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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

Yousef Saadat[1], Seyed Ali Ghafouri[2*], Farshad Tehrani[2], Arash Ghalyanchi Langeroudi[3]

[1]*Bushehr Veterinary Organization, Bushehr, Iran*

[2]*Iranian Veterinary Organization, Tehran, Iran*

[3]*Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

PEER REVIEW

Peer reviewer

Dr.Vahid Karimi (D.V.M, D.V.Sc), Associate Professor, Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Tehran, Iran.
Tel: 021-61117000
Fax: 021-61117000
E-mail: vkarimi@ut.ac.ir

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.
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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 1 530 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

*Corresponding author: Seyed Ali Ghafouri, Iranian Veterinary Organization, Tehran, Iran.
Tel: 00982188953400
Fax: 00982188962392
E-mail: s_ali_ghafouri@yahoo.com
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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.