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Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State

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SUMMARY

A study of Zika virus infections was carried out in four communities in Oyo State, Nigeria. Virus isolation studies between 1971 and 1975 yielded two virus isolations from human cases of mild febrile illness. Haemagglutination-inhibition tests revealed a high prevalence of antibodies to Zika and three other flaviviruses used. The percentages of positive sera were as follows: Zika (31%), Yellow fever (50%), West Nile (46%), and Wesselsbron (59%). Neutralization tests showed that 40% of Nigerians had Zika virus neutralizing antibody. Fifty per cent of Zika virus immune persons had neutralizing antibody to Zika alone or to Zika and one other flavivirus. A total of 121 sera had antibody to Zika virus; of these 48 (40%) also showed antibody to two other flaviviruses, and 12 (10%) had antibodies to three or more other viruses. The percentage of neutralizing antibodies to other flaviviruses in Zika virus immune sera was 81% to Dengue type 1, 58% to Yellow fever, 7% to Wesselsbron, 6% to West Nile and 3% to Uganda S.

INTRODUCTION

Dick, Kitchen & Haddow (1952) reported the first isolations of Zika virus from the blood of a sentinel rhesus monkey and from a pool of *Aedes africanus* mosquitoes in Zika forest, Uganda. Virus surveillance elsewhere has yielded more Zika virus isolations from other species of mosquitoes (Marchette, Garcia & Rudnick, 1969; Lee, 1969).

The first evidence of human infection by this virus was provided in 1952 when Smithburn demonstrated the presence of neutralizing antibodies in human sera collected from East Africa. Later Simpson (1964) reported the isolation of Zika virus from a human being in Uganda. The first report of the occurrence of this virus in Nigeria was made in 1975 when Moore *et al.* reported three isolations of Zika from the blood of febrile patients attending the outpatient clinic of the University College Hospital, Ibadan. Serological surveys by several workers in Nigeria (Boorman & Draper, 1968; Macnamara, Horn & Porterfield, 1959) indicated that Zika and other flavivirus antibodies were commonly encountered in sera of Nigerians. Studies have been carried out by various workers to determine the pattern of these flavivirus infections among Nigerians (Monath *et al.* 1974;

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Fagbami, Monath & Fabiyi, 1977). This paper presents data on virological and serological investigation on Zika virus infections in Oyo State, Nigeria.

MATERIALS AND METHODS

Study areas

The areas covered in this survey included Oshogbo, Igbo-Ora, Ibadan and Oyo. These four localities have been previously described by Guyer (1972), Monath *et al.* (1974) and Fagbami *et al.* (1977).

Blood samples

Blood samples were collected for virus isolation and serology. The method of collecting samples for virus isolation has been discussed by Moore *et al.* (1975) and Fagbami (1977). Blood for virus isolation was fingerprick blood in heparinized capillary tubes collected routinely from the Outpatients Department of the University College Hospital and from Igbo-Ora. Blood for serology was collected by venipuncture from voluntary donors in hospitals. Some of the sera tested in this study have previously been used for Dengue serosurvey. Sera were separated by centrifugation at 2000 rev./min for 15 min and stored at −20 °C in plastic tubes or bijou bottles before use.

Antigens

The viruses used to prepare seed lots and antigens were Zika, Yellow fever, Dengue type 1, West Nile, Wesselsbron and Uganda S viruses. Virus seeds were prepared as 10% suckling mouse brain suspension in 0·75% bovine albumen in phosphate buffered saline (BAPS) containing 10% normal mouse ascitic fluid and stored at −60 °C before use. Antigens were prepared by sucrose acetone extraction according to the method of Clarke & Casals (1958).

Virus isolation

Virus isolation was carried out in suckling albino Swiss mice. Blood was diluted 1/5 in BAPS containing 2000 units of penicillin and 600 μg of streptomycin per ml, or in Eagle's minimal essential medium (MEME) supplemented with 2% fetal calf serum. Diluted blood samples were then inoculated intracerebrally (i.c.) into a litter of suckling mice in 0·02 ml volumes. Mice were observed for 14–21 days. Sick suckling mice were harvested and their brains further processed for virus identification. Specificity of virus isolates was determined by the complement fixation test (CFT).

Serological tests

The complement fixation test was performed in microtitre plates as described by Weinbren (1958).

The haemagglutination inhibition test was performed on kaolin-treated sera by the microtitre method of Clarke & Casals (1958). Sera were tested at 1/10 dilutions

against four flavivirus antigens: Zika, Yellow fever (YF), West Nile (WN) and Wesselsbron (WSL).

Neutralization tests

Human sera were screened for Zika virus antibody by neutralization test in suckling mice. The standard constant virus – constant serum technique was used. All Zika virus immune sera were further tested by neutralization (N) tests against five other flaviviruses: Yellow Fever (YF), West Nile (WN), Wesselsbron (WSL), Dengue type 1 (Den-1) and Uganda S (US). Selected sera positive to Zika and two or more flaviviruses were further tested to obtain endpoints. Endpoint titres were calculated by the method of Reed & Muench (1938) and expressed in Dex (Haldane, 1960).

RESULTS

Virus isolation

Two Zika virus isolations were made from 10778 heparinized blood specimens collected between 1971 and 1975. One was obtained in July 1971 from the blood of a male child aged 2½ years. The second was made in May 1975 from the serum of a 10-year-old boy in Igbo-Ora (Fagbami, 1977). The two virus isolations were obtained in early and peak rainy seasons – the first patient had mild nondescript febrile illness and no other clinical details were obtainable. The second patient reported with fever, headache and body pains.

Haemagglutination-inhibition test

One hundred and eighty-nine persons were tested for HI antibodies; 20 children and 169 adults (Table 1). Percentages of positive sera were as follows: Zika (31%), Yellow fever (50%), West Nile (46%) and Wesselsbron (59%). A total of 130 persons (69%) had HI antibodies to one or more flavivirus used in the test. The highest percentage of HI positive sera (100%) was found in the 40 years and above age group.

Neutralization test

Neutralization tests performed on 130 HI positive sera showed that 49 (38%) contained Zika N antibody. Tests on 59 HI antibody negative sera showed that two (3%) were positive for N antibody to Zika virus. In all, 300 sera were subsequently tested by neutralization tests. One hundred and twenty-one were positive, 15/55 children (0–19 years) (27%) and 106/245 adult (43%). All 121 Zika immune sera were further tested for N antibodies to other flaviviruses in an attempt to define the sera with specific Zika virus antibody (Table 2). The percentages of Zika virus antisera containing antibody to other flaviviruses are as follows: Yellow fever (55%), Dengue type 1 (81%), Wesselsbron (7%), West Nile (6%) and Uganda S (3%). Eleven persons (9%) had monotypic Zika virus antibody; 50 (41%) were positive to Zika and one other flavivirus. Sera containing antibody to Zika and to Zika and one other flavivirus were more frequently encountered in

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Table 1. *Results of tests for flavivirus haemagglutination-inhibition and Zika virus neutralizing antibodies in human sera, Oyo State, Nigeria*

Age in years	No. tested	No. (%) HI positive					No. (%) Zika NT positive
		Zika	YF	WSL	WN	Total flavivirus positive	
0–9	9	0	0	0	0	0	0
10–19	11	3 (27)	5 (45)	6 (55)	5 (45)	7 (64)	4 (36)
20–39	166	54 (33)	87 (52)	102 (61)	79 (48)	120 (72)	67 (40)
⩾ 40	3	2 (67)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33)
All ages	189	59 (31)	95 (50)	111 (59)	87 (46)	130 (69)	72 (38)

Figures in parentheses indicate percentages of those tested in each age group.

Table 2. *Results of tests for other flavivirus N antibodies in Zika immune human sera, Oyo State, Nigeria*

Age in years	No. Zika N positive sera tested	No. (%) positive Zika only	No. (%) positive to Zika and one other flavivirus					
			YF	Den-1	WN	WSL	US	Total
0–9	1	1 (100)	0	0	0	0	0	0
10–19	14	3 (21)	3 (21)	2 (14)	0	0	0	5 (36)
20–39	100	7 (7)	9 (9)	36 (36)	0	0	0	45 (45)
40+	6	0	0	0	0	0	0	0
All ages	121	11 (9)	12 (10)	38 (31)	0	0	0	50 (41)

Age in years	No. (%) positive to Zika + 2 other viruses	No. (%) positive Zika and 3 or more other viruses	Total No. (%) NT positive and				
			YF	Den-1	WSL	WN	US
0–9	0	0	0	0	0	0	0
10–19	5 (36)	1 (7)	7 (50)	7 (50)	1 (7)	2 (14)	0
20–39	39 (39)	9 (9)	54 (54)	85 (85)	7 (7)	4 (4)	3 (3)
40+	4 (67)	2 (33)	6 (100)	6 (100)	1 (17)	1 (17)	1 (17)
All ages	48 (40)	12 (10)	67 (55)	98 (81)	9 (7)	7 (6)	4 (3)

Table 3. *Results of endpoint titration on selected sera containing N antibody to Zika and one or more viruses*

Locality	No. tested	No. (%) with highest LNI* to						Undiagnosable†
		Zika	YF	Den-1	WN	WSL	US	
Ibadan	6	2 (33)	0	2 (33)	0	0	0	2 (33)
Oyo	4	1 (25)	0	0	0	0	0	3 (75)
Oshogbo	5	0	1 (20)	2 (40)	0	0	0	2 (40)
Total	15	3 (20)	1 (7)	4 (27)	0	0	0	7 (47)

* Log neutralization index.

† Equal LNI to two or more flaviviruses.

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the younger age groups. Sixty sera (50 %) of Zika antisera were positive to Zika and two or more other flaviviruses. Endpoint titrations of such selected sera are shown in Table 3. Three (20 %) of 15 tested sera had highest neutralization indices to Zika virus.

DISCUSSION

The role of flaviviruses and other arthropod-borne viruses as aetiological agents of human illnesses in Nigeria has been highlighted by Moore *et al.* (1975). The present survey showed that Zika virus immunity is prevalent among Nigerians; 40 % of persons tested had neutralizing antibodies. Since 50 % of Zika-immune individuals tested had monotypic Zika N antibody or antibodies to Zika and one other flavivirus, the immunity reported in this study cannot be attributed to immunological cross reaction within the flavivirus serogroup. Sera containing monotypic antibodies were found more frequently among the younger age groups. Persons in this group are less likely to have multiple flavivirus infections, which are often responsible for broad heterologous cross reactions.

The results of the haemagglutination inhibition test showed that HI antibodies to Zika and related viruses were prevalent among Nigerians, 69 % of persons tested were positive. It has been shown that neutralizing antibodies develop earlier than HI antibodies (Southam, 1956). The detection of Zika neutralizing antibody in sera of persons negative for flavivirus HI antibody suggested that such persons probably had had recent Zika virus infection.

Apart from the serological evidence of Zika virus activity in Nigeria, previous (Moore *et al.* 1975) and the present virus isolations from human blood further confirmed the active transmission of this virus infection in the country. Although only a few isolates were obtained in past and present studies, the demonstration of a high rate of immunity to the virus in all the areas studied and elsewhere (Boorman & Draper, 1968; Macnamara *et al.* 1959) suggested that Zika virus infection is more widely spread. The low isolation rate may be attributed to the fact that infected persons often present with mild, nondescript illness and it is conceivable that such cases may not report to hospital clinics.

Clinically Zika virus infection is manifested by fever, headache, body pains and rash (Simpson, 1964; Moore *et al.* 1975). In the present investigations, similar manifestations were observed in the two patients from whom the virus was isolated. In Nigeria and many other developing countries, malaria is the common cause of febrile illness (Downs, 1975). The similarities in the clinical presentation of malaria and many arthropod-borne viral infections often result in misdiagnosis especially in places where laboratory facilities are lacking.

Although clinical Zika virus infection is mild, the high prevalence of immunity in Nigeria has some epidemilogical significance. It was shown by Fabiyi & Macnamara (1962) that better antibody response was found in persons without preimmunization flavivirus antibody following yellow fever vaccination. The high percentage of persons immune to Zika virus and other flaviviruses may influence the outcome of a yellow fever immunization programme. It was also shown by Henderson *et al.* (1970) that such immunity to heterologous flaviviruses might

even modify the course of yellow fever infection. Yellow fever is known to be endemic in Nigeria; severe epidemics have been reported in several parts of the country (Carey *et al.* 1972; Monath *et al.* 1973; Fagbami *et al.* 1976). However, there had not been any documented outbreak of this disease in the areas studied. The high prevalence of antibody to Zika and other related viruses might contribute to the absence of yellow fever in these areas.

Zika virus has been isolated from *Aedes africanus* (Dick *et al.* 1952; Weinbren & Williams, 1958). In Nigeria the isolates were obtained from *A. luteocephalus* (Lee, 1969). This mosquito is a forest *Aedes* species; however, the immunity to Zika virus has been demonstrated in all the urban communities studied. It is probable that other species of mosquitoes, possibly *A. aegypti*, play an important role in the transmission of the virus in Nigeria.

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