NEUTRALIZING ANTIBODIES FOR CERTAIN VIRUSES IN THE SERA OF HUMAN BEINGS RESIDING IN NORTHERN NATAL*

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In previous surveys of immunity to arthropod-borne viruses in the Union of South Africa[1] the highest incidence of protective antibodies was found among residents of the Simbu Pan area in northern Natal. Intensified studies in this region were subsequently made during an expedition in which various approaches to the virus problem were undertaken.[2] During this expedition several viruses were isolated, among them 3 which proved to be hitherto unknown, and sera were collected from human beings and domestic animals for survey purposes. Subsequently, the surveys were extended southward to link up with others previously carried out in the Eshowe-Stanger-Durban region. The results of mouse-protection tests on the latter specimens and on those taken from human beings in various localities of the Natal coastal area are the subject of the present report.

Materials and Methods

Sera. The selection of donors was made in the manner previously described[1] in order to obtain persons who had not travelled and were therefore representative of the areas of residence. Approximately 15 children (0 to 14 years of age) and 15 adults (15 years and over) were sampled in each locality. All blood specimens were taken in sterile vacuum tubes, the collection, labelling and documentation being done by one or other of the authors. As soon as the blood was clotted, the specimens were placed in iced vacuum flasks and kept chilled continuously until they reached Johannesburg, where the sera were separated and stored in the frozen state.

Viruses. The Semliki Forest and West Nile viruses used in these studies were the same strains and at approximately the same passage levels as were employed in our previous South African studies.[1] A number of other viruses recently isolated in South Africa were used to initiate studies of their range of infectivity in the Union. These included strains of Sindbis,[3] Bunyamwera and Rift Valley fever,[4] Pongola,[5] Simbu,[6] Spondweni,[7] Wesselsbron,[8] Middelburg,[9] a virus of Casals' group B isolated from a sick youth and provisionally designated H 336, and another agent—probably hitherto unknown—obtained from mosquitoes and provisionally designated AR 136. All the latter strains were isolated by this team in Tongaland. Tests were also made with a strain isolated by Gear,[10] and identified by one of us as Chikungunya virus. Frozen or lyophilized stocks of the lowest available passages of these agents were employed in the tests.

Technique and Interpretations

Table I lists the viruses used in the studies and indicates the route of inoculation and age of mice employed. When

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TABLE I. STRAINS AND LINES OF VIRUSES, AGE OF MICE AND ROUTES OF INOCULATION

Virus, type	*Strain*	*Passage*	*Procedure in test*		*Month and year of Tongaland isolations*
			Route of inoc.	*Age of mice*	
Middelburg	AR 749	6	I-P	0-4 days	May, June '57.
Sindbis	AR 86	60	I-C	adult	
Sindbis	AR 166	6	I-C	0-3 days	
Chikungunya	Original (*a*)	169	I-C	adult	
Chikungunya	Ver. (*b*)	6	I-C	0-3 days	
Semliki	Original (*c*)	8	I-C	adult	
RVF	Neuro. (*c*)	115	I-P	0-2 days	
RVF	AR 118	6, 7	I-P	0-5 days	May '55.
Simbu	AR 53	4, 20	I-C	adult	Apr. '55; Nov. '57.
AR 136	AR 136	29	I-C	0-3 days	Jan. '56 (*d*).
AR 136	AR 344	4	I-C	0-3 days	Mar. '56 (*d*).
Pongola	AR 1	3, 6, 10	I-C	adult	Apr., May '55; Nov. '57, May-June '58.
Bunyamwera	Original (*c*)	44	I-C	adult	
Bunyamwera	AR 11	8-11	I-C	adult	Apr. '55; May, June '57.
Spondweni	AR 94	4, 8, 11	I-C	adult	May '55, Mar.-May '58.
Wesselsbron	H 177	11-13	I-C	adult	Apr., May '55; Nov. '57.
H 336	H 336	7, 9	I-C	adult	Mar. '56 (*d*).
West Nile	B 956 (*c*)	34	I-C	adult	Apr., May '58 (*d*).

(*a*) Supplied by Dr. A. J. Haddow, Director, Virus Research Institute, Entebbe.
(*b*) Isolated by Dr. J. H. S. Gear in South Africa.[10]
(*c*) Strains isolated in Uganda.
(*d*) Isolations in South Africa to be reported.

different lines or different strains of the same virus were employed in the tests, the choice was usually determined by the availability of appropriate mice and with prior knowledge that results with the different lines would be comparable.

All serum-virus mixtures were incubated for 1 hour in a 37° bath before inoculation. Techniques were otherwise the same as we have previously employed,[1] and interpretations likewise. Table II shows the range of dosage in the 140 protection tests. It should be emphasized that the tests were planned to employ a challenge of 100 LD_{50} of virus. When the dose proved to be less than 50 LD_{50} all sera except those which were clearly non-protective were re-examined.

TABLE II. DATA CONCERNING DOSAGE OF VIRUS IN THE TESTS

Virus	No. tests	Dose, LD_{50}			Tests with less than 50 LD_{50} (a)
		Mean	Maximum	Minimum	
Middelburg	6	265	500	100	0
Sindbis	4	321	400	250	0
Chikunygunya	11	156	500	13	2
Semliki	11	227	720	3	2
Rift Valley fever	7	500	1,480	24	1
Simbu	10	439	3,162	11	3
AR 136	7	106	250	32	2
Pongola	16	151	316	40	1
Bunyamwera	16	235	740	16	3
Spondweni	11	359	871	28	3
Wesselsbron	21	94	355	3	9
H 336	14	466	890	10	3
West Nile	6	258	690	28	1

(a) Only negative results were accepted from such tests. Sera giving other results were retested against 50 or more LD_{50}.

Results were accepted as positive only in cases in which the actual dose, as determined by internal controls within the tests, was 50 LD_{50} or greater. This was done because it was thought more important to be certain that sera recorded as positive actually contained antibody, than it was to detect the precise percentage of persons who were immune in any particular group. The resulting severity of the tests may have resulted in some sera containing antibody being classified as negative.

RESULTS

The map in Fig. 1 shows the area covered by the survey and the localities which were sampled in it. Ingwavuma, Gwaliweni, Ubombo and Tshaneni are located on the Lebombo mountain range at an altitude of about 2,000 feet. All the other localities are in the coastal lowland area from sea level to about 500 feet.

In Tables III and IV the localities are arranged according to altitude and relation to permanent bodies of water and grouped into mountain, riverine, and coastal plain zones. Within each zone the localities are arranged from north to south; in these tables some of the data pertaining to 4 localities—Mtubatuba, Eshowe, Stanger and Durban—are taken from a previous report[1] and are repeated here for comparison.

Middelburg virus. Tests were made on sera from residents of 9 localities and only one protective serum, from an adult, was found (Table III). It may be significant that the 2 isolations of this virus from mosquitoes caught in our field study area in Tongaland (Table I) were made after the sera used in the survey were collected. Studies will be made to determine whether human beings in this region have more recently been infected with this virus.

Sindbis virus. Specimens from residents of 8 localities were tested against this agent. Sera of one child from Gumede's Kraal, one adult from the N.R.C. Camp area, 2 from Mkunduzi and one from Nibela were protective, but all the others were non-protective (Table III). Since the localities selected for testing include several which are among the most favourable for other viruses, the results seem to indicate that this agent does not occur commonly in Tongaland.

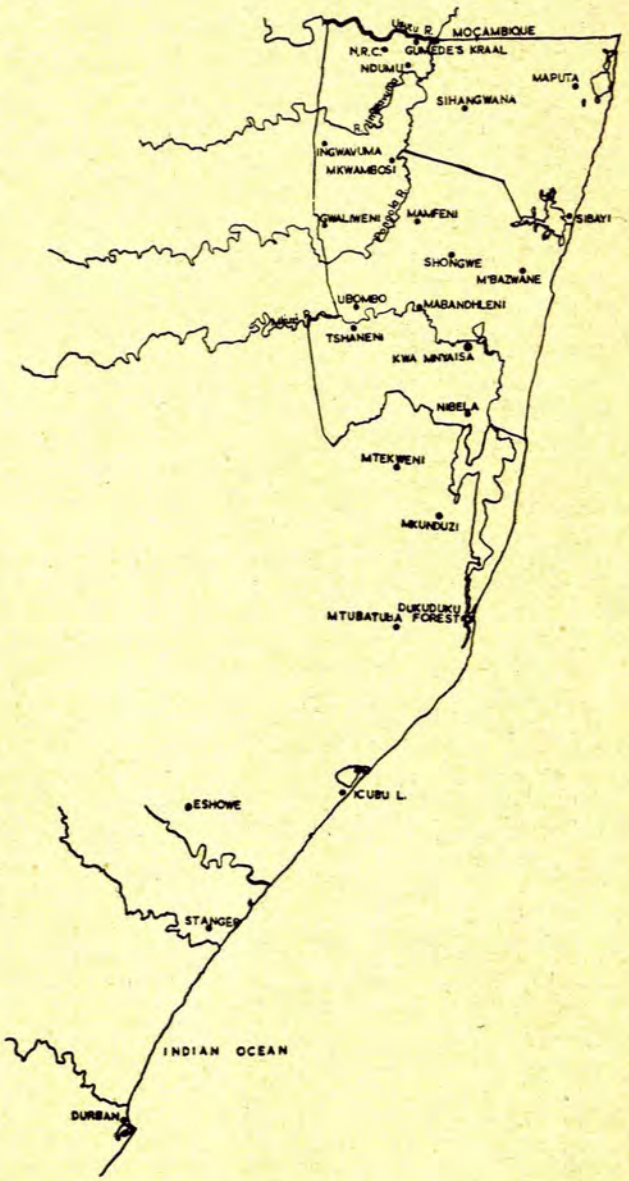

Fig. 1. Map of area of Natal coast covered by the survey.

Chikungunya virus. Sera from residents of 17 localities (Table III) were tested against this agent. At Gumede's Kraal 8 of 22 adults had protective sera, but all the tested children from this locality were non-immune. Immune adults were found also in 11 other localities, but immune children only at Maputa. The results seem to indicate that there probably was an outbreak of infection with Chikungunya virus in the lowland area some years ago, but they also seem

to show that infection with this agent has not been common in the tested localities in recent years.

Most of the sera tested against Chikungunya virus were collected before the isolation of the agent in the Union by Gear and Reid.[10] The positive results obtained with these indicate that the virus was present in the Union at least a year before it was first isolated, and point to the probability that the outbreak in the north-eastern Transvaal did not represent a new introduction of the virus to the Union.

Semliki Forest virus. Sera from 32 residents of Ubombo and 12 from Tshaneni in the highland region of Tongaland were non-protective. In the Tonga lowlands 20 sera out of 306 from adults were protective. None of 284 sera from children in the same area was protective. The results seem to indicate that Semliki Forest virus has not commonly been active in Tongaland in recent years, but may have been 15 or more years ago.

Rift Valley fever virus. Sera from persons residing at Gumede's Kraal, N.R.C. Camp area, Ndumu, Mkwambosi, Mamfeni, Mkunduzi, Maputa and M'Bazwane have been tested, as well as 11 adult residents of the Icubu Lake area (Table III). At Gumede's Kraal 3 of 25 children and 6 of 22 adults had protective sera. Immunes were also found among the adult population in all other localities tested, except Icubu Lake. Thus the results of tests on 138 sera from children and 145 from adults seem to indicate that infection with Rift Valley fever virus is fairly prevalent among human beings in the Tonga lowlands and possibly endemic in the Gumede's Kraal, N.R.C. Camp and Ndumu areas.

Simbu virus. Sera from residents of 3 highland localities in Tongaland were tested against this agent and only one, from an adult resident of Ubombo, possessed neutralizing antibodies. Sera from 216 children and 223 adult residents of 14 lowland localities yielded only one which was protective (Table III). Specimens from residents of Gumede's Kraal and the N.R.C. Camp area were taken 9 months after the virus was first isolated, and those from Kwa Mnyaisa, Nibela, Mkunduzi and Dukuduku Forest were taken 18 months after the first isolation of the agent. It is obvious that this virus had not commonly attacked human beings in either the lowland or highland areas in northern Natal at the time these specimens were collected.

AR 136 virus. Results of tests on sera from residents of 8 lowland localities against this virus showed none of them to be protective. The blood collections at 5 localities were made before the virus was isolated, but those from residents of Nibela, Mtekweni and Dukuduku Forest were taken 9 months after the virus was isolated in the N.R.C. Camp area. Whatever its relation may be to infection in man, it appears that the AR 136 agent has not recently attacked human beings in the area surveyed.

Pongola virus. From the results shown in Table IV it will be seen that only one of 44 children and 2 of 52 adults residing in the highland areas of Tongaland possessed neutralizing antibodies against this agent. It may also be seen that in the lowland areas of Tongaland infection with this agent is apparently very prevalent indeed. In the zone of rivers and pans 50 of 154 sera from children and 72 of 142 from adults neutralized this virus. In the coastal-plain area 11 of 101 sera from children and 35 of 141 from adults were protective. It is thus obvious that the infection rate is high throughout the lowland area, but highest in the localities which are intimately related geographically to the rivers and pans system of the region.

No immunes were found among either children or adults in the Icubu Lake area and it seems likely that the southern coastal region of the sampled area of Natal is not very favourable for this virus. A number of localities, especially in the area of rivers and pans, yielded results which indicate either recent widespread epidemic activity with this virus, or else endemic infection. The great prevalence of the agent in the Simbu Pan area in 1955, as manifested by the fact that 10 strains of Pongola virus were isolated from *Aedes circumluteolus*, is evidence enough that there has been a recent period of high prevalence of infection. It seems likely that there are endemic foci as well.

TABLE III. RATIOS OF SERA PROTECTIVE AGAINST GROUP A AND OTHER VIRUSES

Zone and locality	Month and year collected	Middelburg		Sindbis (a)		Chikungunya (a)		Semliki (a)		RVF		Simbu		AR 136 (b)	
		Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.
Mountain															
Ingwavuma	5/55					0/11	0/22					0/11	0/22		
Gwaliweni	1/57					0/17	0/17								
Ubombo	4/55							0/17	0/15			0/17	0/15		
Tshaneni	4/55							0/ 5	0/ 7			0/16	0/15		
Riverine															
Gumede's	1/56	0/23	1/18	1/ 7	0/13	0/27	8/22	0/27	2/22	3/25	6/22	0/24	0/20	0/15	0/19
N.R.C. Camp	1/56	0/22	0/15	0/24	1/16			0/24	1/16	1/24	4/16	0/22	0/16	0/22	0/15
Ndumu	4/55					0/17	0/13	0/17	2/14	2/17	3/14	0/17	0/13		
Mkwambosi	4/55	0/14	0/13	0/15	0/16	0/15	1/14	0/15	0/16	0/16	3/16	0/15	0/16	0/11	0/13
Mamfeni	4/55					0/15	1/16	0/15	1/16	0/15	1/16	0/15	0/15		
Mabandhleni	4/55	0/ 7	0/15			0/ 2	0/ 5	0/ 8	2/16			0/ 8	0/16	0/ 7	0/15
Kwa Mnyaisa	10/56							0/13	1/10			0/13	0/10		
Nibela	10/56			0/19	1/17	0/19	1/17	0/19	6/17			0/19	0/17	0/19	0/17
Mkunduzi	10/56	0/ 3	0/14	0/15	2/14	0/15	2/15		1/15	1/15	2/15	0/15	0/15		
Coastal Plain															
Maputa	4/55	0/12	0/18			2/12	1/19	0/12	0/19	0/12	1/19	0/12	0/19	0/12	0/19
Sihangwana	5/55					0/12	2/19	0/12	1/19			1/12	0/19		
Sibayi	10/56					0/11	2/18								
Shongwe	5/55					0/16	1/15	0/16	1/15			0/16	0/15		
M'Bazwane	5/55	0/14	0/16			0/14	3/16	0/14	0/16	1/14	1/16	0/14	0/16		
Mtekweni	11/56	0/ 9	0/21			0/ 9	5/21							0/ 9	0/21
Dukuduku For. ..	10/56	0/14	0/16			0/14	3/16	0/14	1/16			0/14	0/16	0/14	0/16
Mtubatuba	10/54			0/15	0/16			0/15	0/16						
Icubu L.	10/56						0/17				0/11				
Eshowe	10/54			0/ 9	0/11			0/16	0/16						
Stanger	10/54							0/15	0/17						
Durban	10/54			0/24	0/21			0/32	1/30						

Ch. = persons 0-14 years of age; Ad. = 15 years of age and over.
(a) These 3 viruses have not been isolated in Tongaland.
(b) AR 136 is the provisional name of the prototype strain; some tests were done with AR 344 strain, which is identical.

TABLE IV. RATIOS OF SERA PROTECTIVE AGAINST GROUP B, PONGOLA AND BUNYAMWERA VIRUSES

Zone and locality	Month and year collected	Pongola		Bunyamwera		Spondweni		Wesselsbron		H 336		West Nile	
		Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.	Ch.	Ad.
Mountain													
Ingwavuma ..	5/55	0/11	1/22	0/11	2/22	0/11	0/22	0/11	1/22	0/11	0/22	0/11	0/22
Gwaliweni ..	1/57	0/17	0/17	1/17	0/17			1/17	1/17	0/17	2/17	0/17	0/17
Ubombo ..	4/55	1/17	1/15	0/17	1/15	0/17	0/15	0/17	1/15				
Tshaneni ..	4/55	0/16	0/15	0/16	1/15	0/16	0/15	1/16	0/15				
Riverine													
Gumede's ..	1/56	10/27	14/22	7/27	5/22	0/26	1/21	6/27	14/22	6/27	10/22	2/27	0/21
N.R.C. Camp	1/56	6/24	15/16	0/24	7/16		0/16	2/24	7/16	0/24	4/16	1/23	0/15
Ndumu ..	4/55	11/17	9/14	4/17	9/14	2/17	1/14	5/17	8/14	0/17	0/13		0/13
Mkwambosi ..	4/55	12/16	11/16	8/16	7/16	0/16	1/16	5/16	9/16	1/15	0/16	0/15	1/16
Mamfeni ..	4/55	5/15	4/16	0/15	7/16	0/15	0/16	0/15	10/16	0/15	0/16	0/15	0/16
Mabandhleni ..	4/55	3/ 8	6/16	2/ 8	12/16	0/ 8	4/16	1/ 8	13/16		3/15		0/15
Kwa Mnyaisa	10/56	3/13	4/10	8/13	7/10	0/13	1/10	4/13	3/10	7/13	5/10	0/13	0/10
Nibela ..	10/56	0/19	5/17	5/19	13/17	0/19	5/17	0/19	7/17	4/19	7/17		0/17
Mkunduzi ..	10/56	0/15	4/15	2/15	11/15	1/15	8/15	3/15	9/15	1/15	7/15		0/15
Coastal Plain													
Maputa ..	4/55	2/12	8/19	3/12	3/19	0/12	0/19	1/12	9/19	1/12	5/19		
Sihangwana ..	5/55	4/12	10/19	1/12	4/19	0/ 6	1/12	5/12	5/19	2/12	1/19		
Sibayi	10/56	1/11	3/18	1/11	7/18			0/11	2/18	0/11	2/18	0/11	0/18
Shongwe ..	5/55	1/16	3/15	2/16	2/15			0/16	4/15	0/16	3/15		
M'Bazwane ..	5/55	1/14	4/16	0/14	1/16			0/14	4/16	0/14	2/16		
Mtekweni ..	11/56	0/ 9	3/21	0/ 9	5/21	0/ 9	2/21	1/ 9	9/21	0/ 9	3/21	0/ 9	0/21
Dukuduku For.	10/56	2/14	4/16	6/14	10/16	0/14	6/16	2/14	6/16	9/14	13/16	0/14	0/16
Mtubatuba ..	10/54			0/15	7/16 (*a*)							0/15	0/16
Icubu L. ..	10/56	0/13	0/17	1/13	3/17		1/17	0/13	4/17	2/13	5/17		
Eshowe ..	10/54			0/16	0/16			1/16	1/16	0/16	0/16	0/16	0/16
Stanger ..	10/54			2/15	2/17			0/15	5/17			0/15	0/17
Durban ..	10/54			1/32	3/30			0/32	0/26			0/32	0/30

Ch. = persons 0-14 years of age: Ad. = 15 years of age and over.
(*a*) One inconclusive in published paper[1] reported not protective was later re-tested and found clearly positive.

Bunyamwera virus. One of 61 children and 4 of 69 adults residing in the mountain zone of Tongaland possessed neutralizing antibodies against this virus. Despite the careful selection of donors it is quite possible that the adults had acquired infection with this agent in the nearby lowland areas. At any rate, if infection occurs at all in the highland localities, it is certainly much less prevalent than in the lowland regions.

In the area of rivers and pans, sera from 36 of 154 children and 78 of 142 adults contained neutralizing antibodies (Table IV). In the coastal-plain area, sera from 17 of 179 children and 47 of 220 adult donors were protective. It is thus evident that infection rates in the region of rivers and pans are considerably and significantly higher than in the coastal-plain region. Nevertheless, the agent apparently occurs throughout the latter area and as far south as Durban. In the vicinity of Mkunduzi, Kwa Mnyaisa, Nibela and Dukuduku Forest, for example, the prevalence of infection is great. The results seem to indicate that in some localities at least there is probably endemic infection with this agent.

Most of the present knowledge concerning the distribution of Bunyamwera virus throughout the world is derived from anti-viral immunity surveys. These indicate that the agent exists, not only in East Africa,[11] where it was originally found,[12] but also in South Africa (Table IV) and probably in North Borneo.[13] Proof that this virus is a human pathogen in the coastal region of Natal has been obtained recently by isolating the agent from a Native child suffering from a febrile illness and demonstrating a specific antibody rise in the serum of this individual during his convalescence.[14] In view of the evident prevalence of this agent in northern Natal and of the fact that experimental inoculation with it[15] has shown that it can induce severe infection in man, it should seem justifiable to direct attention toward it as a probably important human pathogen in the coastal region of south-east Africa.

Spondweni virus. Sera of 44 children and 52 adults residing in highland localities were all non-protective against this agent (Table IV). Among lowland residents, significant numbers of immunes were found at Ndumu, Mabandhleni, Nibela, Mkunduzi and Dukuduku Forest areas. Several localities, including Gumede's Kraal, Mkwambosi and Maputa, in which immunity to other viruses was quite prevalent, yielded either no immunes at all or very few. The spotty distribution of humoral immunity to this agent is of considerable interest, and the reason for it is not as yet known. It may be associated either with the distribution of some non-human host or with whatever arthropod is the vector for the agent among human beings. Furthermore, the agent may have become active in this area only recently. That the virus can cause clinical illness in man is known from the fact that 2 infections with it have occurred among our staff, probably as the result of exposure in the laboratory (unpublished data).

Wesselsbron virus. In the highland area of Tongaland, sera from 2 of 61 children and 3 of 69 adults were protective. This is of some interest as it appears[16] that this agent is active among domestic animals of the Tonga highlands, as well as in man. Nevertheless, it will be seen that infection with the agent is far more prevalent in the lowland regions. The results in Table IV show that sera from 26 of 154 children and 80 of 142 adults residing in the area of rivers and pans in the lowlands were protective against Wesselsbron virus. In the coastal-plain area 10 of 164 children and 49 of 200 adults also exhibited neutralizing antibodies. From this it appears that the agent very commonly attacks human beings in the whole lowland area in northern Natal, and that it is much more prevalent in the region of the rivers and pans than in the drier coastal-plain area.

In certain lowland localities the results obtained with this agent are of special interest. At Gumede's Kraal, 6 of 27 children and 14 of 22 adults yielded protective sera. The N.R.C. Camp area, which is only about 5 miles distant from Gumede's Kraal, yielded only 2 of 24 children and 7 of 16 adults with sera which were protective. This point is of

special interest since significant seasonal differences have been found in the mosquito faunae of these two particular localities.[17] During the winter months of 1956, at which time the total numbers of *Aedes circumluteolus* (probably the principal vector of this virus in Tongaland) were very low in most other localities, including the N.R.C. Camp area, it was found that goodly numbers of this species could be caught in the environs of Gumede's Kraal. The latter area is one of tall-grass savannah in which there are a number of large fig trees and some small banana plantations; the area lies between the Usutu River and Banzi Pan and in the season of heavy rains is often completely cut off from surrounding land areas by overflow of water from the Usutu through Banzi Pan and back to the Pongola or Usutu Rivers. The N.R.C. Camp area is also well watered, there being small pans nearby, but it is a region in which there is dense thornbush with no tall grass and few fig trees. It is believed that the environmental conditions of Gumede's Kraal area permit the winter breeding of *Aedes circumluteolus*, and that this makes possible the existence here of an endemic and/or enzootic state with probable periodic peripheral spreads of infection into adjacent zones at times when the propagation of vector mosquitoes in these adjacent zones leads to suitable population levels.

Other lowland localities in Tongaland yielded results which are of special interest with reference to how recently infection has occurred in these areas. At Mamfeni and Nibela, for instance, significant numbers of adults were found to have protective sera, but the children were all non-immune. These results are interpreted as indicating that Wesselsbron virus has occurred at these localities, but not within the last 15 years. Several localities in the coastal plain region gave similar results (Table IV). From the results shown in this table it appears that this agent has not been present in the Durban area within the lifetime of persons now living there. In localities south of Tongaland and intermediate between Tongaland and Durban the total number of immunes is considerably less than in Tongaland proper, and it would seem that this southern portion of the survey area does not present an environment especially favourable for Wesselsbron virus infections in man.

H 336 *virus*. This agent was isolated from the blood of a sick Native child in the Tongaland area in 1956. It is known to be related to, but probably distinct from, Uganda S virus.

Antibodies against this virus were extremely prevalent in the sera of residents of Gumede's Kraal area, Kwa Mnyaisa, Nibela and the Dukuduku Forest (Table IV). No immunes were found among the residents of Eshowe and it seems likely that infections in man with this agent do not occur there.

As with Wesselsbron virus, the differences between Gumede's Kraal and N.R.C. Camp area are noteworthy in the tests with H 336 virus. In the latter region only 4 out of 16 sera of adults were protective and none of 24 children possessed neutralizing antibodies. In the Gumede's Kraal area, however, the total incidence was 16 out of 49 donors, and nearly one-quarter of the children had protective antibodies (Table IV). These differences are probably related to the factors mentioned above.

West Nile virus. Against this virus 544 specimens were tested from residents of 2 highland, 9 riverine and 7 coastal plain localities. Only 4 were protective. This result indicates that West Nile virus has not been prevalent in this coastal zone in recent years. However, it is worth noting that the virus has been isolated from a sick human being in this region in 1958[18] and that evidence of its activity elsewhere in the Union was already at hand.[19] Its presence in Tongaland in 1958 may have been due to a recent introduction of the agent from some other region.

SPECIFICITY OF THE TESTS

A study of this sort, in which large numbers of specimens are tested against a spectrum of viruses, affords opportunity for critical scrutiny to determine the degree of specificity of the results. Of the viruses used in the current study, 4 belong to Casals' group A, 4 belong to group B, and 5 others are unrelated either to group A, group B, or to each other, so far as is known. Results from Nibela have been selected for specificity analysis because this locality afforded relatively more group A immunes than any other. In Table V the results of all the tests on individual specimens from this locality are shown and the group relationships of the viruses are also set forth. The reference keys in the right-hand column of the table and the appended footnotes set forth certain facts pertaining to the results on the basis of which the following comments seem justified.

Evidence of protection against specific viruses without intra-group cross-reaction indicates that 7 different agents have attacked Nibela residents, including Sindbis, Semliki, Spondweni, Wesselsbron, H 336, Pongola and Bunyamwera.

Group A viruses—including Sindbis, Chikungunya and Semliki Forest—are related to each other, and group B viruses—including Spondweni, Wesselsbron, H 336 and West Nile—are also interrelated, but agents of one group are not known to be related to those of the other group. If cross-reactions were to occur in studies of this sort it would be expected that they should occur most commonly within group A or group B.

Among the Nibela donors plural reactivity is more frequent with increasing age and more commonly involves unrelated than related viruses; hence it is more commonly an expression of increased exposure than of cross-reactivity.

DISCUSSION

The results of preliminary protection-test surveys[1] pointed to the Simbu Pan area of Tongaland as a locality favourable for arthropod-borne virus infections. Investigations undertaken on the basis of those surveys and continued to the present time have led to the isolation of 48 strains of virus belonging to more than 10 (3 strains not yet categorized) viral types. The surveys reported here were undertaken for 3 purposes: (1) to acquire information pertaining to potential health problems in the area; (2) to ascertain whether various viruses isolated in Tongaland or elsewhere in Africa attack human beings in this virus-prevalent coastal zone; and (3) to acquire information pertaining to the ecology of the region as related to viral infections.

From isolations of the viruses from sick human beings it is known that Wesselsbron, H 336, Bunyamwera and West Nile viruses cause overt illness in Tongaland. From field isolations elsewhere in the Union (Chikungunya, Rift

TABLE V. PROTECTION-TEST RESULTS ON SERA FROM NIBELA RESIDENTS ANALYSED FOR CROSS-REACTIVITY

Serum No.	Age	Sex	Virus used in test												
			Group A			Group B				Ungrouped						
			Sind.	Chik.	SFV	Spon.	Wess.	H 336	WN	Pong.	Bun.	Simbu	AR 136			
S 2332	30	F	—	—	P	P	P	P	—	P	P	—	—	A		
2333	35	F	—	—	P	P	P	P	—	P	P	—	—	A		
2334	17	F	—	—	—	P	—	—	—	—	—	—	—	*	B	
2335	45	F	—	—	—	P	P	P	—	—	P	—	—			U
2336	25	F	—	—	—	—	—	—	—	—	—	—	—			
2337	30	F	—	—	P	—	P	P	—	—	P	—	—	A		U
2338	34	F	P	—	—	P	—	—	—	P	P	—	—	A	B	
2339	25	F	—	—	—	—	P	—	—	—	—	—	—	*	B	
2340	40	F	—	—	P	—	P	—	—	—	P	—	—	A	B	U
2341	35	F	—	—	—	—	—	—	—	—	P	—	—	*		U
2342	36	F	—	—	—	—	—	—	—	—	P	—	—	*		U
2343	34	F	—	—	—	—	—	—	—	—	P	—	—	*		U
2344	39	F	—	—	P	—	P	P	—	P	P	—	—	A		
2345	38	F	—	—	—	—	—	—	—	—	P	—	—	*		U
2346	40	F	—	P	P	—	—	—	—	—	P	—	—			U
2347	18	F	—	—	—	—	—	P	—	—	P	—	—		B	U
2348	30	F	—	—	—	—	—	P	—	P	—	—	—		B	U
2349	11	F	—	—	—	—	—	P	—	—	—	—	—	*	B	
2350	10	F	—	—	—	—	—	—		—	—	—	—			
2351	7	M	—	—	—	—	—	P		—	—	—	—	*	B	
2352	10	F	—	—	—	—	—	—		—	—	—	—			
2353	9	F	—	—	—	—	—	—		—	P	—	—	*		U
2354	6	M	—	—	—	—	—	—		—	—	—	—			
2355	6	M	—	—	—	—	—	—		—	—	—	—			
2356	7	F	—	—	—	—	—	—		—	—	—	—			
2357	7	F	—	—	—	—	—	—		—	—	—	—			
2358	6	F	—	—	—	—	—	—		—	P	—	—	*		U
2359	5	F	—	—	—	—	—	—		—	—	—	—			
2360	12	F	—	—	—	—	—	P		—	P	—	—		B	U
2361	14	F	—	—	—	—	—	—		—	P	—	—	*		U
2362	14	F	—	—	—	—	—	—		—	—	—	—			
2363	11	F	—	—	—	—	—	—		—	—	—	—			
2364	4	F	—	—	—	—	—	P		—	—	—	—	*	B	
2365	4	F	—	—	—	—	—	—		—	—	—	—			
2366	4	F	—	—	—	—	—	—		—	—	—	—			
2367	4	F	—	—	—	—	—	—		—	P	—	—	*		U

*=Single positive result.
A=In group A neutralizes only 1 virus (6/7 group A pos.).
B=In group B neutralizes only 1 virus (10/15 group B pos.).
Among children there are 4 group B positives but no plural Bs.
U=In ungrouped neutralizes only 1 virus (15/19 ungrouped pos.).

1/7 A-positives neutralizes 2 A Viruses.
7/7 A-positive sera neutralize one or more B or ungrouped viruses.
10/15 B-positive sera neutralize one or more A or ungrouped.
5/15 B-positive sera neutralize 2 or more B viruses.
11/19 ungrouped positives neutralize 1 or more A or B viruses.
4/19 ungrouped positives neutralize 2 ungrouped viruses.
Only 1/8 child immunes is plurally immune (B and ungrouped).
10/16 adult immunes are plurally immune.
Of 11 plurally positive sera, 1 neutralizes 2 A viruses.
Of 11 plurally positive sera, 6 neutralize 1 A and 1 or more unrelated viruses.
Of 11 plurally positive sera, 5 neutralize 2 or more B viruses.
Of 11 plurally positive sera, 9 neutralize 1 or more B and 1 or more unrelated.

Valley fever) and from laboratory-acquired infections (Spondweni) it is known that 3 of the other agents used in the studies can cause illness in man. The results of the present studies indicate that with the exception of West Nile, each of these agents has commonly attacked human beings in this coastal zone in recent years. In addition, although it has thus far been isolated only from mosquitoes, Pongola virus apparently often attacks human beings, for many lowland residents exhibit neutralizing antibodies in their sera. Few or no immunes were found for 3 viruses which have been isolated in Tongaland, namely Middelburg, Simbu and the agent designated AR 136. These may have natural vertebrate hosts other than man. Semliki Forest virus has not been isolated either in Tongaland or elsewhere in the Union, yet several sera were found which neutralized this and no other group A agent included in the tests.

The diminished evidence of infection with several viruses in the southern portion of the study area, including Eshowe, Stanger and Durban is probably due to the efforts of man. In this area the land has been cleared and much of it brought under European agricultural practices—including especially the cultivation of sugar cane. The thornbush—so prevalent in the northern area—does not now exist in the south, and much has been done to control the water. With this agricultural development, Native habitations have come to be grouped into farm compounds and urban locations less exposed than their former homes to suitable harbourage for mosquitoes. Moreover, the mosquito control programmes—especially in relation to malaria eradication schemes—doubtless have a profound effect on some of the vectors of virus disease. If comparable developmental schemes are applied to the northernmost area of Natal, reduction of the incidence of viral infections may also occur, but those who proceed first to the area will certainly be at risk.

Chikungunya disease, as seen in the first recorded outbreak,[20] was an epidemic, urban-type disease in which the virus[21] was transmitted by *Aedes aegypti*.[22] The vector in the one proved South African outbreak[10] was not discovered, but the infection has been transmitted experimentally in these laboratories[23] by *Aedes calceatus*, another of the Stegomyia sub-genus. These facts, together with the observation that most of the sera in our survey which were protective for this virus were from adults, may indicate that this infection characteristically occurs in epidemic form. If this is true, the coastal area of Natal is apparently a region which can support the virus, but in which no significant outbreak has occurred in very recent years.

The fact that none of these agents has attracted attention as the cause of outbreaks of fatal illness probably indicates that they do not commonly cause death in man. Nevertheless, from what is known of the effects of some of them, and from the frequency with ntly attack which they evidehuman

beings, the probability is high that they are responsible for a great deal of morbidity in man. In addition to Rift Valley fever and Wesselsbron viruses, it is possible that some others may be important in veterinary medicine.

SUMMARY

Blood specimens from human beings residing in 4 highland and 21 lowland localities of the Natal coastal area from Durban northward to the Moçambique border were examined in mouse-protection tests to study the distribution of antibodies to arthropod-borne viruses. Sera from representative areas were tested against 13 agents, 10 of which have been isolated within the zone covered by the survey. The results of the tests indicate that:

1. Certain localities in this coastal zone are capable of supporting Sindbis, Chikungunya and Semliki Forest viruses, but these agents apparently have not been active there in recent years.

2. A considerable number of persons were found to be immune to Rift Valley fever; the existence of immunity in young children in some areas is taken as possibly indicating that the virus is endemic or enzootic there.

3. Pongola and Bunyamwera viruses obviously attack man with considerable frequency in the riverine area of the coastal lowlands. Bunyamwera infection has evidently occurred in man as far south as Durban.

4. Among group B viruses West Nile appears not to be common in the surveyed area (although it was isolated from man at Ndumu in 1958[18]). Spondweni, Wesselsbron and H 336 viruses apparently attack human beings with considerable frequency in the lowland areas covered by the survey. The distribution of immunity to Spondweni virus is very spotty, whereas immunes to the other 2 were more evenly distributed. Both Wesselsbron and H 336 appear not to occur in the southernmost localities sampled and the indications are that this zone is less favourable for them than the lowland areas farther to the north.

5. A considerable amount of morbidity in human beings in the coastal lowlands of northern Natal is probably caused by some of these arthropod-borne viruses.

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