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SURVEY FOR ANTIBODIES AGAINST ARTHROPOD-BORNE VIRUSES IN THE SERA OF INDIGENOUS RESIDENTS OF ANGOLA*

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Introduction

As noted in the companion paper (KOKERNOT et al., 1965), which reports the results of a serological survey among residents of the Caprivi Strip and Bechuanaland Protectorate, this Unit has been studying the geographical distribution of arthropod-borne viruses in southern Africa since 1953. The present survey among indigenous residents of Angola was carried out in May-June, 1960.

Angola is situated on the west coast of Africa, and is bounded on the north and north-east by the Republic of the Congo, on the east by Northern Rhodesia (Zambia) and on the south by South-West Africa and the Caprivi Strip (Figure). The territory is roughly square in shape, with a small detached portion, known as the enclave of Cabinda, lying north of the mouth of the Congo River.

Angola has an estimated area of about 785,000 square miles and a coastline of over 1,300 miles. The littoral zone is narrow and from it the terrain rises sharply to a central plateau that extends over the rest of the province. The altitude of this vast expanse of country ranges from 2,000 to over 6,000 feet above sea level.

Climatic conditions within Angola vary considerably. Along the littoral zone there is a marked influence of the cold Benguela current, with resultant cool west

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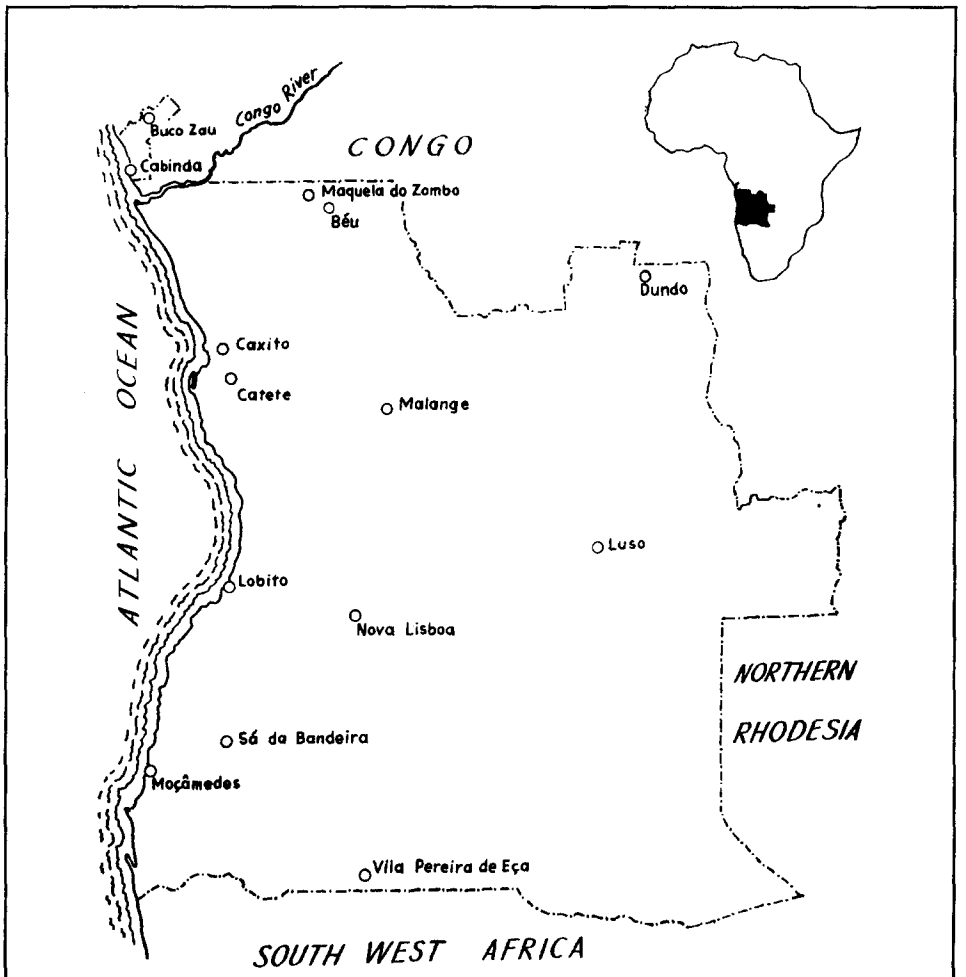

FIG. Map showing localities sampled in Angola

winds. The interior plains, almost as far north as latitude 10° S., are temperate, while in the northern part and the enclave of Cabinda the climate is tropical.

The rainfall pattern, as in most of southern Africa, is characterized by a dry period in the winter followed by varying periods of rainfall during the warmer season. In the south-west, under the influence of the Benguela current, there is rain for only 2 months of the year, while in the north the rainy season lasts almost 8 months. Although total annual rainfall in the littoral zone is low (3 to 13 inches), a great deal of surface water is present, especially in the north-west. Rivers with sources on the plateau often overflow on reaching the lowlands in this area, forming large lakes, swamps and fertile deltas. In the uplands of the interior rainfall varies from 35 to 65 inches a year.

The climatic differences are also reflected in the different floral zones. Cabinda is in a tropical rain forest, while the south-west coastal strip is desert contiguous with the Namib Desert in South-West Africa. The central plateau is characterized by parkland or savannah zones on sandy terrain.

Over 98% of Angola's 4,145,000 inhabitants belong to the large Bantu tribe. Their chief agricultural crops are manioca, maize and beans, while the Europeans have plantations of sugar cane, oil-nut palms, coffee, citrus and, in Cabinda, cacao. Cattle raising is carried on in some sections but in others nagana is a limiting factor.

Materials and methods

Details of the general procedure followed and of the methods used in selecting localities and donors, and in performing and interpreting haemagglutination-inhibition (HI) and neutralization (N) tests, have been given in the companion paper (KOKERNOT et al., 1965).

The 14 localities sampled are shown on the map in the Figure. For the purpose of this presentation, these have been divided into 3 general regions (Table I) which represent differences in physico-geographical and climatological features, but also overlap to a considerable extent.

TABLE I. Localities sampled in Angola, showing altitude, rainfall and number of specimens collected

Locality by region	Feet above sea level	Av. annual rainfall (inches)	Number specimens collected	
			Children	Adults
North-western coastal and plateau				
Buco Zau	1135	76·4	29	21
Cabinda	65	47·4	20	25
Maquela do Zombo	2975	34·8	20	20
Beu	2755	33·5	25	25
Caxito	325	9·8	15	17
Catete	485	9·2	20	15
South-western coastal and plateau				
Lobito	40	9·6	15	15
Sa da Bandeira	5720	37·9	14	16
Mocamedes	145	3·4	15	15
V. Pereira de Eca	3745	14·5	15	15
Eastern and central plateau				
Dundo	2500	64·8	19	11
Malange	3700	56·4	15	15
Luso	4315	43·2	15	15
Nova Lisboa	5525	50·4	15	15

The numbers of serum specimens collected from children and adults in each locality are given in Table I. To compensate for the relatively small number of localities surveyed, additional donors were bled in some instances. Sera were collected in sterile 20 ml. vacuum tubes and immediately placed in 1 gallon glass-lined Thermos flasks containing water ice that was carefully maintained. The flasks were dispatched to the Johannesburg laboratory by air.

Details concerning 19 of the 23 virus types used have been given in the companion paper. The 4 additional viruses employed in this survey were as follows:

1. Mayaro, TRVL 4675 strain (ANDERSON et al., 1957); haemagglutinating (HA) antigen prepared from 11th passage infant mouse brain by sucrose-acetone (SA) technique; N test done with 10th brain passage virus in infant mice inoculated intracerebrally (i.c.).
2. Zika, prototype strain isolated by DICK et al. (1952); HA antigen prepared from 149th passage infant mouse brain by acetone-ether extraction; N test done with 148th brain passage virus and adult mice inoculated i.c.
3. Ntaya, prototype strain isolated in 1943 by SMITHBURN and HADDOW (1951); HA antigen prepared from 20th passage infant mouse brain by SA; N test done with 20th passage material and adult mice inoculated i.c.
4. Lumbo, AR 1050 strain (KOKERNOT et al., 1962); N test done with 2nd, 4th and 42nd passage infant mouse brain, using adult mice inoculated i.c. This virus has been shown to belong to the California complex (WHITMAN and SHOPE, 1962). Irrespective of HI results, all 32 sera collected at Caxito were tested in N test with Wesselsbron, H 336, Spondweni, West Nile and, except for 3 sera from adults, with yellow fever and Zika viruses.

In the single series of yellow fever N tests, an equal volume (0·1 ml.) of normal monkey (*Macaca mulatta*) serum obtained 2-3 hours earlier was added immediately before the test.

Results

The results of initial screening of all sera against 18 antigens in the HI test, and of N tests with the sera that were HI positive, are summarized in Table II according to region and general age group of donor. The number of sera at the head of each column in the table represents the total tested in HI test.

It is apparent from these results that virus activity is most prevalent in the north-western coastal and plateau region. Even though a few more sera were collected from children here than in the other two regions, the proportion of sera showing antibodies was greater along the north-western coast. This level of prevalence in sera from both children and adults is no doubt closely associated with the tropical climate and low elevation characteristic of most of this region.

The over-all results with Group A viruses are brought into clearer focus in Table III, which lists the sera in each locality that were N-test positive for only one virus of the group. Noteworthy as a negative feature of this table is the fact that not one of the 46 sera protective against Mayaro virus protected against that virus alone. As the table shows, however, there is serological evidence that both chikungunya and Semliki Forest viruses have been present in a number of the localities sampled. In view of the close relationship by N test among these two agents and Mayaro (CASALS and WHITMAN, 1957; SPENCE and THOMAS, 1959), it seems probable that these Mayaro neutralizing antibodies are due to immunological overlap and that no infection with this virus has occurred in the population studied. In the north-western localities of Maquela do

TABLE II. Summary of positive HI and N test reactions among sera from children and adults residing in Angola*

Virus type by group	North-western coastal and plateau				South-western coastal and plateau				Eastern and central plateau			
	Children 129 sera		Adults 123 sera		Children 59 sera		Adults 61 sera		Children 64 sera		Adults 56 sera	
	HI	N	HI	N	HI	N	HI	N	HI	N	HI	N
Group A												
Sindbis	5	5	17	15	0	—	3	3	0	—	2	1
Semliki Forest	4	3	46	41	0	—	5	4	1	1	7	7
Chikungunya	6	4	49	40	0	—	6	5	0	—	9	8
Mayaro	4	4	40	33	0	—	5	4	0	—	6	5
Middelburg	3	1	34	5	0	—	0	—	0	—	3	0
Group B												
West Nile	40	2	53	10	0	—	6	4	1	0	2	0
H 336	44	4	74	25	1	1	7	0	0	—	1	0
Wesselsbron	42	11	73	35	2	1	13	3	2	1	4	3
Spondweni	44	4	80	10	1	0	15	5	4	0	6	1
Yellow fever	66	25	82	33	1	1	4	0	3	1	2	0
Zika	40	2	71	26	0	—	20	13	0	—	2	1
Ntaya	41	0	76	0	4	0	10	0	12	0	14	0
Dengue 1	2	0	23	0	0	—	4	0	0	—	0	—
Dengue 2	8	0	43	0	0	—	5	0	0	—	1	0
Group C												
Oriboca	3	0	17	1	1	1	2	0	2	0	5	0
Bunyamwera group												
Bunyamwera	10	10	42	34	1	1	5	5	0	—	6	5
Germiston	8	4	33	26	0	—	2	1	1	1	6	4
California complex												
Lumbo†	—	5	—	17	—	0	—	0	—	1	—	3
Ungrouped												
Rift Valley fever	3	1	15	9	1	0	4	2	4	0	5	1
Pongola†	—	10	—	33	—	0	—	1	—	1	—	8

* All sera listed under HI were positive in the 1 : 20 serum dilution or higher; each specimen was tested against all antigens. With a few exceptions, N tests were done only with HI positive sera.

† N tests with Lumbo virus were done with 121 sera from adults in the north-west and with 49 sera from children and 41 from adults in the eastern and central plateau. N tests with Pongola virus were done with 122 sera from adults in the north-west.

Zombo and Beu, plural protection against the 3 viruses was prevalent among adult donors, and in each locality 2 sera from children also neutralized all 3 agents. Quantitative antibody determinations on these sera showed median titres of 1 : 120 for Semliki Forest, 1 : 220 for Mayaro and 1 : 600 for chikungunya.

The 6 sera N-test positive for Middelburg virus were likewise positive for at least one other member of Group A, and here again it is concluded that the reactions are the result of immunological overlap.

TABLE III. Distribution by locality of sera N-test positive for only one member of virus Group A, B or Bunyamwera*

Locality	Group A			Group B						Bunyamwera Gp.	
	Sindbis	Semliki	Chikungunya	West Nile	H 336	W'bron	Spondweni	Yellow fever	Zika	Bunyamwera	Germiston
North-western coastal and plateau											
Buco Zau	5	2	—	—	1	—	—	15	—	1	—
Cabinda	6	1	—	1	—	3	—	7	—	6	2
Maquela do Zombo	—	—	—	—	—	—	1	16	3	2	—
Beu	—	—	2	—	—	—	—	2	9	2	—
Caxito	1	1	2	—	3	8	—	—	—	3	4
Catete	—	—	1	4	5	1	—	—	—	8	2
South-western coastal and plateau											
Lobito	2	—	—	1	—	—	—	1	4	1	—
Sa da Bandeira	—	—	—	—	1	1	—	—	—	—	1
Mocamedes	—	—	—	—	—	—	—	—	3	—	—
V. Pereira de Eca	—	—	—	—	—	—	—	—	—	5	—
Eastern and central plateau											
Dundo	—	1	2	—	—	1	—	—	—	—	1
Malange	1	—	1	—	—	1	1	—	1	1	—
Luso	—	2	—	—	—	1	—	1	—	—	—
Nova Lisboa	—	—	—	—	—	1	—	—	—	—	—
Ratio†	15/24	7/56	8/57	6/16	10/30	17/54	2/21	42/60	20/42	29/55	10/36
Percentage	62	12	14	37	33	32	9	70	48	52	28

* Ntaya and dengue 1 and 2 viruses not included because no serum was N-test positive. Mayaro and Middelburg viruses not included because no serum was N-test positive for only one of these agents.

† Numerator = number of sera N-test positive for one virus only, denominator = number of sera positive for that virus and one or more others of the same group.

Sera protective only against Sindbis virus were found in 5 localities. As this agent is relatively distinct immunologically from the other Group A viruses reported from Africa, presumably it has been present in each of these localities.

Interpretation of the over-all results with Group B viruses is complicated in certain north-western localities by the fact that some of the donors had been vaccinated against yellow fever in 1959. Although every effort was made to exclude such individuals, this failed completely at Maquela do Zombo and was only partly successful at Buco Zau and Cabinda. Thus, while Table III shows that 38 sera from these localities were protective against yellow fever virus alone, these reactions are not necessarily significant.

Of the 16 sera with neutralizing antibodies for West Nile virus, 6 neutralized that virus only (Table III). 4 of these were from residents of Catete, 2 of them aged 6 and 9 years. This locality, with dense avian populations, ground marshes and lakes, appears to fit in with the known ecology of this virus in the Nile Delta of Egypt (TAYLOR et al., 1955).

54 sera were N-test positive for Wesselsbron virus (Table II), including 12 from Cabinda, all of which were negative for yellow fever, and 19 from Caxito (13 adults and 6 children). Since 8 of these Caxito sera protected only against Wesselsbron (Table III), it is considered probable that this agent or one closely related is endemic in the locality.

In N tests with H 336 virus, sera from 11 adults and 1 child at Caxito were protective. Only 3 of these (2 adults, 1 child) protected against this virus alone (Table III), but since no relationship has been demonstrated by N test between H 336 and Wesselsbron viruses, it is likely that some of the adults at Caxito had been infected with both agents.

As shown in Tables II and III, there is evidence of Zika virus activity in all 3 regions and suggestive evidence of the presence of Spondweni virus. Although the HI results with Ntaya indicate that the antigen used was unusually sensitive, none of the HI positive sera reacted with this virus in N test. N test results with the two types of dengue virus were likewise negative.

30 sera were HI positive for Oriboca, the only Group C virus used in this study (Table II). Taken in conjunction with similar findings in the survey of the Caprivi Strip and Bechuanaland (KOKERNOT et al., 1965), these results suggest that Oriboca or a closely related member of Group C is present in southern Africa.

Sera positive for Bunyamwera and Germiston viruses in the Bunyamwera Group were distributed in all 3 regions, but the greatest prevalence occurred in the north-western region (Table II). As shown in Table III, 29 sera protected against Bunyamwera alone and 10 against Germiston alone. The fact that sera from children at Caxito and Catete were positive for each virus may indicate that both agents are endemic in these localities.

Of the 13 sera positive in N test for Rift Valley fever virus (Table II), 10 were from donors in the north-western region, the youngest a 10-year-old child from Cabinda. N test results with Lumbo and Pongola viruses showed a similar pattern, with one or more adults in each of the 6 north-western localities having antibodies against one or the other agent.

A limited number of sera, some of them selected on the basis of positive results with Pongola virus, were also tested in N test against Bwamba fever virus. Protective ratios were 39/60 in the north-western region, 0/2 in the south-western region, and 12/15 in the eastern and central region. N tests with Witwatersrand virus (315 sera tested) and Simbu virus (180 sera tested) gave negative results.

Summary

Sera collected in 1960 from 492 indigenous residents of 14 widely scattered localities in Angola were tested for HI antibodies against 18 viruses: 5 from Group A, 9 from Group B, 1 from Group C, 2 from the Bunyamwera Group, and Rift Valley fever virus.

Results of these tests indicated that the prevalence of antibodies was greatest in the 6 localities sampled in the north-western coastal and plateau region.

Sera positive in the HI test were further tested with the respective viruses by mouse neutralization test. Analysis of these results according to sera positive for only one virus of Group A, B or Bunyamwera indicated that Sindbis, chikungunya, Semliki Forest, Wesselsbron, Zika, H 336, West Nile, Bunyamwera and Germiston viruses have been active, predominantly in the north-western region, and that Spondweni virus has been present. There was no evidence that Mayaro or Middelburg virus has infected the population sampled, or that Ntaya, dengue 1 or dengue 2 virus has been present. In 3 localities in the north-west interpretation of results with Group B viruses was complicated by the fact that some of the donors had been vaccinated against yellow fever.

HI test results with Oriboca virus suggest that this or a closely related member of Group C exists in Angola.

In additional neutralization tests, one or more sera from children and adults in each of the 6 north-western localities protected against Lumbo (California complex), Pongola or Bwamba fever virus.

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