza virus in the environment, and therefore, these birds would be naturally vaccinated against this virus.

Van Kammen *et al*. (1982) showed influenza A antibodies in sera of free-range village fowls.

Cheng *et al*. (2002) found H_9N_2 avian influenza antibody titers in 26% of human sera and only in 7% of chicken sera, and concluded that human H_9N_2 virus infection would probably derived from chicken H_9N_2 virus.

An investigation was undertaken by Naeem *et al*. (2003) in selected broiler-breeder, broiler and layer flocks, from which nine H_9N_2 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.

In another study conducted by Li *et al*. (2004), anti-H_9N_2 antibodies were found in 12.8% of the chickens and 5.1% of the poultry-farm workers.

In Hong Kong during 2001-2003, the H_9N_2 avian influenza virus had the highest prevalence among live poultry markets (Choi *et al*., 2004).

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 was correlated with flock size and to farms located within the migratory route of migratory wild fowl.

In another study conducted by Nooruddin *et al*. (2006) in Bangladesh, an overall 9.82% seroprevalence of avian influenza was recorded.

In our study, the backyard chickens in the studied areas were reared under semi-scavenging system and were allowed to scavenge with ducks in the yard, in the crop fields near water reservoirs, where there were domestic ducks, wild ducks, and migratory birds, and this may have contributed to the natural infection of the backyard chickens (Alexander, 2003; De Marco *et al*., 2003; Senne *et al*., 2003; Vander *et al*., 2003; Capua & Alexander, 2004). The high prevalence detected in our study was possibly due to the close and frequent contact of village chickens with numerous and different species of migratory waterfowl in the surveyed region. According to the results of the present study, H_9N_2 avian influenza virus is endemic in backyard chickens of Iran, and at different degrees of infection. These birds may be asymptomatic carriers of the influenza virus.

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