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# Investigation on Influenza Virus A Infection in Different Animals in Sudan

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**ABSTRACT**

Influenza A virus infections were reported in poultry and equines in Sudan. To investigate the seroprevalence of this viral infection in poultry, equines, camels and other farm animals; sera were screened for antibodies to influenza A virus using ELISA. A total of 916 blood samples were randomly collected from cattle (n=184), camel (n=184), goat (n=92), sheep (n=92), poultry (n=92), donkey (n=78), and horses (n=194). Samples were collected from Khartoum, Gezira, River Nile, Butana, Kordofan and Darfur. Antibodies to influenza A virus were detected in 100% (92/92) of tested poultry sera 37.6 % of horses and 42.3 % of the donkey's sera. For equine sera, the overall prevalence rate of influenza A virus antibodies was 39% (106/272). The highest overall seroprevalence (73.2%) was observed in Atbara followed by Gazira (41.4%). None of screened cattle, sheep, goats and camel sera were found positive for influenza A virus antibodies. It was concluded that influenza A infection is existing in poultry, donkey and horses.



## INTRODUCTION

Influenza is an acute highly contagious infectious respiratory disease. Influenza viruses are RNA viruses that make up four of the six genera of the family *Orthomyxoviridae* which are Influenza A, B, C and D (Mariette, 2015). Influenza A virus genus has one species. The natural hosts for a large variety of influenza A are wild aquatic birds

(Klenk *et al*., 2008). Occasionally, viruses are transmitted to other species and may cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. The type A viruses are the most virulent pathogens among the three influenza types and cause the severest disease (Klenk *et al*., 2008). After the emerging of avian flu pandemic in 2006, reports describing the prevalence of the disease in poultry, as well as other domestic and wild birds have been published worldwide (Webster *et al.*, 2006, Owoade *et al*., 2008, Li *et al*., 2010, Couacy-Hymann *et al*., 2012,). Beside humans and poultry influenza is known to infect a variety of animals; however, cattle are not commonly recognized as natural hosts for influenza viruses. Some reports suggested sporadic transmission of influenza to ruminants (Norström *et al*., 1999). Horse is a natural host to influenza A virus. Equine H3N8 influenza viruses are circulating in horses' worldwide (Daly *et al*., 1996). Influenza infection is more common in equines worldwide (Yondon *et al*., 2013). Continuous outbreaks of the disease in horses and donkeys are reported annually in different countries, in Australia (Watson *et al*., 2011), India (Virmani *et al*., 2010), China (Wei *et al*., 2010), Mongolia (Motoshima *et al*., 2011) and Ireland (Gildea *et al*., 2013).

In Sudan, the occurrence of the disease was reported in chickens (Manal, 2000, Wegdan and Kheir, 2007a) and equines (Algezoli, and Kheir, 2014). Gafar Elamin and Kheir (1985) reported the detection of influenza ribonucleic protein antibody in sera of camel, goat, sheep and cattle at Kassala, Eastern Sudan using agar gel imuno diffusion (AGID) test. Anti-avian influenza virus A antibodies were detected in 17.8% (500/2816) of domestic fowl sera; the prevalence rate ranged from 11.80 % to 66%; the highest rate was recorded in Blue Nile and South Darfur States; while the lowest prevalence rate was seen in Northern State (Iman *et al*., 2009). Since 2007, no work was done to investigate the prevalence of influenza in different animals in Sudan. This work is intended to investigate the existence of influenza A virus infection in domestic animals in Sudan through the detection of antibodies to the virus.

## MATERIALS AND METHODS

**Sample collection*:*** A total of 916 blood serum samples were randomly collected from cattle (n=184), camel (n=184), goats (n=92), sheep (n=92), poultry (n=92), donkey (n=78) and horses (n=194); from different localities, Khartoum (223 samples), Wad medani (Gezira) (75 samples), Atbara (River Nile) (236 samples), Butana (46 samples), Obeid (Kordofan) (161 samples) and Nyala and Fashir (Darfur) (175 samples). Animals examined were apparently healthy, of both sexes and different ages. The clarified sera were collected, labelled and kept at -20°C till tested for influenza A virus antibodies using ELISA.

**Detection of antibodies against influenza A virus using ELISA*:*** The collected sera (916) were screened for influenza A virus antibodies using ELISA, kits for poultry sera (BioChek, London, UK) and for equine sera (ID-Vet, Montpellier, France). For other species reagents such as anti-camel conjugate for testing camel sera (BIO X Diagnostics, Jemelle, Belgium); and anti protein G conjugate for testing cattle, sheep and goat sera ( Invitrogen, Germany) were used. The test was

performed according to the manufacturer's instructions.

## RESULTS

**Influenza virus antibodies:** Influenza A virus antibodies were detected in all tested poultry sera (100%) collected from Khartoum and Atbara at River Nile State. All screened farm animals (sheep, goats and cattle) and camel sera tested negative for influenza A virus antibodies (Table 1). The overall seroprevalence of influenza A virus in 272 screened horses and donkeys was 39%. Positive results were seen in horses 37.6% (73/194) and 42.3% (33/78) of donkeys sera. The highest overall seroprevalence 73.2% was observed in Atbara followed by 41.4% in Gazira (Table 2).

**Table 1:** Detection of antibodies to influenza A virus in poultry and domestic animals sera using ELISA

| Location | Poultry | | Cattle | | Camel | | Sheep | | Goat | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Tested | +ve | No. Tested | +ve | No. Tested | +ve | No. Tested | +ve | No. Tested | +ve | No. Tested | +ve | %+ve |
| Khartoum | 46 | 46 | 46 | 0 | 46 | 0 | 3 | 0 | 9 | 0 | 150 | 46 | 30.7 |
| Atbara | 46 | 46 | 32 | 0 | 46 | 0 | 29 | 0 | 28 | 0 | 181 | 46 | 25.4 |
| Gazira | - | - | 46 | 0 | 46 | 0 | - | 0 | - | 0 | 92 | 0 | 0 |
| Kordofan | - | - | 30 | 0 | 46 | 0 | 29 | 0 | 27 | 0 | 132 | 0 | 0 |
| Darfur | - | - | 30 | 0 | - | 0 | 31 | 0 | 28 | 0 | 89 | 0 | 0 |
| Total | 92 | 92 | 184 | 0 | 184 | 0 | 92 | 0 | 92 | 0 | 644 | 92 | 14.3 |

**Table 2:** Determination of influenza A seroprevalence in equines using ELISA

| Location | Horse | | | Donkey | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tested sera | +ve | %+ve | Tested sera | +ve | %+ve | Tested sera | +ve | %+ve |
| Khartoum | 72 | 32 | 40 | - | - | - | 72 | 32 | 40 |
| Atbara | 25 | 20 | 80 | 31 | 21 | 67.7 | 56 | 41 | 73.2 |
| Gazira | 11 | 5 | 45.5 | 18 | 7 | 38.9 | 29 | 12 | 41.4 |
| Kordofan | - | - | - | 29 | 5 | 17.2 | 29 | 5 | 17.2 |
| Darfur | 86 | 16 | 18.6 | - | - | - | 86 | 16 | 18.6 |
| Total | 194 | 73 | 37.6 | 78 | 33 | 42.3 | 272 | 106 | 39 |

## DISCUSSION

In this work, antibodies to influenza A were detected in all tested poultry's sera (100%) indicating the wide spread of the infection especially in Khartoum and Atbara. This result is similar to that reported during the emerging avian influenza outbreak in Sudan (91.9%); where the highest prevalence was also noticed in Khartoum (Wegdan *et al*., 2007b). Other work reported lower overall prevalence (8.3%) of influenza in poultry in 14 different States in Sudan (Egbal *et al*., 2013) and other countries, 0.4% in Uganda (Kirunda *et al*., 2014), 2.9% (Gugong *et al*., 2012) 4.4% (Assam *et al*., 2014) and 10.4% (Aiki-Raji *et al*., 2015) in Nigeria. However, some higher prevalence of the disease (34.2%) in Sudan was reported at Sinar (Selma and Ballal, 2013), with comparable results in other countries, 18.9% in Grenada (Sabarinath *et al*., 2011) and 63% in Iraq (Abdul-Sada, 2015). The seroprevalence of avian influenza in Sudan during the last decade showed a very high level, then low level and again increasing level. This variable pattern of infection is most probably due to the difference in the time of sampling

as it is known that the infection increases during cold weather (Lowen *et al*., 2007).

In the present study, antibodies to influenza A virus were detected in 39% of tested equine sera. Detected seroprevalence in horses and donkeys were 37.6% and 42.3%, respectively. These results are far higher than that recently reported (2.3%) in horses and (1.3%) in donkeys in South Darfur, Sudan (Algezoli, and Kheir, 2014). The highest prevalence in this study (73.2%) was observed in samples collected from Atbara then Wad Medani at Gazira (41.4%). This could be attributed to the relatively small size of these cities where equines are used for transport and getting in contact most of time leading to the spread of the infection. However, the detection of antibodies to influenza A virus in all tested areas reflects the wide spread of the infection in Sudan. Detailed work to investigate the prevalence of this infection in other areas of Sudan as well as the determination of the circulating subtypes is highly recommended. The detected influenza A virus seroprevalence in equines in this study is comparable to that reported worldwide; 65.6% of tested donkeys sera in Bulgaria (Chenchev *et al*., 2011), 13 out of 14 horses with respiratory signs in Egypt (Maha *et al*., 2014). 98.6% of 72 horses in Brazil (Mancini *et al*., 2014), 25% of tested horses in Mexico (Loroño-Pino *et al*., 2010). In Turkey, 31.1% of the 623 tested sera were positive; the seropositivity was 41.8%, 12.8% and 9.4% in horses, mules, and donkeys, respectively (Ataseven and Daly, 2007). In the present work, none of cattle, sheep, goats or camels sera tested positive for influenza A antibodies. This result is not expected as many reports describing the existence of antibodies to influenza A virus antibodies in these species. Gaffar Elamin and Kheir (1985) detected antibodies to influenza A virus in camel, goat, sheep and cattle in eastern region of Sudan. In USA bovine sera tested by hemagglutination-inhibition assay using chicken erythrocytes showed that, 51% of sera had detectable antibodies to influenza A virus (Lin *et al*.,2010). Seroprevalence of influenza A virus was detected in cattle sera in Norway and Ireland (Norström *et al*., 2000, Graham *et al*., 2002) and this has been associated with decreased milk production and sometimes respiratory diseases (Crawshaw *et al*., 2008). Detailed study for exploring the seroprevalence of influenza A virus infection in domestic ruminants in Sudan and the characterization of the virus is to be done.

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