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Survey for Antibodies to Arboviruses in the Sera of Children in Portuguese Guinea *

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This paper describes the results of a study of antibodies to several arboviruses in representative groups of children in Portuguese Guinea. The survey was conducted in late 1964 and early 1965, in conjunction with a yellow fever vaccination programme undertaken when this disease appeared in West Africa. Sera were prepared from 1103 blood specimens collected from 10-15-year-old residents in different regions of the territory and were tested against 17 different antigens by means of the haemagglutination-inhibition test. In addition, 51 sera obtained from 20-25-year-old nonresidents were tested. The results show that group B arboviruses, particularly the yellow fever virus, were active in the region. Information on the activity of arboviruses of other groups was also obtained.

Intensive studies of the epidemiology of yellow fever have led to the discovery of many arboviruses (Horsfall & Tamm, 1957), which are now classified according to the system proposed by Casals (1957). The importance of some of these viruses has been established by serological studies of the activity of the yellow fever virus in a particular region conducted by Theiler & Casals (1958).

Several serological surveys have been carried out in Africa—first in East Africa (Strode, 1951) and subsequently in South Africa (Kokernot et al., 1956; Smithburn et al., 1959); Mozambique (Kokernot et al., 1960); Angola (Kokernot et al., 1965); Nigeria, Ghana, and Liberia (Theiler, 1961); Ghana (Fabiyi, 1961); Nigeria (Macnamara et al., 1959); Senegal (Brès et al., 1963); Upper Volta (Brès et al., 1965); Ethiopia (Serié et al., 1964); and Bechuanaland (now Botswana) (Kokernot et al., 1965).

It was felt, however, that further information was needed, and it was decided to investigate the distribution of antibodies to arboviruses in a normal population in West Africa that had not been vaccinated against yellow fever. It should be mentioned that transmission of the yellow fever virus was occurring in West Africa during the period when the blood specimens were collected for this study (*Wkly epidem. Rec.*, 1964) and that four strains of the virus were isolated.

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MATERIALS AND METHODS

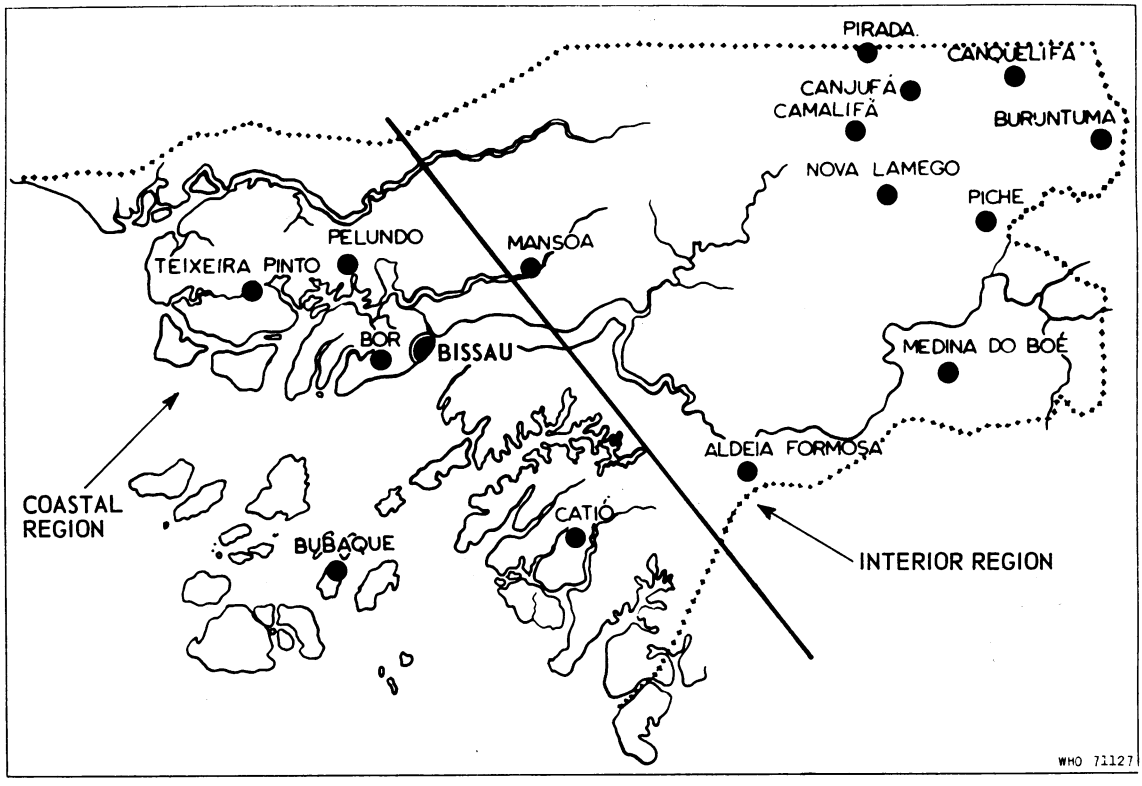
Sera

A total of 1103 blood specimens was obtained from groups of 10-15-year-old residents in the localities shown on the accompanying map. In addition, 51 blood specimens were obtained from 20-25-year-old nonresidents who had been in Portuguese Guinea for less than 2 years, who had not previously lived in a tropical region, and who had been vaccinated against yellow fever. Of the total, 922 specimens were collected in December 1964 and January 1965 and 232 specimens were collected in May 1965. In selecting the localities for the collection of specimens, special attention was given to different climatic conditions and to both coastal and interior regions of the territory. The blood samples were taken with evacuated specimen tubes, which were then kept in the refrigerator while awaiting dispatch to Lisbon by air. In Lisbon the sera were separated under aseptic conditions and were taken to the Yale Arbovirus Research Unit, New Haven, Conn., USA, where the author had the opportunity of studying them by means of the haemagglutination-inhibition test.

Antigens

The viruses used as antigens are listed in Table 1. The antigens were obtained from the large collection maintained in the laboratory of Dr J. Casals and were prepared by the techniques described by Clarke & Casals (1958).

LOCALITIES IN PORTUGUESE GUINEA IN WHICH BLOOD WAS COLLECTED



Haemagglutination-inhibition test

The techniques used were those described by Clarke & Casals (1958). Briefly, the procedure was as follows.

The sera were first treated with kaolin and goose cells for the removal of nonspecific inhibitors and agglutinins and were then serially diluted. Each serum was tested at serial dilutions (starting at 1 : 20) against the antigens of group A and group B, at two dilutions against antigens of the Bunyamwera, Bwamba, and California groups, and at one dilution against antigens of group C. Sera found to be positive in the higher dilutions were further titrated to determine the HI antibody end-point. All the sera were studied for the presence of antibodies to arboviruses of groups A and B; some of them were also checked for the presence of antibodies to the other antigens listed in Table 1.

The HI tests were performed in Linbro trays and the blood cells used were always goose erythrocytes. On the basis of previous experience, the antigens were diluted so as to contain 4-8 units of haemagglutinin per 0.2 ml and were then titrated (preliminary titration).

The diluted sera were distributed in 0.2-ml amounts per well and 0.2 ml of antigen (containing approximately 8 units of agglutinin as calculated by the preliminary titration) was added. Appropriate controls were included. The plates were stored overnight in the cold and the final titrations of the antigens were made the following day. The cell suspension, prepared so as to give the necessary pH and optical density, was then added to each well and the plates were incubated at 37°C or kept at room temperature, depending upon the reactivity of the antigens. Readings were made as soon as sedimentation of the erythrocyte suspension was complete. The HI titre was the highest dilution of the serum that caused complete inhibition of the 4–8 units of the antigen.

Interpretation. In interpreting the serological data obtained, it was borne in mind that there is frequent

TABLE 1
VIRUSES USED IN HI TEST

Group	Viruses	Laboratory references [a]
A	Chikungunya	(Ross late), SABrFD, C-536, 9-6-64
	Semliki	(Prototype), SABrFD, C-465, 26-9-63
	Sindbis	(Eg AR 339), SABrFD, C-612, 19-7-65
B	Zika	SABrFD, C-561, 8-9-64
	Yellow fever	(Asibi), AEBrFD, C-499 24-1-64
	West Nile	(Eg 101), SABrFD, C-456, 18-7-63
	Wesselsbron	(SA H177), SABrFD, C-356, 10-7-62
	SA H 336	SABrFD, C-266, 9-1-62
	Ntaya	SABrFD, C-603, 24-5-65
	Dengue 1	(Hawaii), SABrFD, C-552, 10-8-64
	Dengue 2	(NGB), SABrFD, C-568, 24-9-64
C	Marituba	(Be An 15), SerAEFD, C-610, 30-6-65
	Oriboca	(Be An 17), SerAEFD, C-301, 26-2-62
Bunyamwera	Bunyamwera	(Prototype), SABrFD, C-383, 4-10-62
	Ilesha	(Macnamara), SABrFD, C-394, 19-11-62
Bwamba	Bwamba	(Original), SABrFD, C-376, 25-9-62
California	Tahyna	(Bardos), SABrFD, C-577, 18-11-64

[a] SA = sucrose-acetone extraction; AE = acetone-ether extraction; Br = suckling mouse brain; Ser = suckling mouse serum; FD = frozen-dried.

TABLE 2
SUMMARY OF RESULTS FOR DIFFERENT ARBOVIRUS GROUPS

Virus group	Sera studied (total)	Negative (total)	Positive	
			Total	%
B	1 154	390	764	66
A	920	653	267	29
Bunyamwera	920	788	132	14
Bwamba	846	479	367	43
California	862	809	53	6
C	604	579	25	4
Total	1 154	220	934	81

immunological overlap between antigens of the same group. Specific diagnosis of group B infections was not easily established, owing to the frequency of such overlap. The criterion for specificity in such infections was a 4-fold or greater difference between the titre against a particular antigen and the titres against the other antigens of the same group. Sera that reacted with two or more group B antigens with a similar antibody titre were designated as " multiple-infection " sera. Sera reacting with one group B antigen with a titre of 1 : 20 were not considered " questionable ", but were included in the positives, since their number (except for the yellow fever antigen) was low.

RESULTS

The results of the survey indicate that several arboviruses were active throughout the territory studied. Table 2, which summarizes the data

TABLE 3
DISTRIBUTION OF ANTIBODIES IN SERA FROM COASTAL AND INTERIOR REGIONS

Virus group	Residents, coastal region				Residents, interior				Nonresidents			
	Observed	Neg.	Positive		Observed	Neg.	Positive		Observed	Neg.	Positive	
			Total	%			Total	%			Total	%
B	569	245	324	57	534	132	402	75	51	13	38	75
A	337	286	51	15	533	322	211	40	51	46	5	10
Bunyamwera	337	289	48	14	533	451	82	15	50	49	1	2
Bwamba	323	184	139	43	490	264	226	46	33	31	2	6
California	327	302	25	8	503	475	28	5	32	32	0	0
C	151	148	3	2	376	357	19	5	51	48	3	6
Total	569	152	417	73	534	56	478	90	51	12	39	76

obtained, shows that, of 1154 sera studied, 81% had antibodies (at different titres) to arboviruses. Antibodies to group B viruses were observed most frequently (66%). Viruses of group A and the Bwamba group were also found to be active in the surveyed territory.

Data on the regional distribution of antibodies in the population (Table 3) show that the activity of the arboviruses is probably more intense in the interior of the territory than in the coastal region; this is particularly true of the viruses in groups A and B.

Regarding group B, Table 4 shows that there was a high incidence of " multiple infections " and that the most active virus was that of yellow fever. Of the other group B viruses, Zika and Wesselsbron (or other viruses antigenically close to them) were active in the territory, Wesselsbron being more

TABLE 4
NUMBER OF SERA REACTING WITH GROUP B VIRUSES

Antigen	Total		Residents, coastal region		Residents, interior		Nonresidents	
	No.	%	No.	%	No.	%	No.	%
" Multiple infections "	263	23	88	15	169	32	6	12
Zika	122	11	47	8	74	14	1	2
Yellow fever	223	19	80	14	112	21	31	61
West Nile	10	1	5	1	5	1	0	0
Wesselsbron	125	11	98	17	27	5	0	0
SA H 336	11	1	1	0.2	10	2	0	0
Ntaya	9	1	5	1	4	0.8	0	0
Dengue 1	1	0.09	0	0	1	0.2	0	0
Dengue 2	0	0	0	0	0	0	0	0
Positive	764	66	342	57	402	75	38	75
Negative	390	34	245	43	132	25	13	25
Total	1 154	100	569	100	534	100	51	100

TABLE 5
NUMBER OF SERA REACTING WITH GROUP A VIRUSES

Antigen	Total		Residents, coastal region		Residents, interior		Nonresidents	
	No.	%	No.	%	No.	%	No.	%
" Multiple infections "	41	4	12	4	28	5	1	2
Chikungunya	200	22	33	10	165	31	2	4
Semliki	15	2	4	1	11	2	0	0
Sindbis	11	1	2	1	7	1	2	4
Positive	267	29	51	15	211	40	5	10
Negative	653	71	286	85	322	60	46	90
Total	920	100	337	100	533	100	51	100

frequent in the coastal region. West Nile and SA H 336 were much less active. Nine sera reacted only against Ntaya antigen, but this result is difficult to interpret. There was no serological evidence that the dengue 1 and dengue 2 viruses were active in the territory.

Table 5 summarizes the results obtained with group A antigens. The most active virus, particularly in the interior of the territory, was Chikungunya, or a virus closely related to it antigenically.

The percentage of persons with antibodies to the Bunyamwera virus was, throughout the area, higher than that of persons with antibodies to the Ilesha virus (Table 6).

The serological results obtained with the two antigens from group C (Table 7) suggest that Marituba and Oriboca or related group C viruses were present in the population.

A high percentage of sera reacted with Bwamba antigen (Table 8), showing that this virus (or a closely related virus of the same group) was highly active in the area. The presence of a virus of the California group was also demonstrated.

Table 9 shows the observed HI titres for antibodies to the three most prevalent viruses of group B (yellow fever, Zika, and Wesselsbron). The figures for the yellow fever virus clearly show its importance in relation to the other arboviruses that infect the population.

Tables 10-12 show the HI titres for antibodies to the other arboviruses for which tests were made. From the data in Tables 11 and 12 it is possible to

TABLE 6
NUMBER OF SERA REACTING WITH BUNYAMWERA GROUP VIRUSES

Antigen	Total		Residents, coastal region		Residents, interior		Nonresidents	
	No.	%	No.	%	No.	%	No.	%
" Multiple infections "	6	1	3	1	2	0.4	0	0
Bunyamwera	98	11	34	10	64	12	1	2
Ilesha	28	3	11	3	16	3	0	0
Positive	132	14	48	14	82	15	1	2
Negative	788	86	289	86	451	85	49	98
Total	920	100	337	100	533	100	50	100

TABLE 7
NUMBER OF SERA REACTING WITH GROUP C VIRUSES

Antigen	Total		Residents, coastal region		Residents, interior		Nonresidents	
	No.	%	No.	%	No.	%	No.	%
"Multiple infections"	0	0	0	0	0	0	0	0
Marituba	14	2	1	1	13	3	0	0
Oriboca	11	2	2	1	6	2	3	6
Positive	25	4	3	2	19	5	3	6
Negative	579	96	148	98	357	95	48	95
Total	604	100	151	100	376	100	51	100

TABLE 8
NUMBER OF SERA REACTING WITH VIRUSES OF THE BWAMBA AND CALIFORNIA GROUPS

Antigen	Total		Residents, coastal region		Residents, interior		Nonresidents	
	No.	%	No.	%	No.	%	No.	%
Bwamba:								
Positive	367	43	139	43	226	46	2	6
Negative	479	57	184	57	264	54	31	94
Total	846	100	323	100	490	100	33	100
Tahyna:								
Positive	53	6	25	8	28	5	0	0
Negative	809	94	302	92	475	95	32	100
Total	862	100	327	100	503	100	32	100

evaluate the activity of Bwamba and Bunyamwera viruses in the surveyed territory.

The results obtained in the study of the small group of nonresidents give evidence of recent activity on the part of several arboviruses. As previously noted, all the individuals in this group had been vaccinated against yellow fever and had been in the territory for less than 2 years. However, the results obtained from this group show that yellow fever virus was circulating and that, at the same time, other arboviruses were probably active also.

The activity of the viruses of groups C, Bunyamwera, Bwamba, and California was apparently uniform in both the coastal region and the interior. However, this was not true of the group A viruses, which were more active in the interior.

DISCUSSION

From the results of this survey it seems possible to conclude that arboviruses were actively circulating in Portuguese Guinea. Similar results were reported by Theiler (1961) in Nigeria, Ghana, and Liberia; by Brès et al. (1963) in the Republic of Senegal; and by Brès et al. (1965) in Upper Volta.

Of the group A viruses, Chikungunya virus seems to have been the most active, as was found in Senegal. In group B the yellow fever virus was the most active, this finding being corroborated by isolation of the virus from patients. Among the

TABLE 9
TITRES OBSERVED AGAINST GROUP B ARBOVIRUSES

Titre	Positive for yellow fever		Positive for Zika		Positive for Wesselsbron	
	No.	%	No.	%	No.	%
1 : 20	27	12	6	5	8	6
1 : 40	27	12	12	10	26	21
1 : 80	51	23	39	32	61	49
1 : 160	41	18	22	18	15	12
1 : 320	31	14	23	19	4	3
1 : 640	13	6	4	3	1	1
1 : 1 280	13	6	9	7	4	3
1 : 2 560	14	6	7	6	5	4
1 : 5 120	6	3	—	—	1	1
Total	223	100	122	100	125	100

other B viruses, Zika and Wesselsbron infections were most frequent, a finding that is similar to those of other surveys conducted in this region.

Bunyamwera infections have been detected in West Africa by Casals & Theiler (cited by Horsfall & Tamm, 1957) and by Brès et al. (1963, 1965), but the frequency reported by these investigators was higher than that observed in this survey. The

TABLE 10
TITRES OBSERVED AGAINST GROUP A ARBOVIRUSES

Titres	Positive for Chikungunya		Positive for Semliki		Positive for Sindbis	
	No.	%	No.	%	No.	%
1 : 20	10	5	5	34	3	27
1 : 40	39	20	2	13	3	27
1 : 80	44	22	2	13	1	10
1 : 160	47	24	1	7	2	18
1 : 320	43	21	2	13	2	18
1 : 640	10	5	2	13	—	—
1 : 1 280	6	3	1	7	—	—
1 : 2 560	1	—	—	—	—	—
Total	200	100	15	100	11	100

TABLE 11
TITRES OBSERVED AGAINST GROUP C ARBOVIRUSES AND BWAMBA VIRUS

Titre	Positive for Oriboca		Positive for Marituba		Positive for Bwamba	
	No.	%	No.	%	No.	%
1 : 20	8	73	14	100	94	26
1 : 40	3	27	—	—	107	29
1 : 80	—	—	—	—	106	29
1 : 160	—	—	—	—	42	12
1 : 320	—	—	—	—	12	3
1 : 640	—	—	—	—	4	1
1 : 1 280	—	—	—	—	1	—
Total	11	100	14	100	366	100

Bwamba group, probably represented by the Bwamba virus, seems to be very active in West Africa. Macnamara et al. (1959) reported that 50% of a group studied in Nigeria had antibodies to Bwamba virus; our survey in Portuguese Guinea showed that 43% of the 845 individuals studied had antibodies to this virus.

The finding of antibodies to the group C viruses

TABLE 12
TITRES OBSERVED AGAINST BUNYAMWERA GROUP VIRUSES AND TAHYNA VIRUS

Titre	Positive for Bunyamwera		Positive for Ilesha		Positive for Tahyna	
	No.	%	No.	%	No.	%
1 : 20	41	42	15	53	42	79
1 : 40	33	34	9	32	10	19
1 : 80	15	15	2	7	1	2
1 : 160	8	8	1	4	—	—
1 : 320	1	1	—	—	—	—
—						
—						
—						
1 : 2 560	—	—	1	4	—	—
Total	98	100	28	100	53	100

Marituba and Oriboca in this West African region is of interest. The group C viruses, originally isolated in Brazil (Causey et al., 1961), were first shown to be active in Angola by Kokernot et al. (1965), who found antibodies to the Oriboca virus in sera from residents.

The Tahyna virus or a related virus of the California group is also present in Guinea. Kokernot et al. (1962) found antibodies to the Lumbo virus in 12% of 128 residents of Natal; in our survey, 6% of 862 residents of Portuguese Guinea had antibodies to the Tahyna antigen used in the tests.

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RÉSUMÉ

Une enquête sérologique portant sur la répartition des anticorps suscités par divers arbovirus a été effectuée en Guinée portugaise en 1964 et 1965. Au total, 1103 sérums prélevés sur des autochtones âgés de 10 à 15 ans et 51 sérums recueillis chez des non-résidents permanents âgés de 20 à 25 ans ont été examinés par la réaction d'inhibition de l'hémagglutination. Dix-sept souches d'arbovirus ont été utilisées comme antigènes.

Sur ces 1154 sérums, 81% étaient positifs, avec des titres variables. Les anticorps pour les arbovirus du groupe B étaient les plus fréquents (66%), mais on constatait également une certaine activité des virus des groupes A et Bwamba. L'étude de la localisation géographique des résultats positifs montrait une circulation plus intense des arbovirus dans la région centrale du pays que dans la zone côtière.

En ce qui concerne les arbovirus du groupe B, on notait de nombreuses infections multiples, avec prédominance nette du virus amaril. Les virus Zika et Wesselsbron venaient ensuite en ordre de fréquence. Neuf sérums ont réagi avec l'antigène Ntaya, mais on n'a obtenu aucune preuve sérologique de l'influence des virus de la dengue des types 1 et 2. Dans le groupe A, le virus chikungunya, ou un virus antigéniquement très proche, était le plus actif. Avec les virus du groupe Bunyamwera, le taux de positivité était inférieur à celui signalé dans d'autres régions d'Afrique occidentale. Des anticorps pour les virus Marituba et Oriboca (groupe C) ont été identifiés dans 4% des sérums, cependant que 43% et 6% respectivement des échantillons examinés réagissaient en présence des antigènes Bwamba et Tahyna.

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