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ORIGINAL ARTICLE

Serological Investigation of Equine Influenza Virus in Polo Horses at the 2021 Jos Polo Tournament, Plateau State

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SUMMARY

Equine influenza (EI) is an important respiratory disease of equidae caused by the Equine influenza virus (EIV). The H3N8 and the H7N7 strains of the EIV have been reported in outbreaks of EI. Clinical signs exhibited by infected horses include but are not limited to pyrexia, dry, harsh cough, clear nasal discharge which may turn purulent a few days later, anorexia and lethargy. In this study, 115 polo horses from Kaduna, Nassarawa, Niger, Abuja FCT, Bauchi and Adamawa that participated in the 2021 Jos Polo Tournament were screened for EIV to ascertain the seroprevalence of EIV in these group of horses. Theses sera samples were tested using a specific commercially available indirect Influenza A Virus Antibody ELISA Test Kit (IDEXX Influenza A Test Kit). An overall prevalence of 51.3% was obtained. Subset of ELISA reactive sera were analysed by Haemagglutination Inhibition (HI) for subtype H3 and 6 out of 20 (30%) had up to 3log2 HI titre . In this study, the prevalence of influenza A was highest in female and Argentine horses. The effect of sex breed and location was statistically not significant. Additionally, this study showed that seropositive horses were present in each of the states represented, implying that EIV is still circulating in Nigeria. This study therefore highlights the need for regular monitoring and surveillance of equidae population in Nigeria for EIV to improve our understanding of EI and help in the formulation of national control strategies for EI.

Key words: Equine, Influenza, Seroprevalence, Nigeria, Polo.

INTRODUCTION

Equine influenza (EIV) is amongst the important viruses causing respiratory diseases in horses globally. It is readily transmitted and highly contagious in susceptible hosts (Daly *et al.,* 2020). EIV causes Equine influenza in equidae with the clinical signs exhibited being; fever, lethargy, anorexia, nasal discharge, and a nonproductive dry cough

(Perglione et al., 2016). Mortality rates are generally low during EIV outbreaks; death is most common among foals or equids with poor health or other pre existing conditions. Horses usually recover in 2 weeks with rest, but clinical signs, especially cough, can persist. EIV can result in a secondary bacterial bronchopneumonia, which can be fatal, particularly in young horses.

EIV is classified under the family orthomyxoviridae and belongs to type A influenza viruses with two known subtypes: H3N8 and H7N7, both of which are thought to have originated from avian influenza virus (Chambers, 2020). Influenza A virus has eight single-segment negative sense RNA strands and is sub-typed based on the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which account for 45% of the virus' mass (Lewis et al., 2014). The EIV H3N8 strain, which was first discovered in the early 1960s, continues to pose a serious health and economic threat to the majority of the world's horse populations even though there is now no evidence that the original H7N7 strain of EIV is still in circulation (Oladunni et al., 2021). Transmission of EIV can be direct or indirect through fomites and aerosols. Particularly in naive or uninfected horses, EIV infection is a normally self-limiting respiratory infection marked by fever, lethargy, coughing, dyspnea, and nasal discharge (Firestone et al., 2011).

Despite the low mortality rate associated with EIV infection in horses, affected animals are more susceptible to subsequent bacterial infections, which can cause pneumonia and even death (Back et al., 2016; Jurado-Tarifa et al., 2018). In some horse populations, vaccines are available and frequently used, but due to antigenic drift and other variables, their efficacy is limited. Additionally, the spread of EIV in some susceptible populations has been attributed to vaccinated animals with subclinical infections (Chambers, 2020).

Major outbreaks of EI have been reported in many parts of the world like Asia, Europe, Africa, Australia, South America and North America (Boukharta *et al*., 2015; Alvez Beuttemmüller et al., 2016; Meseko et al., 2016). The first reported major EIV outbreak in Nigeria occurred in 1991 when horses from various regions of the country gathered at the Ibadan polo tournament in the south western region (Adeyefa and McCauley, 1994). Recently, the second major epizootic of EI in Nigeria and the sub-region of West Africa caused for the first time by Florida clade-1(Fc-1) virus was reported by Shittu et al., (2020). Several serological studies of EIV in Nigerian horses showing the presence of antibodies to subtypes H3 and H7 have previously been documented (Adeyefa et al., 1996; Olusa and Adeyefa 2009; Meseko et al., 2016; Olufemi et al., 2022). The re-emerging epizootics of EIV in Nigeria without a standardised and sustainable vaccination plan calls for continuous virological surveillance and serological monitoring of horses and other susceptible domestic animals in Nigeria so as to prevent future outbreaks (Olufemi et al., 2022; Omoniwa et al., 2022). In view of this, the present study investigated the seroprevalence of equine influenza in polo horses that participated in 2021 Jos Polo Tournament, Plateau State, Nigeria.

MATERIALS AND METHODS

A total of 115 apparently healthy polo horses (27 males and 88 females) from different locations (Kaduna, Keffi, Zaria, Minna, Abuja, Bauchi and Yola) in Nigeria that participated at the 2021 Jos Polo Tournament, Plateau State (9.929357°N; 8.875879°E) were used for this study after consent was duly obtained from the caretakers. In this

cross-sectional study, blood samples were obtained aseptically via jugular venipuncture on a single occasion into serum gel clot activator tubes which were allowed to clot before serum was harvested into cryovials. These samples were appropriately labeled and thereafter transported to the Influenza Laboratory of the National Veterinary Research Institute, Vom, Plateau State, Nigeria, in an ice-packed box and stored at -20°C until analyzed. Demograhic variables (age, breed, location, and sex) was recorded for each animal at the time of sampling.

The sera samples were tested using ~~a specific~~ commercially ~~available~~ indirect Influenza A Virus Antibody ELISA Test Kit (IDEXX Influenza A Test Kit) following the manufacturer's instructions. This kit is an enzyme immunoassay capable of detecting IgG antibodies to all influenza A viruses in animal serum. Each sample was diluted tenfold (1/10) with the dilution buffer and mixed thoroughly using plain microplates before being assayed. The samples were tested individually in ELISA plates that were pre-coated with influenza A antigens. The absorbance of the samples and the controls was measured and recorded at 650 nm. The validity criteria of the test were determined as the mean value of the negative control ($\bar{NC}$) ≥ 0.600 and the mean value of the positive control ($\bar{PC}$) ≤ 0.50. The presence or absence of antibodies to Influenza A in the samples was determined by determining the ratio of the sample OD to the mean OD value of the negative control (S/N). The cutoff for negative samples was determined as $S/N \geq 0.50$ while the cutoff for the positive samples was determined as $S/N < 0.60$.

Treatment of test sera to remove nonspecific agglutinins and nonspecific inhibitors was carried out according to WHO protocol (WHO, 2002) before analyses of twenty subset of ELISA positive samples using the HI protocol previously described by Meseko et al., (2016) to test against the HA antigen of subtype H3.

Figure I: Map of Nigeria showing the states of origin of horses sampled during the Jos polo tournament

Results were presented in tables, Percentage (%), the prevalence was calculated as number of positive/total number of samples X 100, also Chi square was used to test for degree of association by sex, breed and location, value of $p \leq 0.05$ was considered significant using GraphPad Prism® 4.0.

RESULTS

A total of hundred and fifteen (115) samples were analyzed composed of 27 males and 88 females. The result showed that there were more seropositive horses than seronegative ones with an overall 51.3% seropositivity (Figure II and Tables I, II and III). Six out of 20 (30%) sera had up to 3log2 HI titre for subtype H3. The influenza A seropositive percentage by sex, breed, and location from where the horses were brought to Jos was determined as shown in Tables I, II, and III.

*Not Significant (>0.05)

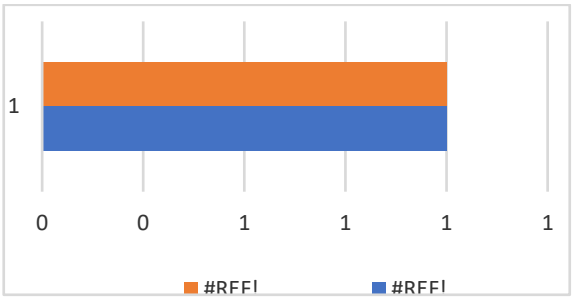

Figure II: Chart showing number of animals positive and negative to EIV

TABLE I: Equine influenza virus (EIV) seropositivity by sex

Sex	Number Sampled	Number Positive	% Positive	P Value	95% CI	Odds Rati
Male	27	12	44.4	0.4149*	0.2932-1.6610	0.6979
Female	88	47	53.4			
Total	**115**	**59**	**51.3**			

*Not Significant (>0.05)

TABLE II: Equine influenza virus (EIV) seropositivity by location

Location	Number Sampled	Number Positive	% Positive	P value
Jos	17	8	47	0.2694*
Kaduna 1	13	9	69	
Kaduna 2	11	7	63.9	
Nasarawa	14	6	42.9	
Abuja	19	13	68.4	
Niger	3	1	33.3	
Bauchi	9	5	55.6	
Yola	23	8	34.7	
Total	**115**	**59**	**51.3**	

(Adamawa and Niger) and were transported to the tournament, this calls to contemplation the rising worries regarding the transboundary introduction

TABLE III: Equine influenza virus (EIV) seropositivity by breed

Breed	Number Sampled	Number Positive	% Positive	P value
Argentine	25	14	56	0.3798*
Arewa	10	4	40	
Sudanese	71	38	53.5	
South African	1	1	100	
Tallon	8	2	25	
Total	**115**	**59**	**51.3**	

*Not Significant (>0.05)

DISCUSSION

We investigated the seropositivity of one hundred and fifteen (115) horses that were brought from different locations across Nigeria for a polo tournament at Jos City, Plateau State. We detected EI antibody in 51.3% of the horses implying that the higher proportion of our study subjects showed serological evidence of equine influenza virus. This study include details of the field status of EI in a number of states in northern Nigeria. Virological and serological evidence of EI has been documented in Nigeria in previous investigations (Olusa et al., 2010; Meseko et al., 2016; Shittu et al., 2020; Olufemi et al., 2022). The geographical locations included in this study contain a significant population of horses. As some of the samples utilised in this investigation were taken from horses that reside in border states and reintroduction of EI to Nigerian horses (Shittu et al., 2020; Diallo et al., 2021) remain relevant. The seroprevalence of 51.3% in this research supports a previous discovery by Adeyefa et al. (1996), which proved that equine influenza existed among equids in all areas of Nigeria. Though, the prevalence observed in this study is less than previous reports from other parts of northern Nigeria (Meseko et al., 2016; Olufemi et al., 2022), a recent broad exposure to the equine influenza virus may be attributable for the predominance seen. More female horses showed higher seropositivity than male horses which is consistent with previous reports by Nyaga et al., (1980). The incidence for polo horses found in this study supports earlier results by Olufemi et al. (2022), who hypothesized a high prevalence due to the movement frequently associated with

equestrian activity. Given that Argentina is a regular source of equine importation into Nigeria (Ministerio De Agricultura, Exportaciones Agentinas de Équidos. 2019), along with other sources like Europe and South Africa, due to the growing interest in equestrian activities in Nigeria, the high prevalence of EI observed in the Argentine breed in this study may potentially play a role in the widespread exposure to equine influenza virus. There was no statistically significant correlation between location and prevalences across states. The findings further support the idea that EIV is widespread because positive animals were found in states like Niger that had not previously been examined. This research suggests that EI is widespread throughout Nigeria's various regions. The study limitation includes not being able to subtype all the influenza A positive sera but a limited number screened suggest circulation of H3 serotype of EI in the stables. While there are no evidence of wide scale vaccination programme for EI in Nigeria even after the 2018-2019 outbreak that was caused by subtype H3N8, this study could not differentiate antibody due to vaccination or infection.

CONCLUSION

This study has demonstrated that even though there have been reports of EI infections in horses in Nigeria, exposure to the virus has been proven by serology, as revealed by the seroprevalence rates attained from all of the stables These results imply that EIV is still circulating in Nigeria and more research is still required in the study of the epidemiology, isolation and characterization of the EIV as well as the study of influenza viruses in other domesticated animal species to inform policy on control measures including vaccination strategy.

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