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An active serological survey of antibodies to newcastle disease and avian influenza (H9N2) viruses in the unvaccinated backyard poultry in Bushehr province, Iran, 2012–2013

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PEER REVIEW

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Comments

This is valuable work on unvaccinated backyard poultry in Bushehr province, South of Iran, which demonstrated Newcastle disease and avian influenza, H9N2 antibodies in serum of chickens. HI test was used as serological assay to evaluate seroprevalence of newcastle disease and avian influenza infection. The result of this study shows that newcastle disease virus and avian influenza (H9N2) are widely distributed in backyard areas of Bushehr province. Details on Page S215

ABSTRACT

Objective: To test the antibodies against newcastle disease virus (NDV) and avian influenza virus (AIV, H9N2) in the unvaccinated backyard poultry in Bushehr province, Iran from 2012 to 2013.

Methods: A total of 1530 blood samples from unvaccinated backyard chickens in Bushehr province, south of Iran, were tested for antibodies against NDV and AIV (H9N2) by hemagglutination inhibition test according to International Epizootic Office (OIE) recommendation.

Results: Of these, 614 (40.13%) and 595 (39.00%) were positive for NDV and AIV (H9N2) respectively.

Conclusions: The findings of the present study indicated that NDV and AIV (H9N2) were endemic and widely distributed in backyard areas of Bushehr province which should be incorporated in the control strategies. Further studies are needed to identify the circulating virus genotypes, model their transmission risk, provide adapted control measures and design proper and applicable vaccination program.

KEYWORDS

Newcastle disease, H9N2, Iran, Backyard chicken

1. Introduction

Newcastle disease virus (NDV) and avian influenza virus (AIV) (H9N2) are the causative agents of serious avian diseases that can result in significant economic losses to the poultry industry. NDV is classified as a member of the *Avulavirus*, *Paramyxoviridae*. NDV strains have been isolated

from all types of commercially reared poultry, ranging from pigeons to ostriches[1]. Avian influenza has emerged as a disease with significant potential to disrupt commercial poultry production often resulting in extensive losses. AIV is a member of the family *Orthomyxoviridae*, containing negative sense single stranded RNA[2]. The AIV H9N2 subtype was first reported to infect turkeys in the United States in

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1966 and has been panzootic in Eurasia^[3]. Generally, the affected chickens show mild to severe respiratory signs, edema of the head and the face (head and face edema), and decreased egg production accompanied with soft-shelled or misshaped eggs. The mortality is usually about 5–30% depending on the type of husbandry^[4]. Newcastle disease is endemic in Iran and every year we have some reports for incidence of Newcastle disease in Iran. In the past few decades, implementation of extensive vaccination programs in commercial poultry farms, and to some extent in small rural poultry farms have reduced the number of epizootics outbreaks of Newcastle disease in Iran^[5,6]. In recent year so many reports for emerging of velogenic Newcastle disease in commercial farms has been reported. Rural and backyard poultry breeding is the prevalent form of poultry production in the developing countries. Also, in Iran (Ghazvin province), the H9N2 (subtype influenza A virus of low pathogenicity) virus was first isolated from chickens in 1998 and it's the most prevalent subtype of influenza virus in poultry industry in Iran till now^[7]. In 2006, H5N1 was reported in swans in northern of Iran firstly, but to this time we don't have official report from industrial flocks in Iran. Although Fereidoni S *et al.* report detection of other subtypes of AIV from migratory birds in Iran^[8]. The presence of specific antibodies to NDV and AIV (H9N2) in the serum of birds in backyard flock helps experts to monitor Newcastle disease or avian influenza in out of industrial poultry flocks and assist veterinary organization to design proper surveillance program to design national and regional poultry health policies^[1]. The aim of the current study was to determine seroprevalence rate of Newcastle disease and AIV (H9N2) in the backyard poultry in Bushehr province, Iran, 2012–2013.

2. Materials and methods

2.1. Study area and sampling

Bushehr province is one of the 31 provinces of Iran (28° 55' 6.24" N, 50° 50' 17.52" E). It is in the south of the country, (Persian Gulf) (Figure 1). In total, 1530 blood samples have been collected from rural and backyard chicken in six counties (32 villages) of Bushehr province according to each backyard flock population. Blood samples (1 mL) were collected from a wing vein by using the appropriate needles and syringes. Serum was separated from the clot by centrifugation at 4000 r/min for 10 min and stored at -7 °C. The procedure was done according to ethical protocols in animal research.

2.2. Serological survey

2.2.1. Newcastle disease

The haemagglutination inhibition (HI) test was used for the detection of the presence of the antibodies against

NDV according to The Office International des Epizooties (OIE) Manual (2012)^[9]. The haemagglutinin (HA) titres of the Newcastle disease La Sota antigen were determined as described by Allan and Gough (1974) and diluted to contain 4–HA units^[10]. This concentration was used for the HI test. The HI titer for each bird was determined and expressed in log₂, and the mean for each birds was calculated. A titer greater or equal to 4 Log₂ was considered positive based on OIE recommendation. Laboratory results of Newcastle disease were entered and managed using Microsoft Excel (Windows 2010). Descriptive statistics for the HI antibody titers were performed using the same program.

2.2.2. Avian influenza (H9N2)

The HA/HI test was performed according to the OIE (2000) manual, using a reference antigen for AIV H9 subtype (A/Ch/Iran/772/99) (H9N2). The HI assay was performed using 96 'U'-well microtiter plates, doubling dilution in phosphate buffer solution, 1% v/v red blood cells, and 4 HA units of AIV antigen. Positive flocks had at least one serum sample with titer >4.



Figure 1. Location of Bushehr province within Iran.

3. Results

Results of the investigation revealed that all six counties had unvaccinated chickens that were positive for antibodies to NDV and AIV (H9N2). The overall seroprevalence rate of NDV antibodies was 40.13% (614/1530) and average HI titer was 5.75. The highest prevalence (56.20%) and HI titer (6.02) for NDV occurred in Tangestan County. Also, the antibody (mean) and seroprevalence and titer of AIV (H9N2) were recorded 39.00% and 5.61, respectively. All data has been shown in Table 1.

Table 1Seroprevalence of NDV and AIV (H9N2) antibody (HI test; Log₂) in unvaccinated backyard chickens in Bushehr province, Iran, 2012–2013.

Counties	No. of villages	No. of blood samples	Newcastle disease		Avian influenza (H9N2)	
			No. of positive samples	HI titer	No. of positive samples	HI titer
			[n (%)]	(mean; Log ₂)	[n (%)]	(mean; Log ₂)
Dashtestan	11	541	222 (41.00%)	5.89	248 (45.84%)	5.68
Bushehr	5	225	70 (31.10%)	5.76	57 (25.33%)	5.79
Kangan	3	204	102 (50.00%)	5.73	69 (33.83%)	5.55
Dashti	5	166	17 (10.24%)	5.41	17 (10.24%)	5.35
Gonaveh	6	225	108 (48.00%)	5.63	134 (59.55%)	5.34
Tangestan	2	169	95 (56.20%)	6.05	70 (41.42%)	6.41
Total	32	1530	614 (40.13%)	5.75	595 (39.00%)	5.61

4. Discussion

This study is a part of the Newcastle disease and avian influenza surveillance and prevention program in Iran, which includes other birds' populations such as commercial poultry and all the controls for importation and exportation of live birds. Backyard poultry flocks chickens play an important role in the spread of the virus among industrial poultry flocks. The samples were collected from unvaccinated, apparently healthy birds (no clinical symptoms), suggesting that the infections were due to circulating avirulent NDV strains and H9N2 subtype. For interpretation of NDV results, whether these are originated from vaccine strains or another source, for example Class I or Class II lineage 1 Newcastle disease viruses, which are common in wild birds, is unknown^[11].

Our results are in agreement with the findings of other research groups particularly who did experiment on Newcastle disease in other provinces of Iran. A serological survey of NDV antibody in backyard chicken around Maharlou Lake, Hadipour (2009) showed that antibody titer and seroprevalence of NDV was recorded 5.21 and 37.56%, respectively^[12]. Rezaeianzadeh *et al.* (2011) carried out a survey on NDV prevalence in village chickens of Fars province using molecular and serological tests. Results showed that chickens in 13 villages (61.9%) were seropositive, but all of reverse transcription–PCR results were negative^[13]. A study carried out in rural chickens by Vui *et al.* showed a 33.9% prevalence of antibodies against NDV in backyard flocks in Vietnam^[14], while 63% seroprevalence was reported by Orajaka *et al.* in south eastern Nigeria^[15]. Also, NDV antibodies were found in 10.2% of backyard poultry populations in Switzerland^[16]. In another study which conducted by Musako *et al.* on Zambian backyard chicken flocks, seroprevalence varied amongst the five provinces sampled, ranging from 48.3% to 82.6% in different geographical regions^[17].

The overall HI titer and seroprevalence against H9N2 of backyard chickens around Maharlou Lake in Iran were 7.73% and 81.6%, respectively^[18]. Surveillance of scavenging ducks for H9N2 showed that seroprevalence of H9N2 were 80.92%^[19]. Ducks and backyard chickens of Shiraz, Southwest of Iran, were checked for antibodies against H9N2. The HI titers and

seroprevalence in ducks and backyard chickens were 8.3, 5.7 and 78.4, 62.9%, respectively^[20]. In a similar study on 700 backyard chicken around Caspian sea in north of Iran, seroprevalence against H9N2 was 72.98%^[18].

This survey shows that the Newcastle disease and avian influenza (H9N2) viruses are circulating in the Bushehr provincial backyard chickens where there may be temporary risk windows for introduction to industrial poultry farms resulting in economic impacts. It is therefore so important that further detailed studies focus on NDV and AIV (H9N2) strains identification so that preventive and control programs particularly with emphasis to start vaccination (ring vaccination) in this area can be formulated.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In recent year so many reports for emerging of velogenic Newcastle disease in commercial farms were reported. Rural and backyard poultry breeding is the prevalent form of poultry production in the developing countries. Also, in Iran (Ghazvin province), the H9N2 (subtype influenza A virus of low pathogenicity) virus was first isolated from chickens in 1998 and it is the most prevalent subtype of influenza virus in poultry industry in Iran till now.

Research frontiers

Newcastle disease and AIV (H9N2) is endemic in Iranian

poultry industry. Surveillance programs are necessary to determine prevalence of infection (particularly in backyard poultry and live bird market as critical epidemiological source). Results of such surveys are beneficial in designing effective control strategies against infection.

Related reports

A total of 1530 blood samples from unvaccinated backyard chickens in south of Iran, Bushehr province were tested for antibodies against NDV and AIV (H9N2) by HI test according to OIE recommendation.

Innovations and breakthroughs

HI test is a specific and sensitive serological test that commonly used in diagnostic laboratories to detect Newcastle disease and avian influenza titers. Determination of titers at backyard poultry herds is vital to determine Newcastle disease and avian influenza status in flock and effectiveness of control strategies.

Applications

The data help Iranian Veterinary Organization to design proper and applicable vaccination and surveillance program.

Peer review

This is valuable work on unvaccinated backyard poultry in Bushehr province, South of Iran, which demonstrated Newcastle disease and avian influenza, H9N2 antibodies in serum of chickens. HI test was used as serological assay to evaluate seroprevalence of newcastle disease and avian influenza infection. The result of this study shows that NDV and avian influenza (H9N2) are widely distributed in backyard areas of Bushehr province.

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