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Sero-epidemiological Survey of Anti-Avian Influenza Virus Antibodies in Five Avian Species in the Sudan

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ملخص البحث

أجريت هذة الدراسة لمعرفة وجود الأضداد لفيروس انفلونزا الطيور ضمت عدة طيور عقب ظهور الوباء في السودان عام 2006. جمعت 2862 عينة مصل دم من الدجاج, الاوز, البط, دجاج غينيا و الدجاج الحبشي من 12ولاية بشمال السودان وتم فحصها باختبارى المقايسة المناعية المرتبطه بالانزيم غير المباشره (iELISA) والتنافسيه (cELISA) واختبار تثبيط التلازن الدموى (HI). أوضحت النتائج وجود الأضداد للنوع ا(A) فى 17.70% من العينات،12.60% منها تحمل أضداد لـ (Subtype H5) تحت النوع. اما بقية العينات فقد كانت سالبه 82.30%. كما أوضحت النتائج عدم وجود اضداد للنوع (N1) في العينات عند اختبارها بواسطة المقايسة المناعيه التنافسية (cELISA) .

Summary

A Sero-epidemiological survey of anti-avian influenza (HPAI) virus antibodies was conducted in five avian species in 12 states of Northern Sudan following the highly pathogenic avian influenza (HPAI) outbreak that occurred in the year 2006. A total of 2862 blood sera were collected from the domestic fowl, geese, ducks, guinea fowls and turkeys and tested by the indirect ELISA (iELISA), competetive ELISA (cELISA) and the haemagglutination Inhibition (HI) tests. The prevalence rate of type A sero-type antibodies was 17.70%. Of these, 12.60% were positive for anti-H5 antibodies subtype viruses while 82.30% were negative for antibodies AI. No N1 antibodies were detected by cELISA.

Introduction

Avian influenza (AI) is caused by infection of the viruses of the family Orthomyxoviridae, genus influenza viruses type A (Cox *et al*,2000). Influenza A viruses are the only orthomyxoviruses known to infect birds. Many avian species have been shown to be susceptible to infection with type A viruses. Aquatic birds were considered the major reservoir of these viruses, but the overwhelming majority of viruses isolated from them are of low pathogenicity for chickens and turkeys. Influenza type A viruses are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens.

In the Sudan, an outbreak of a highly pathogenic avian influenza strain H5N1 occurred in 2006 (Najat *et al*, 2007).

An ELISA assay has been developed to detect anti-avian influenza virus antibodies while HI and NI tests are used to determine the HA subtype. (Swayne and Halvorson, 2003). This study was undertaken to determine the antibody to AI status in five avian species in the Sudan following the outbreak of the disease in 2006.

Materials and Methods

Study area:

Serum samples were collected from twelve States in the Northern Sudan (Khartoum, Gazira, The White Nile and Sennar States), Northern (The River Nile and Northern States), Eastern (Kassala, The Red Sea States) and The Blue Nile and Western States (North kordofan, South kordofan and South Darfur).

Serum collection:

A total of 2842 blood sera were collected, 2816 of sera were collected from the domestic fowl (*Gallus gallus domesticus*) and the remaining 26 from other avians including 17 ducks, 7 geese, one turkey and a guinea-fowl. The birds were adult, of different breeds, raised under open system farms and were not vaccinated against AI. The sera were transported on ice to the Central Veterinary Research Laboratories Centre in Khartoum and kept at -20°C until tested.

Serological Tests:

ELISA:

An indirect ELISA (iELISA) kit was used for the detection of anti-virus AI type A antibodies in chicken sera. It was performed as described by the manufacturers (Biocheck, Holland). The antibody titre in serum samples was calculated by reference to the positive control expressed as sample/ positive control (S/P) ratio. Samples with S/P values equal to or greater than 0.5 were considered positive. For the detection of anti-AI type A virus antibodies in ducks, geese, Turkeys and guinea fowls sera, cELISA (Anigen AIV) was used as described by the manufacturer (Anigen–Korea). Antibody titre in serum samples was calculated using the below formula percent inhibition (PI).

PI value = {1-(OD sample/mean OD control negative)} ×100. Where OD= optical density.

Samples showed PI values ≥50 % in duck and geese or ≥85% in turkeys were considered positive.

Specific detection of anti- N1 influenza subtype virus antibodies in positive sera was carried out using cELISA kits (ID.Vet, France) .The competition percentage was obtained by the following equation:

Competition % = Optical density (OD) of sample/ Optical density (OD) of negative control X100

Samples presenting competition % less than 60% were considered negative.

Heamagglutination inhibition test (HI):

Positive sera for anti-AI virus type A antibodies were further tested for the presence of H5 sub type specific antibodies by virus HI test using H5 inactivated antigen (OIE/FAO Laboratory for A1 and NDV).

Results

Anti-avian influenza virus-type A antibodies were detected in 500 out of 2816 (17.75%) domestic fowl sera. The prevalence rate ranged from 11.80 % to 66% (Table 1). The highest rate was recorded in The Blue Nile and South Darfur States while The Northern State showed the lowest prevalence rate. Sixty-three chicken sera out of 500 were found positive for anti-H5 sub type (12.6%), the highest rate (29.5%) was detected in El Gezira State followed by The White Nile state 25% and The River Nile 14.4%. However, the lowest (3%) was recorded in South Darfur State. The mean HI (log2) titre ranged from 1 to 5.3 (Table 2). Three out of 26 Sera of other birds were positive for anti virus type A antibodies (Table 3).The results of cELISA revealed that, none of the H5 positive sera had anti-N1 subtype antibodies (Table 2).

Table 1: Prevalence rate of anti-AI virus type A antibodies in the domestic fowl (*Gallus gallus domesticus*) in twelve states of Northern Sudan as measured by iELISA.

State	No. Sera collected	No. Positive	% Positive
Khartoum	1373.00	164.00	11.90
El Gezira	0365.00	078.00	21.40
The White Nile	0054.00	016.00	29.60
Sennar	0048.00	011.00	22.90
The River Nile	0664.00	090.00	13.60
The Northern	0039.00	010.00	25.60
Kassala	0051.00	026.00	50.90
The Red Sea	0042.00	012.00	28.50
The Blue Nile	0050.00	033.00	66.00
North Kordofan	0050.00	015.00	30.00
South Kordofan	0030.00	013.00	43.30
South Darfur	0050.00	033.00	66.00
Total	**2816**	**500**	**17.7**

Table 2: **Prevalence rate and titre of positive sera for H5 HI test ad prevalence rates for NI antibodies by cELISA.**

State	H5 Antibodies			N1 antibodies
	No. Positive/ Total	%	HI titre (log 2)	
Khartoum	15/164	09.10	4.70	0.00
El Gezira	23/78	29.50	4.70	0.00
The White Nile	4/16	25.00	5.30	0.00
Sennar	1/11	09.10	3.80	0.00
The River Nile	13/90	14.40	5.60	0.00
The Northern	1/10	10.00	2.00	0.00
Kassala	2/26	07.70	3.50	0.00
The Red sea	1/12	08.30	1.00	0v
The Blue Nile	0/33	00.00	0.00	0.00
North kordofan	2/15	11.80	3.00	0.00
South kordofan	0/12	00.00	0.00	0.00
South Darfur	1/33	03.00	2.80	0.00
Total	**63/500**	**12.60**	**-**	**0**.00

Table 3: Number of positive sera samples for Anti-avian influenza virus in some antibody avian species in twelve states of Northern Sudan.

State	Ducks Positive/TN	Geese Positive/TN	Turkeys Positive/TN	Guinea fowls Positive/TN
The White Nile	0/2	0/2	-	-
Sennar	1/4	0	-	-
South Kordofan	0/2	1/1	-	01
North Kordofan	1/5	0/1	0/1	-
The Red sea	0/4	0/3	-	-
Total	**2/17**	**1/7**	**0/1**	**0/1**

TN= Total Number.

Discussion

The current investigation was carried out in the year 2007 after the occurrence of the HPAI outbreak of 2006 in the Sudan. It provides information on anti-AI virus antibody status covering twelve states of Northern Sudan. States of Khartoum, Al Gezira and The River Nile which had experienced HPAI (H5N1) outbreaks showed lower rates of anti-AI type virus antibodies (Table 1).This might be due to the implementation of rigorous control measures after the outbreak in which all infected farms were depopulated, besides the sanitary measures that had been taken to eliminate the virus. Therefore, the new population of birds were free from anti-H5N1 antibodies as indicated by the low titres of antibodies detected during this study. A few numbers of birds were raised under the intensive system in other states, consequently small numbers of sera were collected and had higher rates of antibodies compared to large size chicken flock farms in Khartoum, El Gezira and The River Nile States in which large numbers of sera were tested. Other states may have also experienced continuous exposure to AI virus, suggesting circulation of the virus among birds in these states.

Previous records of high titre of anti-AI virus type A antibodies using iELISA have reported 86.4% before HPAI outbreak in 2006 (Wigdan *et al*, 2006) and 28.4% by cELISA in Khartoum State (El Amin, 2000) .

Infection of ducks as indicated by the presence of antibodies is of considerable importance due to their ecological role as natural reservoirs of influenza virus A (Alexander, 1982). Other avian species are included in this study and 3 out of 26 had anti-influenza virus-type A antibodies and none had H5 sub-type antibodies.

Although small numbers of birds were tested, and low antibody titres were obtained, the results of this study may indicate that, the virus circulation in the domestic fowl (*Gallus gallus domesticus*) and some wild avians.

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