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SEROLOGIC EVIDENCE OF EQUINE H7 INFLUENZA VIRUS IN POLO HORSES IN NIGERIA

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INTRODUCTION

Influenza is an acute epizootic viral respiratory disease that affects a wide variety of host species including humans, horses, pigs, sea mammals and birds. In horses, influenza virus is an important cause of infectious upper respiratory tract disease (IURTD) with serious economic losses particularly in the racing industry. The disease is characterized clinically by a frequent dry harsh cough, fever and serous nasal discharge, which later turn mucopurulent. The mode of spread is by aerosol with high morbidity and low to moderate mortality that could sometimes be very high in naïve equine populations. It is also associated with the destruction of epithelial cells lining the upper respiratory tracts, trachea and bronchi (Dulbecco & Gunsbers, 1988).

The influenza viruses belong to the family Orthomyxoviridae of which there are five genera: influenza A, B and C, Thogovo and Isavirus (Palase & Shaw, 2007). They are segmented, negative sense, single stranded RNA viruses classified in mammals and birds as serotypes A, B and C based on antigenic differences in their nucleoprotein (NP) and matrix (M1) protein (Akkina *et al.,* 1991; Murphy & Webster, 1996). Although influenza C viruses have been isolated from pigs in China, serotypes B and C usually infect humans only while influenza A subtype viruses infect a wide variety of host species including man, horses, pigs, sea mammals and birds (Easterday, 1975; Guo *et al.,* 1983). Two different subtypes of influenza A virus, equine–1 prototype [(A/equine/Praque/1/56 (H7N7)] and equine–2 prototype [(A/equine/Miami/1/ 63 (H3N8)] have been isolated from horses (Guo *et al.,* 1992; 1995). Although both viruses produce similar disease signs in horses, the infection produced by equine-2 viruses is typically more severe because of the propensity of these viruses to invade the lower respiratory tract with the attendant secondary bacterial infection (Guo *et al.,* 1995).

Transmission of influenza A viruses in mammals is by aerosols generated during coughing and sneezing but spread by direct contact is also possible (Knight, 1973; Webster *et al.,* 1992; Wright *et al.,* 2007). Immunity is subtype specific and depending on the extent of antigenic drift, immunity to natural infection has been estimated to last between 1 and 5 years while vaccination with current vaccines provides limited protection (Wood & Mumford, 1992; Chamber, 1992; Adeyefa & McCauley, 1994; Adeyefa & McCauley, 1997a; Adeyefa *et al.,*; 1997b; Bayly, 1998). Diagnosis of equine influenza is based on traditional techniques such as virus isolation in embryonated eggs or tissue culture and or the detection of significant titre in paired serum samples (Anested & Maagard, 1990), although molecular techniques are also currently used (Adeyefa *et al.,* 1994; 1996b; 1997b; 2000). Enzyme Linked Immunosorbent Assay (ELISA) may also detect viral antigen in respiratory secretions (Anested & Maagard, 1990; Adeyefa & McCauley, 1997a; Adeyefa *et al.,* 1997b).

Nigeria has an estimated horse population of 200, 000 (Bourn, 1992; FAOSTAT, 2008) and majority are use for playing the game of polo. Polo is a very organized sport in Nigeria and the Nigerian Polo Federation (NPF) governs its activities. In mid January 1991, an influenza outbreak caused by H3N8 subtype viruses was reported in Ibadan Polo Club stables during the annual polo tournament among horses from various polo clubs across the country (Adeyefa & McCauley, 1994). Although equine-1 (H7N7) subtype has never been isolated in Nigeria nor anywhere in the world since 1979 (Webster, 1993) and influenza outbreak due to H3N8 subtype viruses has not been reported in the country since 1991, there have however been some reports of antibodies against H7 viruses in human, pig, avian and horses' sera in the country (Olaleye *et al.,* 1990; Adeniji *et al.,* 1993; Adeyefa, 1995). Moreover, importation of horses into Nigeria by the various polo clubs from all parts of the world especially Europe and South America has continued and transmission and spread of disease could be enhanced by congregation of horses during the annual polo tournaments and sales (Adeyefa & McCauley, 1994; Olusa & Adeyefa, 2009). The degree of antigenic variation within equine influenza viruses is of considerable interest (Adeyefa *et al.,* 1996b, 2000). Since the current commercial vaccines which although trivalent have been reported to produce very short lived immunity in vaccinated horses (Chamber, 1992; Wood & Mumford, 1992; Adeyefa & McCauley, 1994; Adeyefa *et al.,* 1997a) and several outbreak of diseases have occurred despite recent vaccination and strict quarantine measures (Lai *et al.,* 1994). There is therefore the need to assess the current circulating status of H7N7 subtype equine influenza viruses in Nigeria, where a combination of absence routine vaccination, poor vaccine storage and lack of quarantine protocols could pose a serious problem of rapid antigenic drift of the viruses. This study was therefore designed to investigate if H7N7 subtype influenza virus is still circulating among polo horses in the country.

Eighty horses were randomly selected from across the country during the 2004 annual polo tournament in Lagos, southwestern Nigeria. The horses were distributed as follows; from the North: Kano 14; Kaduna 15 and Katsina 11 while from the South: Ibadan 12; Lagos 18 and Port-Harcourt 10. About 10 ml of whole blood were collected via jugular venipuncture from each animal into plain universal bottle. Collected samples were mixed up and transported to the laboratory on ice pack and allowed to clot overnight. Sera were harvested and clarified by centrifugation in a bench top Denley centrifuge and stored in 2ml aliquots at -20^{0}C until analyzed. Nasopharyngeal swabs were also obtained from forty horses and placed in Hanks balanced salt solution (HBSS) medium and stored at -4^{0}C until analyzed.

Influenza A viral antigens (A/eq/Sao Paulo/76 [H7N7] and A/eq/Connought Detroit/64 [H7N7]) and control antiserum were obtained from Professor Adeyefa's repository, in the Department of Veterinary Medicine, University of Ibadan while embryonated eggs and African green monkey kidney (Vero) monolayer cells were obtained respectively from Ajanla Hatcheries Nigeria Limited and WHO Collaborative Centre for Influenza, Centres for Disease Control and Prevention, Georgia, USA through the Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

HI and VN tests and virus isolation in embryonated hen eggs and tissue culture were carried out on test sera and nasopharyngeal swabs respectively according to the WHO standard using 1% turkey red blood cells (rbc) as indicator for HI and foetal calf serum as control for VN (CDC 1981; Olaleye *et al.,* 1990; Adeniji *et al.,* 1993; Olusa & Adeyefa, 2009).

The HI titre for the control antiserum used in the HI test was 640 while the HI titres of the test sera ranged from 40- 20,480. Fourteen samples (35%) had HI titres between 40 and 320 while eighteen samples (45%) were from 640- 2,560. Seven samples (17.5%) had antibodies levels of 5,120-20,480 and only one sample (2.5%) was negative.

Cytopathic effect (CPE) was found in the Vero monolayer cells for thirty-three samples (82.5%) and the dilution at which CPE was observed was determined to be the neutralization titre. Neutralizing antibodies were not found in seven samples (17.5%). Viral isolation in embryonated hen eggs from the nasopharyngeal swabs was negative. None of the paired allantoic fluid harvested from the two control eggs was positive on haemagglutination test.

The presence of high HI and neutralizing antibodies in the test population is an indication of continuous exposure of the polo horses to H7 subtype of influenza viruses. In Nigeria, horse grooms and handlers keep local chickens/chicken feeds, ducks and other domestic animals in polo stables and horse that attract feral birds which could serve as a source of exposure for the horses. Since no equine H7 virus was isolated from the sterile swabs collected at the time the horses were bled for sera, it is not known with certainty whether the H7 viruses were of equine or avian origin. The horses might have been exposed to either a low pathogenicity equine H7 subtype virus or the highly/lowly pathogenic avian influenza virus strains. This should not be unexpected since avian influenza viruses are known to cross species barrier to infect new hosts (Horimoto & Kawaoka, 2001). Most recently, HPAI H5N1 viruses infecting man and other species have been reported in Asia, Europe, Middle East and Africa (Chen et al., 2006; Normile, 2006; Salzberg, 2007; Monne et al., 2008). Moreover, Guo et al., (1992) reported the infection of horses in Southern China by novel avian H3N8 influenza virus to which the horses where naïve and which caused high mortality and serious economic losses.

In Nigeria, horses are generally not vaccinated routinely against equine influenza. Although some owners who travel abroad occasionally purchase and administer influenza vaccines (Adeyefa & McCauley, 1994), the high antibody titres observed in this study cannot be accounted for by the low proportion of vaccinated horses if any as at the time of sampling. Moreover, immunity would wane after vaccination if there is no virus challenge (Adeyefa & McCauley, 1994). Although it is probable that the high antibody titres reported here may confer some degree of protection on these valuable horses, this may be short lived in view of antigenic variation and reassortment of genes common with two different influenza viruses (Wiley *et al.,* 1981; Skehel *et al.,* 1984; Adeyefa *et al.,* 1994; Horimoto & Kawaoka, 2001; Monne *et al.,* 2008). It is our opinion that the horses sampled were exposed to low pathogenicity equine or avian influenza viruses which may still be circulating among these horses.

Influenza is an economically important disease of horses as well as other host species. And in the light of its potential for rapid global spread and propensity for interspecies, continuous surveillance, both passive and active and emergency preparedness plans should be established globally and in Nigeria in particular to forestall the serious economic impact of the disease. This is very pertinent in view of the recent HPAI H5N1 outbreaks and the most recent new 2009 swine origin H1N1 pandemic virus (Chen *et al*., 2006; Normile, 2006; Ducatez *et al.,* 2007; Garten *et al.,* 2009; Smith *et al*., 2009). Further studies are recommended in order to isolate and characterize H7 influenza viruses from equine and avian host species which may prove to be pathogenic to horses. These could then be incorporated into currently available commercial vaccines for horses. Further studies are also required to determine host and viral factors responsible for interspecies transmission and the horses' immunological responses to influenza A virus invasion.

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