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Original Article

Seroepidemiological investigation of influenza type A (a zoonotic disease) in native turkeys in East Azerbaijan

Ali Reza Dehnad[1,2], Behrooz Naghili[2*], Mohammadreza Ghorani[3]

1- Biotechnology Department, East Azerbaijan Research and Education Center Agricultural and Natural Resources (AREEO), Tabriz, Iran
2- Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3- Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
*** Corresponding Author**: naghili_b@yahoo.com

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Summary

Influenza is one of the acute respiratory viral diseases. The purpose of this study was seroepidemiology of type A flu in native turkeys in East Azerbaijan province, Iran. During 18 months, 350 blood samples were taken randomly from the turkey flocks in East-Azerbaijan, Iran. The collected sera were stored at -70°C for further analyses. To determine the influenza antibody level, the *Haemagglutination inhibition (HI)* and *single radial immunodiffusion (SRD)* tests were performed. Firstly, the mean HI titers of each flock and area were recorded separately, which subsequently compared with the SRD data. Interestingly, all of the serum samples were negative for the H5 and H7 antigens. However, some of them were positive for H9. There were significant differences in the mean titers of HI in vaccinated and non-vaccinated herds against influenza, different age groups, and the flocks with and without clinical symptoms ($p < 0.05$). Taken to gather, it seems that vaccination against influenza virus and an increase in age can increase serum titration of influenza virus. Regarding the presence of influenza virus in the turkey flocks with the various antigenic features and high mutation rate, it should be considered in relation to public health.

Keywords: Seroepidemiology, Influenza type A, East Azerbaijan, Turkey.

Introduction

The influenza virus belongs to the orthomyxoviridae family, which are categorized into types A, B, and C according to the nucleoprotein and matrix protein similarity. Among these, type A is the most important as the pathogenicity in humans and animals, which is responsible for fatal influenza pandemics (Swayne et al., 2013; Al-Natour, 2005). Importantly, it has been isolated from mammals such as humans, pigs,

horses, minks, and whales in addition to birds. Influenza is one of the acute contagious viral pulmonary diseases observed in sporadic, epidemic and endemic forms (Al-Natour, 2005). Of note, it can infect domestic birds such as poultry (Swayne et al., 2013), ducks (Brown and Stallknecht, 2006), pheasants (Humberd et al., 2007), and turkeys (Halvorson, 2000), which causes high economic loss poultry industry (Swayne et al., 2013).

It was previously reported that influenza virus infections in the birds were 11%. The prevalence of the virus in birds' populations in lakes was much higher and were about 20 to 60% that can show the potential role of the migratory birds in the whole world (Swayne et al., 2013).

In recent years, some flu viruses were isolated from turkeys in countries with advanced poultry industry (Irvine et al., 2010). Commercial flocks of ducks also show infection with influenza viruses. However, they rarely show clinical signs due to resistance to disease (Chen et al., 2010; Eggert and Swayne, 2010). Most species of birds are susceptible to flu, and the virus was isolated from ducks, geese, quails, turkeys, guinea fowls, partridges, pheasants, ostriches, and other wild birds like swallows, sparrows, swans, pelicans, and seagulls (Alexander, 2000a).

Increasing evidence suggested two main classification for influenza viruses: "low-pathogenic" and "high pathogenic" (Alexander, 2000b). There is a variety of the biological and pathogenic properties of poultry influenza viruses and cannot predict by host identity and antigenic HA subtypes. Subtypes of H5 and H7 cause severe disease in chickens, turkeys, ducks and seagulls However, just antigenic condition is not determinative for pathogenesis. The pathogenesis of any virus depends on the reaction between the host, and the virus and a pathogenic virus is not pathogen for other pathogens (Alexander, 2000a).

There is a wide spectrum of pathogenesis between poultry influenza viruses. These viruses cause two completely different types of diseases that one of them is common and mild, and the other is rare and highly lethal. The signs of the mild one are just messy and ruffled feathers, declining in egg production or mild signs in the respiratory system. The prevalence of this type of disease can be too rare to detect just by repeated examinations. However, the symptoms of the second type that is uncommon but highly pathogen, is completely evident (Swayne et al., 2013).

It should be mentioned that the morbidity of the influenza infection depends on the virus strain, species and age of infected birds, immune system, and the presence of other simultaneous disease like colibacillosis and Newcastle disease (Alexander, 2000b). In California and Minnesota states with intensive turkey husbandry farms and migratory birds, influenza infections are regularly seen (Swayne et al., 2013). Growing evidence suggested that a bird's flu virus may alter and get the ability to infect humans and is transmitted from person to person (Alexander, 2000a). The aim of this study was seroepidemiology evaluation of influenza type A in native turkeys of East Azerbaijan province.

Materials and methods

During 18 months, 350 blood samples were taken randomly from the turkey flocks (17 vaccinated and 18 un-vaccinated flocks, and 10 samples in each flock) in East-Azerbaijan, Iran. The blood samples were centrifuged with speed rpm 3000 at 4°C for 15 minutes. The collected sera were stored at -70°C for further analyses. To determine the influenza antibody level, the *haemagglutination inhibition (HI)* and *single radial immunodiffusion (SRD)* tests were performed (Stohr et al., 2012). The mean HI titers of each flock was recorded separately, which subsequently compared between vaccinated and un-vaccinated flocks. Also, four age groups were considered, including three, six, nine, and 18 months old. Besides, we compared the mean of the titers between the flocks with and without clinical signs.

Haemagglutination inhibition (HI) test:

1- 0.025 ml of PBS was poured in every well.
2- 0.025 ml of suspect serum was poured to the first well.
3- 0.025 ml of diluted based on $\frac{1}{2}$ serum was poured to the other wells.
4- 0.025 ml of virus suspension containing 2 units of hemagglutination was added to every well and discarded for 30 min at room temperature (20 °C) and 60 min at 4°C.
5- Then 0.025 ml of 1% chicken RBC suspension was added to every well, and shake it slowly and waited for 20 min until the RBCs sediment. In this stage, we considered one well as a for RBC control.
6- In the wells that hemagglutination inhibition occured, the fall of the red blood cells was similar to RBC wells control.
7- To achieve valid results, negative control serum was used.

Single radial immunodiffusion (SRD) test

1- Nucleocapsid antigens was prepared by freezing and melting the chorioallantoic membrane of 10-day embryonated eggs inoculated with the virus three times after crushing and homogenizing them.
2- Then they were centrifuged, and the supernatant was deactivated by 0.1% formalin or beta propiolactone. They have centrifuged again, and the obtained liquid was used as an antigen.
3- The test was done by using 1% agar gel in phosphate-buffered saline (PBS) at the PH 7.2.
4- Agar was poured on the microscope lam of 2-3 mm size. Some wells with diameters of 5 mm, which were 2-5 mm away from each other, were created by special borer on the agar.
5- Then suspect serum was poured in a well, and positive control serum and antigens were poured in the neighbor wells. This action caused a similar continuous line between positive control serum and suspect serum and ribonucleoprotein antigen.
6- About 50 µl of the indicators as mentioned earlier was added to every well. The created sedimentary lines were recognizable after 24 to 48 hours, and this time depended on the concentration of antibody and antigen.

Statistical analyses

The present data were analyzed using SPSS software (SPSS, version 14 for windows, USA), presented as mean ± SD, and a $P < 0.05$ were considered significant. Also, the diagrams were drown by SPSS 14.

Results

On the base of the present results, the turkey serum samples were negative for infection with subtypes H5 and H7, but infection with subtype H9 was evident in some flocks. The average HI titration in flocks that had received influenza vaccines and in the flocks without vaccination, was 6 ± 0.25 and 3.45 ± 0.15, respectively. This represents a significant difference ($p < 0.05$) and the positive effect of vaccination against the influenza virus in turkey flocks. The comparative evaluation of age with titration indicates that an increase in age increases serum titration (fig. 1A). As more details, there was a significant difference between 3 months age and other age groups. Also, there was a significant difference ($p < 0.05$) between the mean titration of the flocks with and without clinical signs (Fig. 1B).

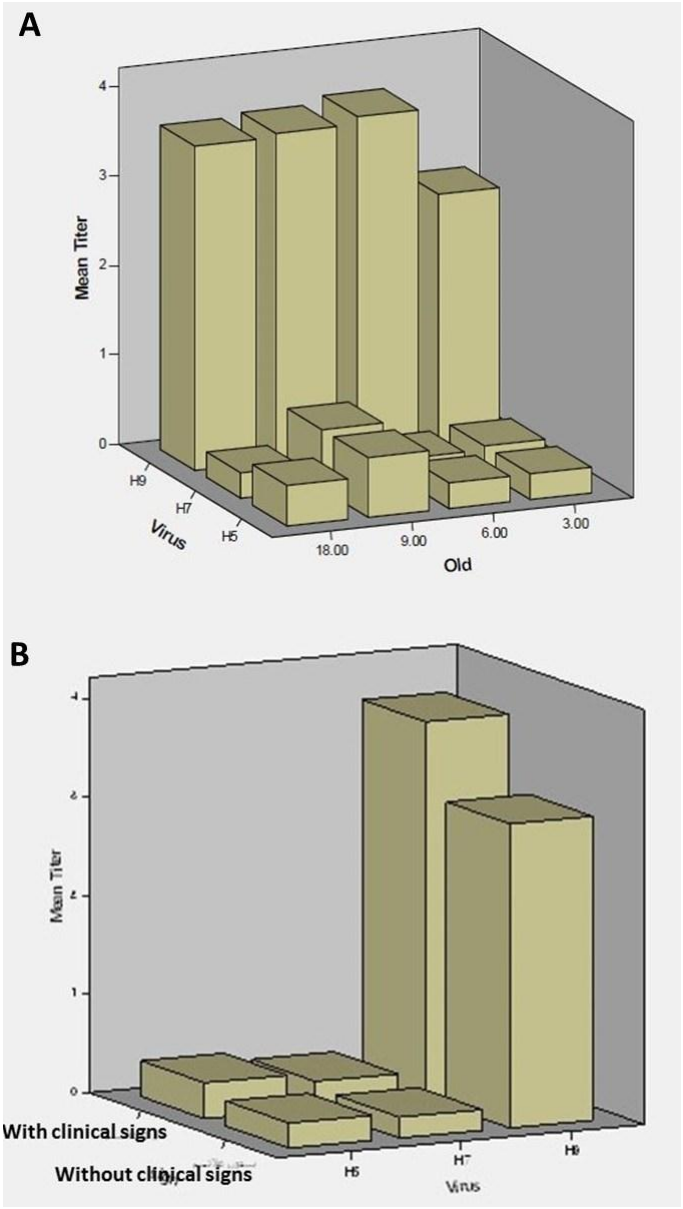

Fig. 1. The mean titers of influenza virus in the turkey flocks. There were the significant differences between various age groups (A) and flocks with and without clinical symptoms ($P < 0.05$).

Discussion

Several methods are used for serological identification. The most common method for identification of antibodies related to haemagglutinin is the HI test. The antibodies related to NP are similar in turkeys and pheasants, but they may be unrecognizable in infected ducks (Eggert and Swayne, 2010;

Brown and Stallknecht, 2006). In addition, like other species, ducks` antibodies may be stimulated, but HI tests are malfunction for their detection (Eggert and Swayne, 2010; Brown and Stallknecht, 2006). Since 1963 that the first report of the influenza virus isolation of turkeys was reported, the most turkey breeder countries had been involving the problems relevant to influenza and the different prevalence patterns has seen there (Swayne et al., 2013). In the US, in 1964, the prevalence of influenza in turkeys was reported from 19 different states. In most states, the prevalence of influenza was in a sporadic form, but in California and Minnesota have the most turkey breeding farms and located in the pathway of migration routes of waterfowls, influenza disease was seen more (Alexander, 2000a). Base on the present results, from 350 serum samples, the turkey serum samples were negative for infection with subtypes of H5 and H7, but the infection with the subtype of H9 was evident in some flocks. It should be mentioned that the titration 1 of H5 and H7 is probably due to the cross interaction that occurs between subtypes and the high level of H9 titration can influence this issue (Swayne et al., 2013), and this is compliance with the results of a previous study that showed the infections with the subtype of H9N2 could cause immune stimulation mediated by cellular and cross-interaction and cause protective effect against subtypes of HPAI like H5N1 that is fatal in normal condition (Seo and Webster, 2001). The average of H9 titration in flocks with and without influenza vaccines was 6 ± 0.25 and 3.45 ± 0.15, respectively that indicating the positive effect of vaccination against influenza in turkey flocks and this is compliance with the results of the previous data (Ellis, 2004).

Comparative evaluation between age and titration indicates that the increase in age can increase the influenza serum titration, and it seems that age is an important risk factor for influenza disease. To evaluate the effect of age on the serum titration, flocks were divided into 4 categories of 3, 6, 9, 18 month-old. The three month-old flocks` serum titration was the least and the serum titrations of 6,9,18 month-old categories were similar. This increase in serum titration may be due to an increase in the risk of turkey exposure to the virus. In a survey by Woo and Parks in Korea, age is considered as an important factor of involvement. Suppressing the immune system and increasing the risk of bird exposures by the increase in age are mentioned as the cause of this issue (Woo and Parks, 2008).

According to the results, the flocks` positive titrations had more clinical manifestation than the flocks` negative titrations, and this is compliance with the findings of Al-Natour

(Al-Natour, 2005). Regarding semi-quantitative data in the SRD, it is occasionally believed that the results of HI method represents the more sensitivity than SRD one. Therefore, we focused on the HI results for the mean titers.

Conclusion

It should be mentioned that the morbidity of the influenza infection depends on the virus strain, species and age of infected birds, immune system, and the presence of other simultaneous disease like colibacillosis and Newcastle disease. Collectively, it seems that an increase in age and vaccination program against influenza virus can increase serum titration of the turkey flocks.

Ethical approval

The experiment was approved by the Research Ethics Committee of Tabriz University of Medical Sciences and performed according to the Helsinki's humanity research declaration.

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Conflict of Interest Statement

There is no conflict of interest.

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