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A Multipurpose Serological Survey in Kenya

2. Results of Arbovirus Serological Tests

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Arbovirus infections are of public health interest in East Africa, where a very widespread epidemic of o'nyong-nyong fever was reported in 1959–60 and where the threat of yellow fever, present in neighbouring areas such as Ethiopia, remains. Sera collected in a serological survey in Kenya were therefore tested for antibodies against 3 group-A arboviruses (chikungunya, o'nyong-nyong and Sindbis), 6 group-B arboviruses (Zika, yellow fever, West Nile, Banzi, Wesselsbron and dengue 1), and Bunyamwera virus. The sera were examined mainly by the haemagglutination-inhibition test but a small proportion were also subjected to virus neutralization tests.

The results showed that the prevalence of arbovirus infection varies markedly from area to area in Kenya. All types of arbovirus infections were more frequent on the coast than on the dry plateau around Kitui and the Lake Victoria area, The only exceptions were o'nyong-nyong and chikungunya, which were found to be just as prevalent on the coast as in Nyanza, where an epidemic was reported in 1959–60. Yellow fever antibodies were found to be present in about half of the people living on the coast but practically absent from the other two areas. It was concluded that the yellow fever antibodies in the coastal area must be due either to vaccination or to cross-reactions with other group-B arboviruses.

A multipurpose serological survey was conducted in Kenya from November 1966 to April 1968. The survey included approximately 1500 randomly selected subjects in each of three different districts: Central Nyanza near Lake Victoria, Kitui District in Central Kenya and Malindi District in the Coast Province.

Each serum specimen was divided into 4 portions in the field: one portion was forwarded to the East African Virus Research Institute (EAVRI) at Entebbe, Uganda, for arbovirus serological testing and the other three were sent to laboratories in Europe for other antibody determinations. The present paper presents the results of the arbovirus serological tests made in Entebbe and a further paper will deal with the antibody determinations made elsewhere.

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SURVEY AREAS AND METHODS

One objective of the survey was to study the geographical variation in prevalence of the various infections for which antibody determinations were carried out. For this purpose, population groups were examined in various parts of the country in such a way that the results would be strictly comparable. The sampling, as well as the survey techniques, are outlined in the first part of this report (Geser, Christensen & Thorup, 1970) which also gives a description of the three survey areas.

During the survey an attempt was made to identify the environmental factors that may be associated with variation in prevalence of infections. Most of the environmental conditions observed in the survey groups were more relevant to diseases transmitted through direct human contact or through contamination of the environment than to those transmitted by insect vectors. Some variables expressing distance to potential insect breeding grounds such as rivers, lakes or dense forest were, however, thought to influence the risk of arbovirus infection in the various

TABLE 1

AGE-SPECIFIC PREVALENCE OF 7 DIFFERENT INFECTIONS, BY SURVEY AREA

Survey area	Age-group (years)	No. examined	No. positive to test [a]							Percentage positive to test [a]						
			CK	SB	BW	ZK	YF	WN	BZ	CK	SB	BW	ZK	YF	WN	BZ
Central Nyanza	0–4	1	1	—	—	—	—	—	—	100.0	0.0	0.0	0.0	0.0	0.0	0.0
	5–9	10	4	—	1	2	1	1	1	40.0	0.0	10.0	20.0	10.0	10.0	10.0
	10–14	165	92	6	10	2	1	1	1	55.8	3.6	6.1	1.2	0.6	0.6	0.6
	15–19	80	36	2	4	2	—	—	—	45.0	2.5	5.0	2.5	0.0	0.0	0.0
	20–29	121	62	7	6	2	—	1	—	51.2	5.8	5.0	1.7	0.0	0.8	0.0
	30–39	134	64	9	10	2	3	5	1	47.8	6.7	7.5	1.5	2.2	3.7	0.7
	40–49	114	63	5	8	1	7	4	1	55.3	4.4	7.0	0.9	6.1	3.5	0.9
	50–59	108	70	8	6	7	4	7	3	64.8	7.4	5.6	6.5	3.7	6.5	2.8
	⩾60	89	57	6	7	9	6	7	5	64.0	6.7	7.9	10.1	6.7	7.9	5.6
	Unknown	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Total	822	449	43	52	27	22	26	12	54.6	5.2	6.3	3.3	2.7	3.2	1.5
Kitui District	0–4	160	1	1	1	—	—	16	4	0.6	0.6	0.6	0.0	0.0	10.0	2.5
	5–9	194	—	13	3	—	—	20	14	0.0	6.7	1.5	0.0	0.0	10.3	7.2
	10–14	87	—	12	5	—	—	8	6	0.0	13.8	5.7	0.0	0.0	9.2	6.9
	15–19	68	—	11	2	—	—	13	6	0.0	16.2	2.9	0.0	0.0	19.1	8.8
	20–29	167	4	31	11	4	2	27	11	2.4	18.6	6.6	2.4	1.2	16.2	6.6
	30–39	150	2	26	16	4	6	24	12	1.3	17.3	10.7	2.7	4.0	16.0	8.0
	40–49	87	—	13	8	—	2	15	5	0.0	14.9	9.2	0.0	2.3	17.2	5.7
	50–59	56	—	10	6	1	1	6	4	0.0	17.9	10.7	1.8	1.8	10.7	7.1
	⩾60	65	3	9	5	5	4	14	7	4.6	13.8	7.7	7.7	6.2	21.5	10.8
	Unknown	8	—	4	1	—	—	1	1	0.0	50.0	12.5	0.0	0.0	12.5	12.5
	Total	1 042	10	130	58	14	15	144	70	1.0	12.5	5.6	1.3	1.4	13.8	6.7
Malindi District	0–4	60	7	1	7	9	11	24	12	11.7	1.7	11.7	15.0	18.3	40.0	20.0
	5–9	108	27	12	12	11	10	41	17	25.0	11.1	11.1	10.2	9.3	38.0	15.7
	10–14	97	53	14	19	7	4	34	7	54.6	14.4	19.6	7.2	4.1	35.1	7.2
	15–19	73	39	15	16	24	18	32	18	53.4	20.5	21.9	32.9	24.7	43.8	24.7
	20–29	160	96	34	53	97	72	108	83	60.0	21.3	33.1	60.6	45.0	67.5	51.9
	30–39	102	57	21	28	81	68	88	79	55.9	20.6	27.5	79.4	66.7	86.3	77.5
	40–49	89	54	20	39	76	63	83	77	60.7	22.5	43.8	85.4	70.8	93.3	86.5
	50–59	68	40	16	19	60	46	64	60	58.8	23.5	27.9	88.2	67.6	94.1	88.2
	⩾60	77	51	24	28	69	58	71	67	66.2	31.2	36.4	89.6	75.3	92.2	87.0
	Unknown	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Total	834	424	157	221	434	350	545	420	50.8	18.8	26.5	52.0	42.0	65.3	50.4

[a] BW = Bunyamwera, BZ = Banzi, CK = chikungunya, SB = Sindbis, WN = West Nile, YF = yellow fever, ZK = Zika.

population groups and were, therefore, observed and correlated with the prevalence of arbovirus infections during analysis of the data.

In the first survey area (Central Nyanza) venous blood was not collected from children under 5 years of age, and only a few sera from children aged 5–10 years were sent for arbovirus serology because it was thought at the planning stage of the survey that it would be difficult to obtain a sufficient quantity of serum from subjects in this age-group. The experience gained in Nyanza proved that satisfactory vein puncture could be carried out even in small children, and in the surveys in Kitui and Malindi, serum was obtained for arbovirus serology from subjects of all ages.

SEROLOGICAL TESTS

All sera were tested for presence of arbovirus antibodies with the haemagglutination-inhibition (HI) test. The number of sera from each survey area that were tested with the various antigens is shown in Table 1. Altogether, 7 antigens were applied to sera from all three areas—namely, chikungunya (E 103), Sindbis (AR 339), Bunyamwera (Aedes '43), Zika (MR 766), yellow fever (FNV), West Nile (MP 22) and Banzi (H 336). HI testing with 3 other antigens—namely, o'nyong-nyong (JAR), Wesselsbron (MP 5965) and dengue 1 (HAW)—was carried out only on selected sera during the initial serological examination.

HI tests were performed according to the method of Clarke & Casals (1958) in microtitre plates. Sera were tested in twofold dilutions beginning at a 1 : 10 dilution. All the sera were tested against 4–8 units of antigen; a serum that inhibited antigen at the initial or higher dilutions was considered to be positive.

In order to gain a better understanding of the results of the HI testing, the more specific neutralization test was carried out with some of the antigens on a limited number of selected sera. Neutralization tests were performed by means of the constant serum–varying dilution technique. A virus dose calculated to provide approximately 100 suckling mouse intracerebral LD_{50} doses was incubated with an equal volume of undiluted serum at 37°C for 1 hour and inoculated by the intracerebral route into white Swiss mice. Yellow fever neutralization tests were performed in 3-week-old mice; West Nile (MP 22), Wesselsbron (MP 5965) and dengue 1 (HAW) neutralization tests were performed in suckling mice. If no more than 1 mouse died in a litter, the test was considered to be positive.

FINDINGS

Geographical variation in the prevalence of infection

The results of the initial HI testing are shown in Table 1 in terms of prevalence of antibodies to 7 different arbovirus antigens as observed in each of the three survey areas, according to age.

It is evident that the prevalence varies considerably from area to area for most of the infections included in Table 1. On the whole, it seems as if there were considerably more arbovirus infection in Malindi District than in the other two areas; this observation applies to all seven infections with the exception of chikungunya which was just as prevalent in Central Nyanza as in Malindi District.

Arbovirus group A. The sera collected during the first part of the survey in Central Nyanza were subjected to HI testing with both chikungunya and o'nyong-nyong. The results of the testing with these two closely related antigens are shown in Table 2, from which it can be seen that practically identical results were obtained. In subsequent testing, therefore, only chikungunya antigen was used

TABLE 2

CORRELATION BETWEEN RESULTS OF HI TESTING WITH CHIKUNGUNYA AND O'NYONG-NYONG ANTIGENS IN SERA FROM CENTRAL NYANZA, BY AGE

Age-group (years)	Positive with both CK and ON[a]	Negative with both CK and ON[a]	Positive with CK, negative with ON[a]	Negative with CK, positive with ON[a]	Total
0–4	1	—	—	—	1
5–9	4	4	—	—	8
10–14	54	42	—	—	96
15–19	27	18	—	—	45
20–29	39	19	—	1	59
30–39	41	23	—	—	64
40–49	40	30	—	—	70
50–59	38	38	1	—	77
⩾60	38	29	—	—	67
Total	282	203	1	1	487

[a] CK = chikungunya, ON = o'nyong-nyong.

TABLE 3

RESULTS OF HI TESTING OF 300 SELECTED SERA FOR WESSELSBRON AND DENGUE 1

Area	Age-group (years)	No. examined	Wesselsbron		Dengue	
			No. positive	% positive	No. positive	% positive
Central Nyanza	0–4	1	0	0.0	0	0.0
	5–9	1	0	0.0	0	0.0
	10–14	18	3	16.7	0	0.0
	15–19	8	0	0.0	0	0.0
	20–29	13	0	0.0	0	0.0
	30–39	17	3	17.6	0	0.0
	40–49	14	1	7.1	0	0.0
	50–59	14	5	35.7	0	0.0
	⩾60	13	5	38.5	2	15.4
	Unknown	—	—	—	—	—
	Total	99	17	17.2	2	2.0
Kitui District	0–4	12	1	8.3	0	0.0
	5–9	17	5	29.4	0	0.0
	10–14	13	4	30.8	0	0.0
	15–19	6	0	0.0	0	0.0
	20–29	20	6	30.0	1	5.0
	30–39	12	4	33.3	1	8.3
	40–49	6	2	33.3	0	0.0
	50–59	5	3	60.0	1	20.0
	⩾60	7	2	28.6	0	0.0
	Unknown	—	—	—	—	—
	Total	98	27	27.6	3	3.1
Malindi District	0–4	8	4	50.0	0	0.0
	5–9	16	7	43.8	1	6.3
	10–14	9	2	22.2	0	0.0
	15–19	7	5	71.4	1	14.3
	20–29	17	13	76.5	6	35.3
	30–39	11	11	100.0	10	90.9
	40–49	17	17	100.0	17	100.0
	50–59	2	2	100.0	2	100.0
	⩾60	9	9	100.0	9	100.0
	Unknown	2	0	0.0	0	0.0
	Total	98	70	71.4	46	46.9

and sera with antibody to chikungunya were considered to be positive also to o'nyong-nyong.

More than half the sera collected from Nyanza (54.6%) and Malindi (50.8%) had HI antibody to chikungunya, whereas only a small proportion (1.0%) of the sera from Kitui were positive. There was no apparent increase in prevalence of chikungunya antibody with age in Nyanza; in Malindi District the percentage of positive sera increased sharply from 11.7% and 25.0% in the two lowest age-groups to more than 50% in the remaining age-groups.

Sindbis HI antibody was detected in all survey areas; the percentage of positive sera was less in Nyanza (5.2%) than in Kitui (12.5%) and highest in Malindi (18.8%). In each area there was a noticeable increase in the percentage of positive sera with age.

Bunyamwera. The prevalence of Bunyamwera antibody was found to be considerably higher in Malindi (26.5%) than in Nyanza (6.3%) or Kitui (5.6%). In Malindi District the percentage of sera with Bunyamwera antibody increased from approximately 11% in the 0–4-years and 5–9-years age-groups to a maximum of 43.8% in the 40–49-years age-group.

Arbovirus group B. In the initial HI testing of sera from Central Nyanza only 4 group-B antigens were used—namely, Zika, yellow fever, West Nile and Banzi. In later HI testing Wesselsbron and dengue 1 antigens were added.

The geographical variation in prevalence of arbovirus infections, reported here (Table 1) is based on the initial HI testing except for Wesselsbron and dengue 1; for these two it is based on the results of HI testing of 100 randomly selected sera from each survey area. These 300 sera were also used for the re-testing which is reported below.

The proportion of positive HI reactions to all group-B antigens was low in Central Nyanza and Kitui District—with the possible exception of West Nile infection in Kitui—and high in Malindi District, as can be seen from Table 1.

With regard to yellow fever, the results of the HI testing (Table 1) show that antibodies to this infection are practically absent in Central Nyanza (2.7%) and Kitui (1.4%) and very prevalent in Malindi (42.0%). The prevalence of yellow fever HI antibodies in Malindi increases with age from about 10% in children under 15 years up to about 70% in people over 40 years of age.

Table 3 shows the results of the HI testing done with dengue 1 and Wesselsbron antigens on the 300 sera selected for the repeat testing. It can be seen that HI antibodies to dengue 1 were prevalent in Malindi District (46.9%) and practically absent in Central Nyanza (2.0%) and Kitui District (3.1%). In Malindi District the prevalence rose from 0 in the youngest age-group to 14.3% in the 15–19-years age-group and reached 100% in those over 40 years of age.

HI antibodies to the Wesselsbron antigen were also more prevalent in Malindi District (71.4%) than in Central Nyanza (17.2%) and Kitui District (27.6%). A clear age progression in Wesselsbron infection can be discerned in Malindi District but is less obvious in the other two areas.

Neutralization test

In order to assess the specificity of the HI tests and thereby obtain a measure of the proportion of false negative and false positive reactions, a small number of selected sera were tested with the, supposedly, more specific neutralization test.

Altogether 111 HI-positive sera were submitted to yellow fever neutralization testing and the results are shown in Table 4; 11.7% of these yellow fever HI-positive sera also reacted to the neutralization test. None of the 38 sera from persons under 20 years of age gave a positive yellow fever neutralization test.

A comparison between the results obtained by the HI and the neutralization tests for West Nile, Wesselsbron and dengue 1 is made for each survey

TABLE 4

RESULTS OF YELLOW FEVER NEUTRALIZATION TESTS ON SELECTED SERA

Age-group (years)	No. examined	No. positive	% positive
0–9	17	0	0.0
10–19	21	0	0.0
20–29	32	2	6.3
30–39	19	6	31.6
40–49	6	0	0.0
⩾50	16	5	31.3
Total	111	13	11.7

TABLE 5

COMPARISON OF RESULTS OF HI AND NEUTRALIZATION TESTS FOR WEST NILE, WESSELSBRON AND DENGUE 1 ANTIGENS ON SELECTED SERA, BY SURVEY AREA

Antigen	Survey area	Neutralization test	HI titre, first examination								
			0	1	2	3	4	5	6	7	Total
West Nile	Central Nyanza	Positive	1	2	3	5	—	1	—	—	12
		Negative	22	2	2	—	—	—	—	—	26
	Kitui District	Positive	—	4	8	17	4	1	—	1	35
		Negative	11	4	5	6	2	—	—	—	28
	Malindi District	Positive	—	4	5	4	4	—	4	—	21
		Negative	28	14	5	8	4	1	1	—	61
	Total	Positive	1	10	16	26	8	2	4	1	68
		Negative	61	20	12	14	6	1	1	—	115
		Total	62	30	28	40	14	3	5	1	183
Wesselsbron	Kitui District	Positive	3	1	1	—	—	—	—	—	5
		Negative	24	4	—	—	—	—	—	—	28
	Malindi District	Positive	0	6	7	9	—	1	—	—	23
		Negative	30	8	5	6	—	2	—	—	51
	Total	Positive	3	7	8	9	—	1	—	—	28
		Negative	54	12	5	6	—	2	—	—	79
		Total	57	19	13	15	—	3	—	—	107
Dengue 1	Malindi District	Positive	1	1	2	3	3	1	—	—	11
		Negative	18	—	—	3	1	—	—	—	22
		Total	19	1	2	6	4	1	—	—	33

area in Table 5 and a summary given in Table 6. Accepting the neutralization tests as specific, it is seen that the proportion of false negative reactions to the HI tests is very small whereas the proportion of false positives is considerable. The small proportion of false negatives indicates that HI tests are well suited for screening for group-B arboviruses since very few actual positives will be missed.

Reliability of the serological testing

In order to estimate the importance of the experimental error involved in arbovirus HI testing, two attempts were made to determine the consistency of the test.

Examination of duplicate specimens. Without the knowledge of the examining laboratory, some specimens were obtained in duplicate by dividing a serum sample into two parts immediately after its collection in the field. The two specimens were given different identification numbers to ensure " blind " duplicate testing in the laboratory. A total of 68 specimens from Kitui and Malindi districts were prepared in duplicate and the two

TABLE 6

SUMMARY OF RESULTS OF HI AND NEUTRALIZATION TESTS FOR WEST NILE, WESSELSBRON AND DENGUE 1 ANTIGENS ON SELECTED SERA

Antigen		Total examined	Positive by both methods	Negative by both methods	Positive by HI but negative by neutralization test	Negative by HI but positive by neutralization test
West Nile	No.	183	67	61	54	1
	%	100.0	36.6	33.3	29.5	0.5
Wesselsbron	No.	107	25	54	25	3
	%	100.0	23.4	50.5	23.4	2.8
Dengue 1	No.	33	10	18	4	1
	%	100.0	30.3	54.5	12.1	3.0

titres which were obtained for each divided specimen have been correlated separately for 8 different antigens (Table 7).

On the whole, the results of the two tests corresponded fairly well. In the case of chikungunya infection, for instance, the proportion of positive reactions was 36.8% on the first examination and 44.1% on the second; a total of 53 specimens (77.9%) yielded identical titres in the two tests. If small differences (<1) between the first and second titres are disregarded, as many as 61 specimens (89.7%) achieved identical classification, in terms of positive or negative, in the duplicate testing for chikungunya.

The agreement with respect to classification into " positive " and " negative " by the two tests for the 8 infections which were included in the duplicate testing is shown in Table 8 for two different criteria.

Re-testing of selected sera after storage. The laboratory investigations were carried out over a period of 18 months. In order to investigate the extent to which variation of reagents over this period caused differences in the test results, samples of the survey sera were subjected to repeated HI testing with arbovirus antigens at the end of the 18-month period. From each of the three survey areas a sample of 100 sera were selected for this re-testing; the second tests were done " blindly ", so that the observer could not know the result of the first test.

The 300 selected sera were tested with 9 different antigens but two of these (Wesselsbron and dengue 1) had not been universally used during the initial testing and the comparisons of two test results could, therefore, be made only for 7 antigens (Table 9). Correlations between the results of the first and second tests are shown in Table 10 for 7 different infections.

Association between prevalence of infection and environmental factors

Factors such as the presence of potential insect breeding grounds and the density of the human population might conceivably influence man–mosquito relationships. Such factors were therefore observed during the survey and correlated with the prevalence of arbovirus infections.

Distance from insect breeding grounds. The distance from the houses included in the survey to potential breeding grounds was estimated and recorded for each household; during the analysis of the survey data the prevalence of each antibody was calculated for people living at various distances from the different types of breeding grounds as follows:

In all three survey areas, people live in rural dwellings scattered among the ubiquitous African bush and all houses were found to be near bush and trees of various kinds. There was thus no variation in this factor and the association between distance to bush and forest and prevalence of infection could not be studied.

For each of the three survey areas tabulations (not given here) were prepared for the prevalence of 7 different arbovirus infections as a function of distance from river (or smaller stream), swamp or lake. The results showed that the prevalence of infection is totally independent of whether the

TABLE 7
CORRELATION BETWEEN HI TITRES OBTAINED FROM DUPLICATE SPECIMENS, SEPARATELY FOR 8 DIFFERENT INFECTIONS

		Titre of first examination									Titre of first examination								
		0	1	2	3	4	5	6	NE[a]	Total	0	1	2	3	4	5	6	NE[a]	Total
		Chikungunya									Yellow fever								
Titre of second examination	0	37	—	1	—	—	—	—	—	38	38	5	—	—	1	—	—	—	44
	1	2	4	1	—	—	—	—	—	7	4	4	1	2	—	—	—	—	11
	2	2	—	3	2	—	—	—	—	7	—	2	2	1	—	—	—	—	5
	3	2	1	2	7	—	1	—	—	13	—	—	3	3	—	—	—	—	6
	4	—	—	—	1	2	—	—	—	3	1	—	—	—	—	—	—	—	1
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NE[a]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total		43	5	7	10	2	1	—	—	68	43	11	6	6	2	—	—	—	68
		Sindbis									West Nile								
Titre of second examination	0	54	3	1	—	—	—	—	—	58	21	3	2	—	1	—	—	—	27
	1	3	2	—	1	—	—	—	—	6	5	4	—	1	—	—	—	—	10
	2	2	1	1	—	—	—	—	—	4	1	2	4	1	—	—	—	—	8
	3	—	—	—	—	—	—	—	—	—	6	—	3	3	—	—	—	—	12
	4	—	—	—	—	—	—	—	—	—	—	—	1	2	1	—	—	—	4
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	4	—	6
	NE[a]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total		59	6	2	1	—	—	—	—	68	33	9	10	7	5	—	4	—	68
		Bunyamwera									Wesselsbron								
Titre of second examination	0	43	4	—	2	—	—	—	—	49	19	4	3	2	—	—	—	—	28
	1	5	2	2	—	—	—	—	—	9	8	2	1	—	—	—	—	—	11
	2	—	2	4	—	—	—	—	—	6	4	—	5	1	—	—	—	—	10
	3	—	—	—	2	—	—	—	—	2	—	—	6	8	—	1	—	—	15
	4	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NE[a]	—	1	—	1	—	—	—	—	2	—	—	—	—	—	—	—	3	3
Total		48	9	6	5	—	—	—	—	68	31	6	15	11	1	1	—	3	68
		Zika									Banzi								
Titre of second examination	0	32	3	2	1	—	—	—	—	38	26	3	2	—	1	—	—	—	32
	1	1	3	4	1	—	—	—	—	9	11	3	2	—	—	—	—	—	16
	2	3	1	5	—	—	—	—	—	9	2	—	2	4	—	—	—	—	8
	3	1	—	2	2	1	—	—	—	6	—	—	—	3	1	—	—	—	4
	4	—	—	1	—	1	2	—	—	4	—	1	2	1	1	—	—	—	5
	5	—	—	—	—	1	—	—	—	1	—	—	—	1	—	1	—	—	2
	6	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	1	—	1
	NE[a]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total		37	7	14	4	3	3	—	—	68	39	7	8	9	3	1	1	—	68

[a] NE = not examined.

TABLE 8
AGREEMENT IN CLASSIFICATION OF DUPLICATE SPECIMENS

Infection	Identical classification		Difference in titres ≤2	
	No.	%[a]	No.	%[a]
Chikungunya	53	77.9	61	89.7
Sindbis	57	83.8	64	94.1
Bunyamwera	51	77.3	64	97.0
Zika	43	63.2	59	86.8
Yellow fever	47	69.1	64	94.1
West Nile	37	54.4	54	79.4
Wesselsbron	35	53.8	55	84.6
Banzi	37	54.4	59	86.8

[a] Percentage of samples that were examined in both the first and second tests.

subjects live close to, or far from, rivers, swamps or lakes. The relationship between prevalence of antibodies and distance from domicile to the coast line was calculated for Malindi District; again, the results showed that the two variables are completely unconnected.

Density of houses. During the field work observations were made on the proximity of houses to each other in the survey groups. This was done by recording for each household how many other houses lay within a distance of 200 m. In the analysis, the houses were grouped according to whether 0, 1–2, 3–5, 6–10 or more than 10 houses were found within a radius of 200 m, and the prevalence of the various arbovirus infections within each group of households was calculated; the results are shown in Table 11, separately for each survey area. A closer scrutiny of Table 11 reveals the interesting fact that the prevalence of infection tends to be higher in dense accumulations of households (more than 10 houses within a radius of 200 m) than among scattered houses with no neighbouring houses, or at most 1–2 houses, within a distance of 200 m. The preponderance of infection in dense accumulations of households can be seen for all 7 infections both in Malindi District and in Central Nyanza. In Kitui District the population was so scattered that accumulations of households beyond 10 houses within a radius of 200 m did not occur in any survey group. The association between population concentration and the risk of arbovirus infection could, therefore, not be investigated in the material collected in Kitui.

The consistent finding of a higher prevalence of arbovirus infection in dense household accumulations is highly significant and leads to the conclusion that people living in dense population clusters are more at risk from arbovirus infections than are people who live in less densely populated, but otherwise similar, conditions.

Size of household. The number of persons living in a household may conceivably affect the risk that each member of a household will be bitten by a mosquito, leading to an arbovirus infection. The prevalence of HI antibodies for 6 of the arbovirus infections examined was calculated separately for people living in households of different sizes; the results did not indicate that there is any association between risk of infection and the size of the household in which a person lives.

DISCUSSION

Certain difficulties are inherent in serological surveys whenever such surveys are used to compare the prevalence of antibodies in different population groups. Some experimental error invariably originates in the laboratory where the classification of a certain serum as "positive" or "negative" is subject to some inaccuracy. The magnitude of the experimental error involved in HI testing was estimated in the present study by repeating the test. Testing of duplicate specimens, blindly and simultaneously, showed that the basic laboratory error is far from negligible in arbovirus investigations.

Another type of difficulty in interpreting results of prevalence surveys originates from the fact that antibodies may persist for a long time in a given individual. The prevalence measured at a given point in time may therefore be a reflection of infections acquired when the subject lived in another locality under different environmental conditions. This is particularly true if the older age-groups are included in the investigations, as was the case in the present survey. The error may be reduced by basing prevalence estimates on observations in young children since their immune reactions must have been acquired during a relatively short time. It was not possible in the present study to improve the data by limiting observations to young children because the number of children examined in the survey was too small to yield sufficiently precise estimates.

TABLE 9
CORRELATION BETWEEN RESULTS OF REPEATED HI TESTING OF 300 SELECTED SERA, SEPARATELY FOR 7 DIFFERENT INFECTIONS

		Titre of first examination										Titre of first examination									
		0	1	2	3	4	5	6	7	NE[a]	Total	0	1	2	3	4	5	6	7	NE[a]	Total
		Chikungunya										Yellow fever									
Titre of second examination	0	165	7	2	—	—	—	—	—	5	179	236	3	—	—	—	—	—	—	6	245
	1	5	8	7	5	—	—	—	—	1	26	7	11	8	—	1	—	—	—	1	28
	2	2	2	5	10	5	—	—	—	1	25	1	1	5	5	1	—	—	—	2	15
	3	2	1	1	20	5	2	2	—	1	34	—	—	1	2	2	1	—	—	—	6
	4	3	—	2	16	1	4	4	—	1	31	—	—	—	1	—	—	—	—	—	1
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NE[a]	2	—	—	2	1	—	—	—	—	5	3	2	—	—	—	—	—	—	—	5
Total		179	18	17	53	12	6	6	—	9	300	247	17	14	8	4	1	—	—	9	300
		Sindbis										West Nile									
Titre of second examination	0	215	3	—	3	1	—	—	—	4	226	200	12	—	—	—	—	—	—	4	216
	1	33	5	4	1	—	—	—	—	4	47	18	4	12	6	2	—	—	—	3	45
	2	2	2	5	3	—	—	—	—	1	13	2	—	3	7	4	3	—	1	1	21
	3	2	2	1	1	—	—	—	—	—	6	—	—	—	2	1	4	—	—	1	8
	4	—	1	—	—	1	1	—	—	—	3	—	—	—	1	—	2	—	—	—	3
	5	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	2
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NE[a]	5	—	—	—	—	—	—	—	—	5	1	1	1	1	1	—	—	—	—	5
Total		257	13	10	8	2	1	—	—	9	300	221	17	17	17	9	9	—	1	9	300
		Bunyamwera										Banzi									
Titre of second examination	0	202	3	—	—	—	—	—	—	5	210	235	19	8	4	1	—	—	—	8	275
	1	29	6	4	1	—	—	—	—	2	42	—	—	4	4	6	2	—	—	—	16
	2	8	5	1	1	—	—	—	—	1	16	—	—	—	2	—	1	—	—	1	4
	3	6	3	2	2	1	—	—	—	1	15	—	—	—	—	—	—	—	—	—	—
	4	1	4	3	2	1	1	—	—	—	12	—	—	—	—	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NE[a]	4	—	1	—	—	—	—	—	—	5	2	1	1	1	—	—	—	—	—	5
Total		250	21	11	6	2	1	—	—	9	300	237	20	13	11	7	3	—	—	9	300
		Zika																			
Titre of second examination	0	234	2	4	—	—	—	—	—	7	247										
	1	3	9	4	1	—	—	—	—	1	18										
	2	—	1	3	6	2	—	—	—	1	13										
	3	1	—	2	3	4	1	—	—	—	11										
	4	—	—	—	2	1	1	2	—	—	6										
	5	—	—	—	—	—	—	—	—	—	—										
	6	—	—	—	—	—	—	—	—	—	—										
	7	—	—	—	—	—	—	—	—	—	—										
	NE[a]	3	—	1	1	—	—	—	—	—	5										
Total		241	12	14	13	7	2	2	—	9	300										

[a] NE = not examined.

TABLE 10
AGREEMENT IN CLASSIFICATION
BY TWO TESTS USING DIFFERENT ANTIGEN BATCHES

Infection	Identical results		Difference in titres ≤2	
	No.	%[a]	No.	%[a]
Chikungunya	199	69.6	256	89.5
Sindbis	227	79.4	274	95.8
Bunyamwera	212	74.1	260	90.9
Zika	250	87.4	275	96.2
Yellow fever	254	88.8	282	98.6
West Nile	209	73.1	263	92.0
Banzi	235	82.2	260	90.9

[a] Percentage of samples that were examined in both the first and second tests.

It holds true for both types of errors involved in serological surveys—namely, the inaccuracy of the antibody determinations and the lack of time relevance of the prevalence figures—that they do *not* produce false results but merely tend to obscure variations and correlations that may truly exist in the populations examined. It follows from this that differences and correlations that can be demonstrated in the survey data attain a higher significance since they have been strong enough to emerge in spite of the obscuring factors involved.

Although the prevalence data lacked precision, the results obtained in the present serological survey clearly revealed a significant geographical variation in the prevalence of arbovirus infections in Kenya. Chikungunya infection showed the most dramatic geographical variation; as many as 50% of all people had chikungunya antibodies in Central Nyanza and in Malindi District, whereas this antibody was virtually absent in Kitui District. The environmental factor most likely to be responsible for this difference seems to be the pronounced dryness that distinguishes Kitui District from the other two survey areas. However, another factor which seems to be associated with the risk of acquiring infection—namely, the density of human dwellings—emerged from the survey. The results showed unequivocally that people who live in houses situated close together stand a greater risk of acquiring arbovirus infections than people who live under less crowded, but otherwise similar, conditions. This fact may partly account for the absence of chikungunya infection in Kitui where the houses are much more scattered than they are in Central Nyanza and in Malindi District. The reason for the higher risk of infection in dense-population groups may simply be that insect vectors and humans thrive in the same environment, where, for instance, streams and other water sources are frequent. In the present survey, however, no correlation could be demonstrated between the risk of arbovirus infection and the nearness of natural waters such as streams, swamps or lakes. There may, therefore, be a special effect of population density and it is possible that the availability of human sources of infection is a critical factor in determining the rate of arbovirus transmission.

Another group-A arbovirus of special interest in East Africa is o'nyong-nyong. This virus cannot be distinguished immunologically from chikungunya virus and these two viruses have been considered as identical in the present report. An o'nyong-nyong epidemic with a very high incidence rate originated in Uganda in 1959 (A. J. Haddow et al., 1960; Williams et al., 1965) and spread to Nyanza Province in Kenya; during 1960 approximately 50% of the Nyanza population was estimated to have been infected. The findings of the present survey are in agreement with a high incidence of o'nyong-nyong infection in 1959–60 in Nyanza but do not support the assumption that the epidemic was of a temporary nature and that it ceased soon after 1960. On the contrary, the finding of about 50% positive HI chikungunya reactions in all age-groups down to the 0–4-years age-group seems to indicate that the transmission of chikungunya/o'nyong-nyong is still continuing unchecked.

At the time of the o'nyong-nyong outbreak in Kenya in 1959–60 it was reported that the epidemic did not reach the coast. This observation appears somewhat at variance with the results of the present survey, which revealed a massive chikungunya immunity in all age-groups in Malindi District, suggesting a continued transmission in the area similar to the transmission which is continuing in Central Nyanza.

O'nyong-nyong was not reported from Kitui District even though anopheline mosquitos which are the vectors of this arbovirus are known to occur there. The survey confirmed that o'nyong-nyong/chikungunya infection is virtually absent from Kitui District and further suggested that the very scattered nature of the human population in Central Nyanza may be, in part, responsible for this lack of transmission.

TABLE 11

PREVALENCE OF 7 ARBORVIRUS INFECTIONS,[a] BY DENSITY OF HOUSES AND BY SURVEY AREA

Survey area	No. of houses within a radius of 200 m	CK	SB	BW	ZK	YF	WN	BZ	No. of persons examined
Central Nyanza	None	39.3	0.0	10.7	0.0	0.0	0.0	0.0	28
	1– 2	61.3	3.6	4.4	2.9	1.5	3.6	0.0	137
	3– 5	53.4	5.0	4.4	3.4	3.4	3.1	2.8	320
	6–10	49.7	6.2	5.1	2.3	1.7	2.8	0.0	177
	>10	59.4	6.9	12.5	5.0	3.8	3.8	1.9	160
	Total	54.6	5.2	6.3	3.3	2.7	3.2	1.5	822
Kitui District	None	1.5	10.6	5.5	2.0	1.0	19.1	9.5	199
	1– 2	0.8	12.4	6.4	1.1	1.4	12.8	5.9	643
	3– 5	1.2	15.9	2.4	1.2	1.8	11.6	5.5	164
	6–10	0.0	9.1	3.0	3.0	3.0	12.1	9.1	33
	>10	—	—	—	—	—	—	—	—
	Total	1.0	12.5	5.6	1.3	1.4	13.8	6.7	1 042 [b]
Malindi District	None	39.1	8.7	21.7	39.1	32.6	63.0	41.3	46
	1– 2	50.0	13.5	23.4	53.1	37.5	55.2	49.0	192
	3– 5	53.8	23.1	30.7	50.7	42.9	64.4	48.6	424
	6–10	35.6	16.9	30.5	55.9	35.6	59.3	47.5	59
	>10	54.0	16.8	24.8	58.4	53.1	81.4	64.6	113
	Total	50.8	18.8	26.5	52.0	42.0	65.3	50.4	834
All areas	None	11.7	9.2	8.8	8.1	6.2	24.5	13.9	273
	1– 2	19.0	11.4	9.5	11.6	8.5	20.9	13.6	972
	3– 5	44.2	15.4	15.2	25.1	21.6	33.3	24.7	908
	6–10	40.5	8.9	10.4	14.1	9.3	16.4	11.5	269
	>10	57.1	11.0	17.6	27.1	24.2	35.9	27.8	273
	Total	32.7	12.2	12.3	17.6	14.3	26.5	18.6	2 698 [b]

[a] BW = Bunyamwera, BZ = Banzi, CK = chickungunya, SB = Sindbis, WN = West Nile, YF = Yellow fever, ZK = Zika.
[b] Including 3 persons for whom density of housing was not recorded.

Bunyamwera virus is one member of a large group of related mammalian viruses and was originally isolated in Uganda (Smithburn et al., 1946). More recently, this virus has been isolated from mosquitos collected near Malindi (D. Metselaar, unpublished data). Antibodies to Bunyamwera virus were present in 26.5% of the population sampled near Malindi, indicating that human infection is not uncommon in this area; only 5%–6% of sera from Nyanza and Kitui were positive to Bunyamwera antigen.

The survey results showed that group-B arbovirus infections are much more prevalent in Malindi District than in the two other survey areas. This was true of all 6 antigens that were included in the HI

testing, with the exception of chikungunya, which was just as common in Central Nyanza.

In group B the greatest interest centres on yellow fever since serious outbreaks of this disease have occurred recently in countries near Kenya—Ethiopia and the Sudan, for example. Recent studies in northern Kenya (Henderson et al., 1968a) showed that approximately 15% of the population in Marsabit and Lokitoung had yellow fever neutralizing antibodies. The results of the present HI testing showed that antibodies to yellow fever are practically absent in Central Nyanza (2.7%) and in Kitui (1.4%) but very prevalent in Malindi (42.0%).

There is no clinical, or other, evidence that yellow fever epidemics have ever occurred in the human population of Kenya. The yellow fever immunity revealed by the HI test must, therefore, be due either to vaccination or to cross-immunity with other group-B arbovirus infections. It is known that some 300 000–400 000 yellow fever vaccinations were carried out in the coastal area of Kenya in 1941 and that some sporadic vaccination has been done in other parts of Kenya since then. Since the total population of the Coast Province amounts to more than a million persons it is impossible that the comparatively few vaccinations made 25 years ago could account for all the HI reactions observed in the present survey in which 69.9% of all people over 30 years of age, and 12.7% of persons under 20 years of age, had positive reactions. It follows, therefore, that some at least of the immunity revealed by the HI test must be due to cross-reactions with other group-B antibodies.

No yellow fever neutralizing antibodies were detected in the survey in persons under 20 years of age and of 73 adults examined only 13 (17.8%) revealed the presence of yellow fever neutralizing antibodies. These findings are compatible with the hypothesis that yellow fever neutralizing antibodies are caused by vaccination.

It is known that *Aedes aegypti* and *Ae. simpsoni* are prevalent in the Kenya coastal area and that these mosquitos are largely absent from Central Nyanza and Kitui District (E. C. C. van Someren, unpublished data). The absence of yellow fever epidemics at the coast in spite of vectors being available and outbreaks of yellow fever occurring in adjacent countries may be explained in terms of cross-immunity in the human population. Recently, it has been shown (Henderson et al., 1968b) that rhesus monkeys immunized with Wesselsbron virus do not manifest viraemia when challenged with yellow fever virus. If these findings can be extended to man, then it is conceivable that the high level of immunity to Wesselsbron infection which was revealed by the present survey in the population of the coastal area of Kenya may interfere with man–mosquito transmission of yellow fever and partly account for the historical absence of yellow fever epidemics from the coast of East Africa.

Recent outbreaks of dengue fever in South-East Asia have been associated with haemorrhagic manifestations (Johnson et al., 1967) and it is, therefore, interesting to consider whether similar outbreaks may be expected in East Africa. The primary vector of dengue, *Ae. aegypti*, is present in the coastal area of Kenya but outbreaks of typical dengue fever have not been reported there. The HI testing in the present serological survey showed that dengue antibodies are prevalent at the coast (47%) but practically absent in the other two survey areas. Whether HI reactions observed in Malindi District are caused by dengue transmission or are due to cross-reactions with other group-B infections is difficult to decide. One-third of the Malindi sera which were tested for dengue 1 neutralization gave positive reactions, indicating that some at least of the dengue-immune reactions may be specific and that dengue transmission has taken place in the coastal area of Kenya in the past. In view of the absence of dengue immunity from more than half of the population at the coast and of the high density of *A. aegypti* there, it seems possible that dengue fever may reappear at the coast and there is a definite need for careful surveillance to detect any recrudescence of this disease.

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RÉSUMÉ

ENQUÊTE SÉROLOGIQUE À FINS MULTIPLES AU KENYA: 2. RÉSULTATS DES EXAMENS SÉROLOGIQUES EN CE QUI CONCERNE LES ARBOVIRUS

Des échantillons de sérums recueillis au Kenya au cours de l'enquête décrite dans l'article précédent ont été envoyés à l'East African Virus Research Institute, à Entebbe (Kenya), où l'on a procédé à la recherche des anticorps actifs contre les arbovirus. Pour les épreuves d'inhibition de l'hémagglutination (IH), on a utilisé trois antigènes du groupe A (chikungunya, o'nyong-nyong et Sindbis), six antigènes du groupe B (fièvre jaune, Zika, Wesselsbron, West Nile, Banzi et dengue 1) et un antigène Bunyamwera. Afin d'évaluer la spécificité de l'épreuve d'IH, on a aussi examiné un certain nombre de sérums en épreuve de neutralisation avec les antigènes fièvre jaune, Wesselsbron et dengue 1. Le degré de l'erreur inhérente à la pratique de l'épreuve d'IH, apprécié par des tests répétés et par le titrage « aveugle » de deux spécimens d'un même sérum, a été reconnu comme non négligeable.

Les résultats ont fait ressortir de très fortes variations de la prévalence des arbovirus suivant les régions. Dans l'ensemble, l'infection était beaucoup plus répandue dans le district de Malindi, sur le littoral de l'océan Indien, que dans les districts de Nyanza (lac Victoria) et de Kitui (centre du Kenya). Seuls les virus chikungunya et o'nyong-nyong ont été décelés avec une fréquence également élevée à Nyanza (54,6%) et à Malindi (50,8%). Le virus amaril était pratiquement absent à Nyanza (2,7%) et à Kitui (1,4%), mais fortement prévalent à Malindi (42,0%). Aucune épidémie de fièvre jaune n'ayant été signalée au Kenya, l'immunité antiamarile ainsi révélée semble devoir être attribuée aux vaccinations ou à une réactivité croisée des sérums avec d'autres antigènes du groupe B.

Le seul facteur de milieu identifié comme responsable d'une plus grande fréquence de l'infection par les arbovirus est la densité des habitations, les personnes vivant dans des maisons très proches les unes des autres y étant plus exposées que les habitants de maisons disséminées.

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