

Serological Survey of Avian Influenza Type a and Subtype a Antibodies in Chickens Sera in Sudan Using AGID and HAI Tests

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Abstract

The aim of this study is designed to determine the prevalence of AI virus type A and subtypes H9, H7 and H5 antibody in different localities in Sudan. During the period of 2003 to early 2006, a total of 1025 sera samples were randomly collected from young, growing and adult, apparently health, unvaccinated chicken flocks at Khartoum, Central, Northern, Eastern and Western States of Sudan. Using AGID test, AI type A antibody was detected in 692 (67.51%) of the tested samples. From type A positive sera, a total of 311 samples (36 sera from each state) were examined to subtypes H9, H7 and H5 by means of haemagglutination inhibition (HAI) test. AI antibodies to subtypes H9 and H7 were detected in 141(45.33%) and 11(3.54%) of tested serum samples respectively. All samples tested negative (0%) for type H5. The risk factors of location, Age groups and strains are statistically associated with seropositivity of Avian Influenza (P-value <0.05). In conclusion: The results obtained confirmed the presence of AI type A antibody and sub types H9 and H7 in Sudan.

Keywords

Avian Influenza, Sudan, Antibodies, AGID, HAI

Received: November 4, 2015 / Accepted: November 25, 2015 / Published online: January 5, 2016

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1. Introduction

Avian influenza (AI) is an infectious disease of birds caused by a strain of influenza A virus. The disease has economical and human health impacts due to its economic losses(decline in egg production , high mortality rate in birds which may reach (100%), decrease in carcass quality and loss of local and international trade and its zoonotic nature cause disease or death in human(Swayne and Halvorson, 2003). Three main tests are used to detect AI antibodies, the agar gel immuno diffusion (AGID) test, haemagglutination inhibition (HAI) test and enzyme linked immune sorbent assay (ELISA) Gough (2004). The AGID test is often used to screen sera for AI group specific antibodies directed against the viral matrix and nucleoprotein antigens which are shared by all influenza A viruses (Beard, 1970, Swayne *et al.*, 1998; Terregino and

Capua, 2009). The sera which were positive by the AGID test were examined by HAI test to specify the sub type's antibodies (Swayne et al, 1998; Swayne and Halvorson, 2003; Gough, 2004). In Sudan, Gafar Elamin and Kheir (1985) reported influenza ribonucleic protein antibody in animal sera from Eastern Sudan-Kassala using AGID test. AI type A antibody was detected in chickens sera in Khartoum State (Manal, 2000). During 2006, a devastating outbreak of AI occurred in AlGazeera and Khartoum States. The outbreak was caused by a highly pathogenic strain of AI virus that caused infection in domestic poultry with clear clinical signs and mortality rate reaching 100% in different farms (Wegdan and Khair, 2007)

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2. Material and Methods

2.1. Antigens

Reference AI antigens (H5, H7 and H9) haemagglutinin inactivated antigens (Veterinary Laboratory Agency, UK) and AI AGID antigen (Deventer, The Netherlands) supplied by (Detasi-company, Khartoum, Sudan) were used.

2.2. Antisera

Reference AI antisera to (H5, H7 and H9) haemagglutinin (Veterinary Laboratory Agency, UK) and AI AGID antisera (Deventer-The Netherlands) supplied by (Detasi-company, Khartoum, Sudan) were used.

2.3. Test Sera

A serological survey of AI type A antibody was done in chickens sera from different localities in Sudan from 2003 to early 2006. A total of 1025 serum samples were randomly collected from apparently healthy, different age groups of un-vaccinated chicken flocks at Khartoum State (Khartoum, Khartoum north and Omdurman cities), Central Sudan (Wadmadani), Eastern Sudan (Kassala and Gadarif) Western Sudan (Nyala) and Northern Sudan (Atbara). Birds were of different breed and all were raised in an open system of management. The collected sera were kept at -20°C until examined by AGID and HAI tests.

2.4. AGID and HAI Tests

The serum samples (n=1025) were screened for presence of AI antibody using AGID test. The test was done as described in OIE (2015), serum samples and positive serum control were tested with reference AI type A antigen. 311 samples were selected from positive sera to AI type A and were examined by HAI test using inactivated AI subtypes H9, H7 and H5 antigens. The test was done as described in OIE (2015). For HA tests reference antigens to subtypes H9, H7 and H5 were examined with 1% chicken erythrocytes. For HAI tests, 4HA units of each reference AI antigens (H9, H7 and H5) were used.

3. Results

The results of AGID test to AI type A antibody are shown in Table 1. From the 1025 chickens sera collected from different localities, AI type A antibody was detected in 692(67.51%).

The results of HAI to subtypes H9, H7 and H5 are shown in Table 2. A total of 311 positive sera to AI type A were tested for the presence of AI subtypes H9, H7 and H5 using HAI test. The results showed that 45.33% (141/311) of the tested sera were positive to subtype H9, 3.54% (11/310) to subtype H7 and none of the tested sera (311) were found positive to subtype H5. Statistically, the risk factors of location, Age groups and strains are statistically associated with seropositivity of Avian Influenza (P-value <0.05).

Table 1. The results of type A AI antibody in serum samples collected from different localities in Sudan as detected by AGID test.

Location	Age / weeks	Date of collection	Number of serum examined	Number of positive samples	Percentage (+ve)
Khartoum State					
Khartoum	5-70	2005, Feb.	128	123	96.09%
Khartoum North	5-70	2003-2005	263	192	73%
Omdurman	5-70	2006 Feb.	67	36	53.73%
Khartoum State	1-2	2006 Feb.	112	47	41.96%
Central Sudan					
Wadmadani	5-70	2005 Sep- Oct.	232	134	57.75%
Eastern Sudan					
Kassala	5-70	2003 Nov.	57	51	89.47%
Gadarif	5-70	2006 Feb.	35	30	85.71%
Western Sudan					
Nyala	5-70	2004 Oct.	93	43	46.23%
Northern Sudan					
Atbara	5-70	2006 Feb.	38	36	94.73%

Table 2. The results of HAI test for avian influenza antibodies to subtypes H9, H7 and H5.

Subtype	Number of serum examined	Number of positive serum	Percentage (+ve)
H9	311	141	45.33%
H7	311*	11	3.54%
H5	311	0	0%

*One sample was discarded

4. Discussion

Influenza type A viruses can infect people, birds, pigs, horses,

seals, whales, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes based on two proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA).

In the present study, a serological survey to AI type A and subtypes A (H9, H7 and H5) antibodies was done in chickens sera from different localities in Sudan using AGID and HAI tests. As stated in the results, 692(67.51%) of the samples were found positive to AI type A antibody by AGID test. Since these birds are not vaccinated against AI, the results indicated that, they were previously exposed to avian influenza viruses; and this is in agreement with Wegdan *et al.* (2007) who reported the presence of type A avian influenza viruses antibodies in serum samples when examined by ELISA test.

On the other hand, the results of HAI test confirmed the presence of AI antibodies to subtypes H9 and H7, but not for subtype H5. The AGID test is often used to detect antibodies to AI type A and the HAI test is the preferred assay to determine the HA subtypes (Swayne and Halvorson, 2003, Gough, 2004, OIE, 2015). The highest percentage level (45.33%) of HAI antibody detected is for H9 followed by (3.54%) for H7. This finding indicated that, AI virus subtype H9 is the most prevalent subtype circulating in poultry farms in the Sudan. this finding agreed with that of Capua and Alexander (2009) who mentioned that H9 infection have been reported in the Middle East, Asia, Europe, USA and Africa.

The presence of low percentages of H7 and absent of H5 antibodies in chickens sera may be due to low pathogenic AI which may be transmitted from migratory birds or small birds to poultry by infected feces; either through direct contact or indirectly through contamination of feed, water or in free-range area. Migratory birds usually spend about 3 months in Sudan (from late November up to February) in different locations on the Nile, along the Nile shores, pools and ponds. This finding is in consistent with that of Stegeman and Bouma (2004).

H5 and H7 subtypes of avian influenza A viruses can be further classified as either highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI). This distinction is made on the basis of genetic features of the virus. HPAI is usually associated with high mortality in poultry. It is not certain how the distinction between “low pathogenic” and “highly pathogenic” is related to the risk of disease in people. HPAI viruses can kill 90 to 100% of infected chickens, whereas LPAI viruses cause less severe or no illness if they infect chickens. Because LPAI viruses can evolve into HPAI viruses, outbreaks of H5 and H7 LPAI are closely monitored by animal health officials. The viruses of low pathogenic AI subtypes H5 and H7 may mutate into highly pathogenic AI strains (Swayne and Halvorson, 2003) (Senne *et al.*, 2006) and may cause pandemic influenza.

In conclusion, the present study showed widespread of AI type A antibody and subtype H9 antibody in different localities in Sudan. The presence of antibodies to subtypes H7 may be due to primary introductions of AI viruses from migratory birds or

small birds to commercial poultry flocks. H5 antibodies were not detected in the serum samples examined.

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