REVUE D'ELEVAGE ET DE MEDECINE VETERINAIRE DES PAYS TROPICAUX

ISSN: 0035-1865

RESEARCH ARTICLE

Enquete serologique nationale sur la grippe equine au Nigeria

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Nationwide serological survey of equine influenza in Nigeria

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Key words

Horse - Donkey - Equine influenza virus - Survey - ELISA - Haemagglutination test - Monoclonal antibody - Immunology - Epidemiology - Nigeria.

Summary

The objective of this work was to examine the incidence of equine influenza viruses in the equine population of an area of tropical Africa where equine influenza virus activity has recently been reported for the first time. A serological survey of sera from horses and donkeys from regions of Nigeria taken from 1990 to 1993 was carried out and the results obtained were com-pared with equine sera from Western Europe (Ireland). The sera were assayed for presence of antibodies by both haemagglutination inhibition (HI) and ELISA using a monoclonal antibody to the prototype H3 equine influenza virus, A/equine/Miami/1/63. The results showed that equine influenza was present in horses and donkeys in all regions of Nigeria.

■ INTRODUCTION

Influenza A viruses are widespread in nature and cause disease in humans and animals, including birds (7). These viruses are classified according to the antigenic differences between their haemagglutinin (HA) and neuraminidase (NA) glycoproteins. So far, 14HA and 9NA subtypes of influenza A virus have been identified (8), none of which are serologically crossreactive. Two subtypes cause clinical disease in horses, the H7N7 and H3N8 subtypes referred to as equine-1 and equine-2, respectively. Both have been reported to circulate individually in horse populations in the world with the exception of Australia (4) while both had also previously co-circulated in some horses (10). Although the last recorded isolation of H7N7 virus from horses in the field was in 1978 (11), there is serological evidence that suggests continued circulation of this virus (Webster R.G., unpublished data). However, the mechanism of virus maintenance in horses is not very clear, but it seems likely that the viruses are passed continuously to susceptible horses (12).

There is paucity of information on epidemiology of equine influenza in tropical Africa. The authors recently reported the first known outbreak of equine influenza in Nigeria among polo horses from which three influenza virus H3N8 subtypes were independently isolated (1). Studies on molecular characterisation of these viruses indicate that their genes are all equine in origin and that HA is most closely related to recent European and Scandinavian isolates (2).

However, we do not know the exact source of the viruses or their previous circulation among Nigerian horse populations prior to the 1991 outbreak of the disease. Durojaiye and Denya in 1982 (pers. comm.) observed antibodies to both equine-1 and equine-2 viruses in some Nigerian horses. On the other hand, it is probable that the viruses were brought into the country with horses recently imported from Argentina or the United Kingdom, where there have been recent outbreaks of equine influenza (13). Also, preliminary investigation in 1989 (C.A.0. Adeyefa, unpublished data) revealed antibodies to equine-2 influenza virus in horses not involved in the outbreak that was recently reported (1). The purpose of this study was therefore to further elucidate the epidemiology of equine influenza through a nationwide serological survey. Comparative studies were also carried out with post-natural infection, pre- and post-vaccination equine sera from Ireland. The results of a seroepidemiological survey and the developed competitive enzyme immunosorbent assay (ELISA) test are reported here.

■ MATERIALS AND METHODS

Virus antigen

Equine-2 prototype A/Eq/Miami/63 (H3N8) virus was grown in 11 day old embryonated hen eggs and purified by diffcrential sedimentation through 15 - 60 % sucrose gradients in Beckman SW 28 and 40.1 rotors. Virus concentration which was 20 mg/ml was determined by the method of Bradford (3). The purified virus was used as ELISA antigen at a concentration of 1 µg/ml.

Equine sera

A total of 375 equine sera were tested for antibodies to equine-2 influenza virus of H3N8 subtype. These comprised 181 horse and 6 donkey sera collected between January 1990 and October 1991 from various parts of Nigeria ; 4 post-natural infection, 45 pre-vaccination and 45 post-vaccination sera collected between January and June 1992 at the Irish equine Center, all tested in Pirbright, as well as additional 42 horse and 52 donkey sera collected between July and December 1993 in Nigeria and tested in Ibadan. These sera originated from 19 sampling locations (*e.g.* markets, veterinary schools, polo clubs, etc.) within 11 town areas throughout Nigeria (figure 1). The number of animals on each site varied greatly, from 2 to 50.

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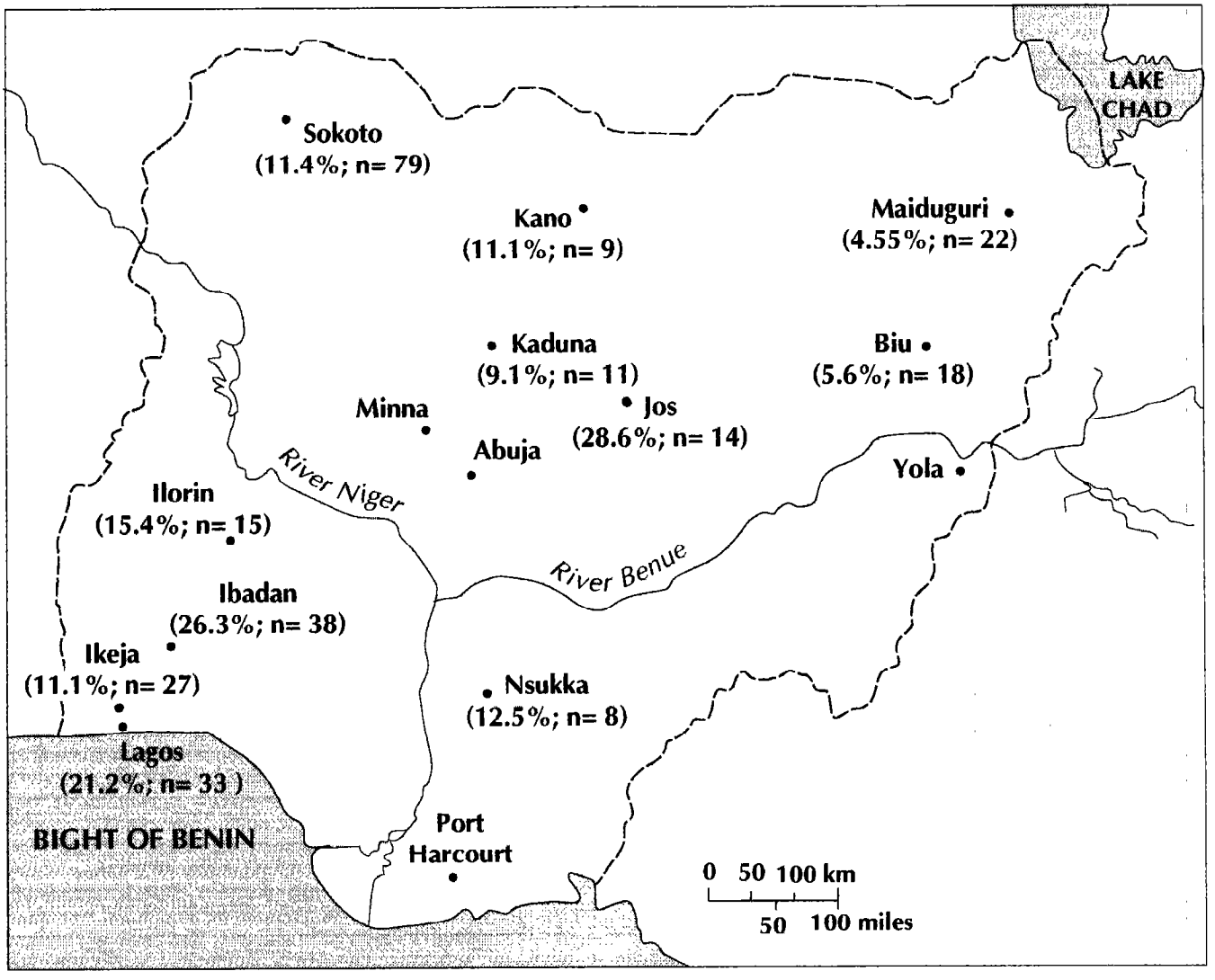


Figure 1: map of Nigeria showing areas of sampling (tested by competition ELISA) and the geographical spread of equine influenza virus activity. Seven horses belonging to private properties have not been included.

n = number of tested sera

Competition ELISA

Monoclonal antibody (Mab) against the HA of A/Eq/Miami/63 (H3N8) was used in competition ELISA developed for this test. The Mab was used at a dilution of 1:250,000. The ELISA was performed in Dynatech® flexible ELISA plates. The optimal dilutions of test antigen and Mab were determined by checkerboard titrations in 0.05 M carbonate bicarbonate buffer pH 9.6. Test sera were used at dilutions of 1:5.

Briefly, 50 µl per well of antigen diluted in carbonate bicarbonate buffer was passively adsorbed onto the solid phase of ELISA plates which were then incubated at 37°C for 2 h. Plates were washed 5 times with PBS pH 7.6 by flooding and emptying the wells which were blotted dry to remove residual washing buffer. 50 µl of 1:5 dilution of each test serum prepared in PBS containing 0.05 % Tween 20 (PBST) blocking buffer was added to duplicate wells of polystyrene microtiter transfer plates. Duplicate wells in second to the last row of wells in the transfer plates also received 50 µl per well of a known strongly positive convalescent antiserum, a weekly positive control antiserum and a negative control horse serum diluted in PBST while the last row of wells received 100 µl of PBS only. Fifty µl Mab diluted 1:250,000 in PBST was simultaneously added to each well in rows 1-11 of the transfer plates and mixed on an orbital shaker for 5 min. The contents of the transfer plates were then transfered into correspondingly marked ELISA antigen plates which were then incubated for 1 h at 37°C on an orbital shaker after which they were washed and blotted dry. To dilute anti-mouse IgG horse raddish peroxidase conjugate at 1:1000, 5 % (W/V) ovalbumin in PBST was used. Fifty µl conjugate/ovalbumin in PBST was added to each well and incubated for 1 h at 37°C on a shaker. After washing, 50 µl/well of orthophenylene diamine/H202 was added. Colour development was stopped after 10 min by addition of 50 µl/well of 1.25 M H_2SO_4. Plates were read spectrophotometrically at 492 nm with Titertek® multiscan ELISA plate reader. The value for no competition (maximum colour) was obtained from the mean absorbance values at 492 nm in the last row of wells containing PBS and measured the interaction between mouse antiserum and influenza virus (5). Positive reactions were recorded when test sera in duplicate wells showed 50 % inhibition of the mean absorbance value recorded in the last row wells (5).

Haemagglutination inhibition test (HI)

HI was performed in microtiter plates with 1 % chicken red blood cells by standard method. The 94 Irish equine sera and the

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additional 94 Nigerian equine sera were tested for HI antibodies against the prototype equine-2 virus, A/Eq/Miami/63. All sera used in HI test were pre-treated with potassium metaperiodate to remove any non-specific inhibitors present in normal serum. HI titers are expressed as reciprocals of the highest serum dilutions inhibiting 4HA units of virus.

■ RESULTS

Twenty nine (16 %) of 181 horse sera collected between January 1990 and October 1991 in Nigeria were positive for ELISA antibodies while none of the 6 donkey sera were positive. However, 6 (14.3 %) of 42 horse sera and 5 (9.6 %) of 52 donkey sera collected between July and December 1993 in Nigeria were positive for ELISA antibodies. Of the 94 Irish horse sera, one (25 %) of 4 natural infection sera, 2 (4.9 %) of 45 pre-vaccination and 39 (86.66 %) of 45 post-vaccination sera were positive by ELISA.

The percentages of positive sera by competition ELISA varied considerably among Nigerian equine sera and ranged between 9.1 and 40 %. Although sera from some sources were negative, positive serum samples could be found anywhere in the country. One of the 2 donkey sera from Ilorin was positive while 2 (40 %) of the 5 horse sera from the University of Ibadan veterinary teaching Hospital were positive, followed by Ibadan Polo Club (29.6 %), Jos Polo Club (28.5 %), Lagos Polo Club (25 %) and Sokoto horse market (19.04 %). Other positive samples ranged from 5.5 - 12.5 % including those from Biu, Kano, Kaduna, Ilorin and Nsukka for which local information indicates that a few of the horses sampled there were brought from Yola on the mid North-Eastern border.

In the HI tests, 6 Nigerian horse and 6 donkey sera were positive for HI antibodies out of the horse and donkey sera positive by ELISA with HI titers ranging from 10-640. With the Irish horse sera, 33 out of 39 positive by ELISA were also positive by HI among the 45 post-vaccination sera while none of the 4 post-infection sera and only 2 of the 45 pre-vaccination sera were positive by HI as they were by ELISA with HI titers ranging from 16-512. Table I shows the correlation between HI and ELISA results of the Irish and Nigerian equine sera. The geographical spread and percentage positives of equine-2 influenza virus antibodies in Nigeria are shown in figure 1.

TABLE I

Correlation between HI and ELISA results of 94 Irish and 94 Nigerian equine sera

		HI		ELISA	
Num. of sera tested		**Num. pos.**	**% pos.**	**Num. pos.**	**% pos.**
Irish equine sera					
Total Num. tested	94	35	37.2	42	44.7
Post-natural infection	4	0	0	1	25
Pre-vaccination	45	2	4.4	2	4.4
Post-vaccination	45	33	73.3	39	86.6
Nigerian equine sera					
Total Num. tested	94	12	12.76	11	11.70
Num. of horse sera	42	6	14.3	6	14.3
Num. of donkey sera	52	6	17.3	5	7.6

■ DISCUSSION

The estimated population density of horses in Nigeria is 208,000 and that of donkey is 1 million, while human population is 88.6 millions from the 1991 census (9). This study survey covered equines aged 6 months to over 25 years located in most regions of the country although it was limited mostly to animals in equine establishments such as polo clubs, race courses, police and military units/formations and university veterinary schools. The number of sera collected was very small (375) compared with estimated equine populations in the country. In spite of this, the results of our nationwide serological survey indicate that equine influenza occurs in most parts of Nigeria as there was no region in the country where no positive serum samples could be found. Although the study of the circulation of equine influenza viruses within horse populations in any geographical area relies mostly on the detection of serum antibodies to these viruses, precise knowledge of the antigenic identity of the viruses prevalent in the areas under study is of inestimable value. We have previously isolated 3 equine-2 (H3N8) influenza viruses from sick horses involved in a recent outbreak of diseases in Ibadan, Nigeria, during a polo tournament in January 1991 (1). Some of the animals involved in this outbreak comprised indigenous and imported horses. Some of the sera collected from these horses and some collected from different parts of the country long before and long after the outbreak of disease showed antibodies to the equine-2 virus. This suggests previous exposure of horses with positive sera to equine-2 (H3N8) influenza virus, which implies that the virus had previously circulated and still circulates in Nigerian equine populations.

Furthermore, a sustained presence of antibodies in peripheral circulation is characteristic of the immunological response to equine-2 influenza virus (6). Our detection of antibodies in sera collected long before the clinical outbreak of disease further supports the possibility of previous exposure of some horses to the virus. Alternatively, the viruses isolated in our study had probably been introduced to susceptible animals in Ibadan by the recently imported horses from endemic regions, with the stress of transportation and the unfavourable dusty weather conditions in Ibadan at the time of outbreak acting as predisposing factors (1).

Of the 94 Irish equine sera, one (25 %) of the 4 post-clinical natural infection sera was positive by ELISA, while only 2 (4.4 %) of 45 pre-vaccination and 39 (86.6 %) of 45 post-vaccination sera were positive by ELISA. These results correlated well with HI results although the ELISA test was more sentitive in detecting antibodies in 6 more sera than the HI test. The competition ELISA described in this study appears to be very useful for detecting acute infection and post-vaccination antibody responses in horses as indicated by the results of the Irish equine sera. However, the low percentages of positive sera detected by the test in the Nigerian sera could be due to the clone of the Mab used which was directed against a region of the HA and thus probably recognised a single or fewer epitopes on the antigen. The A/Eq/Newmarket/76 antiserum raised in rabbit gave a higher percentage of positive sera when used in the ELISA test on a limited number of randomly selected sera (data not shown). The use of polyclonal antiserum is thus recommended by the authors where influenza virus glycoprotein specific Mabs are not available for competition ELISA for more extensive epidemiological investigations of influenza in a variety of host species in this region of the world.

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Acknowledgements

We are grateful to Dr. A.I. Daneji, Department of veterinary Medicine, Utman Dan Fodio University, Sokoto and Dr. V.O. Shoyinka, Department of veterinary Pathology, University of Nigeria, Nsukka for equine sera collected in July - December, 1993. We also thank for his handling assistance Mr. Tunde Mabawonku, Department of veterinary Medicine, University of Ibadan, during the 1990-1991 sample collection. Dr. Adeyefa was supported by the Association of Commonwealth Universities academic Staff Fellowship tenable at the Pirbright Laboratory of the Institute for Animal Health, U.K., from January to November 1992.

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Reçu le 19.01.1996, accepté le 6.05.1996

Résumé

Adeyefa C.A.O., Hamblin C., Cullinane A.A., McCauley J.W. Enquête sérologique de la grippe équine sur le territoire du Nigeria

L'objet de cette enquête était d'examiner l'incidence des virus de la grippe équine dans une population d'équidés d'Afrique tropicale où des foyers de grippe équine ont été récemment reportés pour la première fois. Une enquête sérologique à partir de sérums de chevaux et d'ânes de plusieurs régions du Nigeria, prélevés de 1990 à 1993, a été effectuée et les réultats obtenus ont été comparés avec des sérums provenant d'Irlande. La présence des anticorps a été testée à la fois par le test d'inhibition de l'hémagglutination et par le test ELISA en utilisant un anticorps monoclonal au prototype H3 du virus de la gripppe équine, A/equine/Miami/1/63. Les résultats ont montré que la grippe équine était présente chez les chevaux et les ânes de toutes les régions du Nigeria.

Mots-clés : Cheval - Ane - Influenzavirus équin - Enquête - Test ELISA - Epreuve d'hémagglutination - Anticorps monoclonal - Immunologie - Epidémiologie - Nigeria.

Resumen

Adeyefa C.A.O., Hamblin C., Cullinane A.A., McCauley J.W. Encuesta serológica nacional sobre la influenza equina en Nigeria

El objetivo del presente trabajo fue el de examinar la incidencia del virus de la influenza equina en la población equina de un área tropical de Africa, donde la actividad del virus de la influenza equina fue reportada recientemente por la primera vez. Una encuesta serológica en caballos y burros de las regiones de Nigeria, realizada entre 1990 y 1993, así como los resultados obtenidos, fueron comparados con sueros equinos de Europa del Oeste (Irlanda). Se midió la presencia de anticuerpos en los sueros, tanto por inhibición de la hemaglutinación como por ELISA, utilizando un anticuerpo monoclonal al prototipo H3 del virus de la influenza A/equine/Miami/1/63. Los resultados muestran que la influenza equina estuvo presente en caballos y burros de todas las regiones de Nigeria.

Palabras clave : Caballo - Asno - Virus de la influenza equina- Encuesta - ELISA - Prueba de hemaglutinación - Anticuerpo monoclonal - Immunología - Epidemiología - Nigeria.