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Complement fixing antibodies against arboviruses in horses at Lagos, Nigeria

OLALEYE (O. D.), OLADOSU (L. A.), OMILABU (S. A.), BABA (S. S.), FAGBAMI (A. H.). Anticorps de fixation du complément contre les arbovirus chez les chevaux à Lagos, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 321-325.

Les sérums de 62 chevaux récoltés dans deux écuries à Lagos, Nigeria, ont été testés pour la recherche des anticorps de fixation du complément (FC) contre les antigènes de 8 arbovirus, à savoir : Chikungunya, Igbo-Ora, fièvre jaune, maladie de Wesselsbron, West Nile, Potiskum, Uganda S et la fièvre de la vallée du Rift. Dix pour cent des sérums de chevaux examinés contenaient des anticorps de FC pour un ou plus des antigènes-tests et indiquaient une activité considérable des arbovirus dans les deux écuries. Les réactions aux antigènes des flavivirus étaient très courantes et les titres d'anticorps les plus élevés ont été obtenus avec les virus de la maladie de Wesselsbron et la fièvre jaune. Onze pour cent des sérums examinés ont réagi aux antigènes des α-virus alors que 10 p. 100 ont donné une réaction positive aux antigènes de FC pour le virus de la fièvre de la vallée du Rift. *Mots clés* : Cheval - Anticorps - Arbovirus - Nigeria.

INTRODUCTION

Active surveillance for arbovirus activity in Nigeria started in 1964 when the Rockefeller Foundation established the Virus Research Laboratory at Ibadan. Since that time, surveillance system for arboviruses was carried out by sampling of human blood, mosquitoes, domestic and wild animals (27). Domestic animals mostly examined included cattle, sheep, goats and swine (11, 28).

Apart from a limited serological survey for neutralizing antibody to African horse sickness virus in Nigerian horses (10, 19), little is known about the role of horses in the maintenance and transmission cycle of arboviruses in Nigeria. However studies elsewhere (8) revealed that inapparent infection of horses with arboviruses occurs frequently in many areas. In order to determine the extent of arbovirus activity in the Nigerian horse population, sera collected from horses in two stables in Lagos, Nigeria were tested for CF antibody to 8 arbovirus antigens.

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MATERIALS AND METHODS

Collection of sera

Sixty-two horse sera were collected from two stables at Lagos, Nigeria in June 1987. The horses were all adult males, consisting of Dongola and Arab-Barb breeds.

Blood was collected through the jugular vein using sterile needles and vacuum tubes as described by KEMP *et al.* (11). Sera were separated by centrifugation at 1,800 rpm and stored in screw-capped bijou bottles in a mechanical freezer (-20 °C) until tested.

Virus used

The viruses used to prepare the test antigen were Yellow fever, Wesselsbron, West Nile, Potiskum, Uganda S, Chikungunya, Igbo-Ora and Rift Valley fever (Table I). Virus antigens were infected suckling mouse brain prepared by sonication and sucrose acetone extraction as described by CLARKE and CASALS (3).

Complement Fixation test

Complement Fixation (CF) tests were performed in plastic plates using the modified microtitre techniques of SEVER (22). Sera were inactivated at 56 °C for 30 minutes and tested in two-fold serial dilution with veronal buffer against optimum dilutions (obtained after a checker board titration) of the antigens.

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TABLE I Viruses used in the complement fixation test.

Virus	Virus No.	Passage history	Year of isolation	Location	Source
Chikungunya	Ib-H 35	16	1964	Ibadan	Man
Igbo-Ora	Ib-H 10964	10	1966	Ibadan	Man
Yellow fever	Ib-VIR 114978	10	1902	Ghana (Asibi vaccine strain)	Man
Wesselsbron	Ib-AN 31956	6	1968	Kano	Camel
West Nile	Ib-AN 7019	4	1965	Ibadan	Mouse
Potiskum	Ib-AN 10069	15	1966	Fika	Giant rat
Uganda S	Ib-AN 8829	6	1966	Ibadan	Mouse
Rift Valley fever	Ib-VIR 121535 (Dak Ar B 1976)	6	1969	Bangui (Central African Republic)	Mosquito *(Mansonia africana)*

RESULTS

Out of a total of 62 horse sera tested against 8 arbovirus antigens ; 48 had CF antibody to one or more antigens. Seven out of 62 sera (11 per cent) reacted with Igbo-Ora virus antigen while 5 (8 per cent) had Chikungunya virus CF antibody. Five out of 7 sera (71 per cent) that were positive with Igbo-Ora virus antigen cross-reacted with Chikungunya virus antigen. However, end point titration showed 3 out of 7 alphavirus positive sera (43 per cent) to be specific for Igbo-Ora virus (titre 1:64), while only one of the seven sera (14 per cent) was specific for Chikungunya virus antigen (titre 1:32). CF antibody titres in other sera were 1:8 for both alphavirus antigens.

Complement fixing antibody to the flaviviruses was most frequently encountered in the present serological survey. Forty-eight out of 62 sera (77 per cent) tested were positive to one or more flavivirus antigens used. Percentages of positive sera to individual flaviviruses were : Yellow fever 77 per cent, Wesselsbron 77 per cent, West Nile 71 per cent, Potiskum 62 per cent and Uganda S 71 per cent. Ten out of the 48 sera (21 per cent) showed specific reactions to Wesselsbron virus and 5 (10 per cent) to Yellow fever. Only 5 out of 51 sera (10 per cent) tested for Rift Valley fever virus CF antibody were positive.

TABLE II Complement fixing antibody against arboviruses in horse sera.

Antigen	No. tested	No. and (per cent) positive	No. positive CF antibody at various dilutions					
			4	8	16	32	64	128*
Chikungunya	62	5 (8)	—	—	3	2	—	—
Igbo-Ora	62	7 (11)	—	—	2	2	3	—
Yellow fever	62	48 (77)	—	20	20	3	5	—
Wesselsbron	62	48 (77)	8	20	10	—	5	5
West Nile	62	44 (71)	7	28	9	—	—	—
Potiskum	62	37 (62)	35	2	—	—	—	—
Uganda S	62	44 (71)	9	22	13	—	—	—
Rift Valley fever	48	5 (10)	—	3	2	—	—	—

* Reciprocal of antibody titre.

DISCUSSION

The role of arthropod-borne viruses as aetiological agents of human illness in Nigeria has been highlighted by MOORE *et al.* (15). Many arboviruses have been isolated from domestic and wild animals in the country (10, 11, 23). Antibodies to arthropod-borne viruses such as West Nile (5) and Igbo-Ora (20) viruses have been demonstrated in sera from Nigerian domestic animals other than horses.

The results of the present study showed a low to moderate activity of arboviruses in Nigerian horses. Although it appears that many of the horses showing reactions to flaviviruses might in fact be reflecting cross-reactions to the same infecting agent (17), the most interesting finding in this study was demonstration of CF antibody to Chikungunya, Igbo-Ora, Yellow fever, Wesselsbron and Rift Valley fever viruses in horse sera in Nigeria. Previous studies in the country revealed that Chikungunya virus is an important aetiological agent of human illness (16) probably the most commonly isolated alphavirus (15). There is serological evidence of high activity of Igbo-Ora virus in Nigerian domestic animals (20). Igbo-Ora virus has also been shown to cause human infection in Ivory-Coast (21). An outbreak caused by Igbo-Ora virus involving all age groups was reported in a rural community in Ivory-Coast (13). Strains of the virus were isolated from sick persons and mosquitoes during the outbreak. Although wild reservoirs of the virus could not be identified during the epidemic, the results of this study and earlier report of demonstration of haemagglutination-inhibition antibody to Igbo-Ora virus in small ruminants in Nigeria (19) suggest a transmission cycle of the virus in animals. It is therefore important to determine the role of Igbo-Ora virus in causing animal disease. Further, the detection of CF antibody to the two alphaviruses in horses indicates that horses are being infected by several arboviruses in the Lagos area ; however, overt disease in horses resulting from infection by any of these alphaviruses used in the present test has not been reported. It is possible that such infections may be mild escaping veterinary attention ; infected horses may also serve as reservoir hosts for these viruses.

Hitherto, very little is known about activities of flaviviruses in Nigerian horses. The broad reactivity to these viruses may be due to high endemicity of flaviviruses in Nigeria. Yellow fever is known to be endemic in Nigeria (16), severe epidemics have been reported in several parts of the country (2, 7, 14, 18, 25). Complement fixing antibody to Yellow fever found in horse sera tested may be due to exposure of the animals to Yellow fever virus during the recent (1986/1987) Yellow fever epidemic in the country (26). On the other hand, the virus may be circulating among the horse population thus serving as focus of potential epidemic under favourable conditions. Although Wesselsbron, Potiskum, West Nile and Uganda S viruses were originally isolated from animals, this is probably the first report of demonstration of CF antibody to Wesselsbron virus in horses in Nigeria. It is, however, not surprising since the virus was originally isolated from camel. Horses and camels are commonly kept together, especially in the northern part of the country where large numbers of the two species are kept. Serological surveys by other workers (1, 9) showed high prevalence of CF and haemagglutination-inhibition antibodies to Wesselsbron and West Nile viruses in camel sera in Nigeria.

Rift Valley fever virus strains have been isolated from culicoides and mosquitoes in Ibadan (12). Previous serological surveys for Rift Valley fever virus antibodies in Nigeria showed neutralizing antibodies in man (24), wild and domestic animals other than horse (6). In another related study to determine the activity of Rift Valley fever virus in cattle, sheep, goats, pigs, camel and horses in Nigeria, none of the 26 horse sera collected between 1964 and 1968 by the Virus Research Laboratory, Ibadan, contained neutralizing antibody to Rift Valley fever virus (28). This may be due to small sample size. In this study, 10 per cent of 51 horse sera tested showed CF antibody to the virus. Lack of clinical disease in horses despite the high prevalence of CF antibody may be due to the fact that horses react to Rift Valley fever virus infection only by fever and production of antibodies without clinical disease even after experimental challenge with high dose of the virus (4). Also because of low levels of viraemia reported in horses experimentally infected with the virus (29), the possibility of the animal serving as amplifying host for the virus is very remote. It is however important to note that horses may serve as focus of infection for abattoir workers and other related occupational groups even with low viraemia particularly in Rift Valley fever virus endemic areas. The role of Rift Valley fever virus as an agent of human and animal diseases in Nigeria is currently being investigated in Ibadan laboratory.

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OLALEYE (O. D.), OLADOSU (L. A.), OMILABU (S. A.), BABA (S. S.), FAGBAMI (A. H.). Complement fixing antibodies against arboviruses in horses at Lagos, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 321-325.

Sixty-two sera horse collected from two stables at Lagos, Nigeria, were tested for complement fixing antibody to 8 arbovirus antigens ; Chikungunya, Igbo-Ora, Yellow fever, Wesselsbron, West Nile, Potiskum, Uganda S and Rift Valley fever. Ten per cent of the horse sera examined contained CF antibody to one or more of the test antigens and indicated considerable arbovirus activity in the two stables. Reactions with flavivirus antigens were most common and the highest antibody titres were obtained with Wesselsbron and Yellow fever viruses. Eleven per cent of the sera tested reacted with alphavirus antigens while 10 per cent were positive for Rift Valley fever virus CF antibodies. *Key words* : Horse - Serum - Antibody - Arbovirus.

OLALEYE (O. D.), OLADOSU (L. A.), OMILABU (S. A.), BABA (S. S.), FAGBAMI (A. H.). Anticuerpos de fijación del complemento contra los arbovirus en los caballos en Lagos, Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (3) : 321-325.

Se comprobaron los sueros recogidos en 62 caballos en Lagos, Nigeria, para buscar los anticuerpos de fijación del complemento (FC) contra los antigenos de los 8 arbovirus siguientes : Chikungunya, Igbo-Ora, fiebre amarilla, enfermedad de Wesselsbron, West-Nile, Pokistum, Uganda S y fiebre del valle del Rift. Diez por ciento de los sueros de caballos examinados contenían anticuerpos de FC para uno o más de los antigenos-pruebas e indicaban una actividad considerable de los arbovirus en las dos cuadras. Las reacciones a los antigenos de los flavivirus eran muy corrientes y se obtuvieron los títulos de anticuerpos más elevados con los virus de la enfermedad de Wesselsbron y de la fiebre amarilla. Once por ciento de los sueros examinados han reaccionado a los antigenos de los α-virus mientras que 10 p. 100 tuvieron una reacción positiva a los antigenos de FC para el virus de la fiebre del valle del Rift. *Palabras claves* : Caballo - Arbovirus - Anticuerpos - Nigeria.

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