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Seroprevalence of equine influenza in three southwestern states of Nigeria

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Abstract

Equine influenza (EI) virus is one of the most economically significant pathogens causing respiratory disorders in equids. The recent outbreaks of EI in equids in North Western Nigeria showed EI virus is circulating in the Northern part of the country. This study determined the seroprevalence of EI infection in horses from the Southwestern states of Nigeria. One hundred sera samples from horses kept in three South Western states: Lagos, Ogun, and Oyo were screened for antibodies against influenza A nucleoprotein antigen using the direct ELISA method. Positive sera samples were thereafter examined thereafter further examined for haemagglutination inhibition (HI) antibodies to the H3 subtype of the influenza A virus. Antibodies were detected in 60 of the horses (22 Lagos, 8 Ogun and 30 Oyo). Although significantly higher seropositivity was observed in the male horses, seroprevalence of EI was also higher among 11- 15 years, exotic breeds and patrol horses. High seropositivity of horses in the South Western states of Nigeria to EIV infection is an indication that adequate monitoring of horse movement and continuous surveillance of the virus and vaccination should be strongly recommended.

Keywords: ELISA, Influenza A, Horses, Haemagglutination inhibition, Nigeria

Introduction

Equine influenza (EI) is an infectious disease of the upper respiratory tract of horses caused by the equine influenza virus (EIV) and characterized by a sudden onset of fever, depression, poor performance, harsh dry cough, and nasal discharge, pneumonia and death harsh dry cough, nasal discharge, pneumonia and death (Blitvich *et al.,* 2010). The causative virus belongs to the family Orthomyxoviridae and it is an Influenza A virus (IAV) (Palese & Shaw, 2007). The disease is associated with two subtypes H3N8 and H7N7 (Blitvich *et al.,* 2010). It has been reported that both viruses produce comparable symptoms in horses but the infection caused by H3N8 is usually more severe due to its proclivity for invading the lower respiratory tract and secondary bacterial infection (Guo *et al.,* 1995). Of the two subtypes, a report of disease associated with H7N7 was last seen in 1979. However, in the past

three decades H3N8 has been responsible for most outbreaks worldwide (Adeyefa & McCauley, 1994; Diallo *et al.*, 2021).

EI is an endemic disease in Africa and its outbreak has been reported in some African countries like South Africa (Guthrie *et al.,* 1999), Algeria (Laabassi *et al.,* 2015), Nigeria (Shittu *et al.,* 2020), Niger, Mali, Senegal and Sudan (Diallo *et al*., 2021). In Nigeria, outbreak of EIV was first reported in polo horses in Ibadan, South West Nigeria (Adeyefa & McCauley, 1994). Thereafter, serological evidences of both subtypes of EIV in horses have been reported in different parts of the country (Olusa & Adeyefa, 2009; Meseko *et al.,* 2016). The most recent outbreak reported involving majorly donkeys in some North-Western states in 2018 and 2019 (Shittu *et al.,* 2020) and the subsequent high seroprevalence of EIV infection observed in horses and donkey confirmed that the disease is still circulating in the northern part of the country (Olufemi *et al.,* 2022). It is therefore imperative to investigate the current status of EI in the Southern part of Nigeria. Hence, the aim of this study was to investigate the seroprevalence of equine influenza in horses from three states of the Southwestern, Nigeria.

Materials and Methods

Study area

Three out of the six states of the southwestern zone of Nigeria were selected for the study namely; Lagos (6.5˚N, 3.6˚E), Ogun (6.9˚N, 3.6˚E) and Oyo (8.16˚N, 3.16˚E) (Figure 1). The state's selection was based on the availability of horses and owners' consent to partake in the study. The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/033-0423/18).

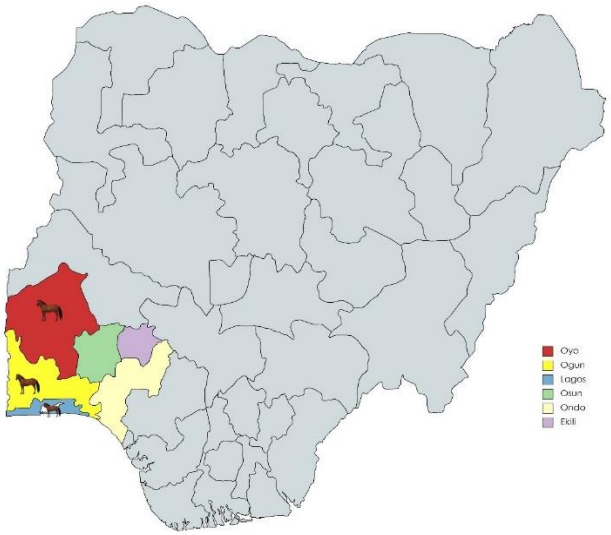

Figure 1: Nigeria map showing the three states of the six Southwestern states sampled. Created using https://mapchart.net//africa-detailed.html

Study animals and sample collection

A total of 100 horses (Lagos n=25, Oyo n=60 and Ogun n=15) were sampled across the three states using convenience sampling method. The age was determined based on either stable records or dentition as earlier described (Wayne & Melvin, 2000). None of the horses had history of vaccination against equine influenza. About 5mL of blood was aseptically collected via jugular venipuncture, and sera were harvested and stored at –20˚C until analyzed.

Enzyme-linked immunosorbent assay (ELISA)

The detection of antibodies to the influenza A virus in the horse serum samples was determined using direct ELISA (Influenza A Ab Test kit, IDEXX Laboratories, USA). The assay was done in duplicates and the procedure was performed in accordance with the manufacturer's instructions. Results were evaluated based on the ratio of mean of sample (cutoff for positive samples was S/N < 0.60; negative samples S/N > 0.50) to mean of negative control ((S/N). Samples with S/N values < 0.60 were considered positive for antibodies to influenza A virus.

Haemagglutination inhibition (HI) test

Positive serum samples for influenza A virus antibodies were subjected to the HI assay in order to determine the influenza A virus subtype. Hemagglutination (HA) test to appropriately determine 4HA units of the H3N8 antigens used (OIE/FAO reference laboratory, Instituto

Zooprofilattico Sperimentale delle Venezie, Padova, Italy). The HI assay was conducted as described in the OIE Terrestrial Manual of the World Organisation for Animal Health (WOAH) (OIE, 2019). The HI titre is the highest dilution of antiserum causing complete inhibition of 4 units of virus hemagglutination. The validity of the result was dependent on a titre of less than 2^3 for 4 HA unit with a negative control serum and a titre of within one dilution of the known titre of the positive control serum.

Statistical analysis

The seroprevalence was expressed in percentage. The association between horse factors (sex, age, breed, purpose) and seropositive for EI was determined by chi-square or Fisher exact test and $p < 0.05$ was considered significant. The statistical analyses were done using GraphPad Prism version 8.0.2.

Results and Discussion

Of the 100 horses screened from the three states of the southwestern zone of Nigeria, the state-based seroprevalence ranged from 50.0% to 88.0%. Overall, 60% (95% CI = 49.7 – 69.7) of the horses were seropositive for EIV in the southwestern zone of Nigeria (Table 1). The ELISA-positive sera samples had antibody titers ranging from $\log_{10} 2^6 – \log_{10} 2^9$ with a mean titre of $\log_{10} 2^{6.12}$ and a geometric mean titer of 686 on HI test for H3 subtype. The seroprevalence of EI reported in the country previously ranged from 9.1 to 60.9% (Adeyefa *et al*., 1994; Olusa *et al*., 2010; Meseko *et al.,* 2016). The present high (60.0%) seropositive of horses to EIV in the southwest is an affirmation that EIV is also circulating in the southwestern region of Nigeria. Thus, this report is very timely in view of the recent outbreak of EI reported in the North-western zone of Nigeria (Shittu *et al*., 2020; Olufemi *et al.,* 2022). The high seroprevalence observed in South West could also be influenced by the current trend of the EI in the Northwest zone since horses particularly those involved in sport activities like polo, racing and durbar procession are commonly transported across different parts of the country to participate in equestrian activities (Olusa *et al*., 2010; Meseko *et al*., 2016).

The seroprevalence of equine influenza virus in relation to horse factors like age, sex, breed and uses is shown in Table 2. Although, there is no certainty on the degree of influence these factors (age, sex, breeds and uses) have on the seropositivity of equids to EI. Nevertheless, we observed significantly higher seroprevalence in the male horses (82.6%) than the female horses (40.7%). No significant differences in the seroprevalence of EI were observed between the other factors like age, breed and uses. However, seropositive to EIV was higher among the older horses (70.0%), exotic breeds (Argentine 90.9%, South African 100% and Sudanese 59.6%) and patrol (66.7%) and polo (65.7%) horses. These findings were similar to the risk factors reported to be associated with high seroprevalence of

EI in the Northwest Nigeria (Olufemi *et al.,* 2022) as well as other previous studies (Jurado-Tarifa *et al*., 2018; Daly *et al*., 2021).

In conclusion, there is a high seroprevalence of EI in horses from South Western States of Nigeria. Thus, viral isolation and characterization and tracing of the origin of the EIV in the South West as well as

Table 1: Seroprevalence of equine influenza virus among horses from three South-western, Nigeria

State	No. of sample	No. positive sample	% seropositive	95%CI	P value
Lagos	25	22	88.00	68.8 – 97.5	
Ogun	15	8	53.33	26.6 – 78.7	
Oyo	60	30	50.00	36.8 – 63.2	0.0042
Total	100	60	60.00	49.7 – 69.7	

Table 2. Seroprevanlence of equine influenza virus in relation to horse age, sex, breed and uses in the South-western, Nigeria

Horse factors		No. of sample	No. positive sample	% seropositive	95%CI	P value
Sex	Male	46	38	82.61	68.6 – 92.2	
	Female	54	22	40.74	27.6 - 55	<0.0001
Age (years)	< 5	15	6	40.00	16.3 – 67.7	
	6 – 10	57	35	61.40	47.6 – 74.0	0.3338
	11 – 15	20	14	70.00	45.7 – 88.1	
	> 16	8	5	62.50	24.5 – 91.5	
Breed	Argentine	11	10	90.91	58.7 – 99.8	
	Sudanese	47	28	59.57	44.3 – 73.6	
	Local	33	18	54.55	36.4 – 71.9	0.0633
	S/African	4	4	100.00	40.0 – 100.0	
	Cross	5	0	0	0 – 52.2	
Uses	Polo	67	44	65.67	53.1 – 76.9	
	Patrol	12	8	66.67	34.9 – 90.1	0.0743
	Recreation	15	7	46.67	21.3- 73.4	
	Teaching/ Research	6	1	16.67	0 – 64.1	

Note: Male (Stallion/colt) and Female (Mare/filly)

monitoring of horse movement and continuous surveillance of the virus and vaccination is strongly recommended.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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