Serological studies on the epidemiology of sandfly fever in the Old World

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Selected human sera from 59 different localities in Africa, the Mediterranean littoral, eastern Europe and Asia were examined by plaque reduction neutralization test against eight sandfly (Phlebotomus) fever virus serotypes (Sicilian, Naples, Arumowot, SudAn 754-61, Karimabad, Salehabad, Gordil and Saint Floris) known to occur in the Old World. Results of these studies provide new information on the geographic distribution and prevalence of human infection with each of the viruses. Specific neutralizing antibodies were detected against all of the agents except Salehabad. Naples and Sicilian antibodies were encountered most frequently and had the widest geographic range; moreover they were found only in areas where Phlebotomus papatasi *occurs. Age-specific antibody rates for several of the viruses are presented. These data and the epidemiology of sandfly fever are discussed.*

Although sandfly (or Phlebotomus) fever has long been recognized as a disease of some public health importance in countries bordering on the Mediterranean and in central Asia, little information is available on the extent of its geographical distribution, the virus serotypes responsible for the illness, or the prevalence of infection among indigenous human populations. To date, eight antigenically distinct Phlebotomus fever virus serotypes (Naples, Sicilian, Arumowot, SudAn 754-61, Karimabad, Salehabad, Gordil, and Saint Floris) have been isolated in the Old World (*1-4*). While the human pathogenicity of the Naples and Sicilian viruses has been well documented (*5*, *6*), the other six serotypes have been recovered only from insects or animals and their ability to infect man is unknown. In order to answer some of these questions, we recently examined human sera from 59 different localities in Africa, the Mediterranean region, eastern Europe, and Asia for neutralizing antibodies against the eight Old World representatives of the Phlebotomus fever group. The present paper reports results of this study and presents new data on the epidemiology of sandfly fever.

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MATERIALS AND METHODS

Source of human sera and populations sampled

Table 1 lists the 59 localities studied, the type of populations sampled, and the year in which the sera were obtained and Fig. 1 shows the approximate geographical locations. The human sera utilized in this study came from many different sources. For this reason and because of the difficulties in transporting sera from different countries, some of the specimens were received frozen, others were lyophilized, and a number were obtained dried on filter paper discs. Most of the sera from a given locality were from populations living within a single geographical region (i.e., city, town or province); in a few cases, however, the exact origin of the donors was unknown or the specimens were collected from several areas within a country. In the latter cases, only the country of origin is listed in Table 1. The sources of the sera are given in Annex 1.

Viruses

The sera were tested against eight different Phlebotomus fever virus serotypes known to occur

Table 1. Prevalence of neutralizing antibodies against 6 Phlebotomus fever virus serotypes among selected human populations in the Old World

	Locality [a]	Population sampled and date	Total no. sera tested	Percentage positive [b]					
				Sicilian	Naples	Arumowot	SudAn 754-61	Karimabad	Salehabad
1	Senegal	Children — 1966	50	0.0	0.0	0.0	0.0	—	—
2	Zigida, Liberia	Mixed — 1972	99	0.0	0.0	0.0	0.0	0.0	—
3	Ghana	Adults — 1963	34	0.0	0.0	0.0	0.0	0.0	—
4	Nigeria	Mixed — 1970–74	94	0.0	0.0	0.0	0.0	—	—
5	Northern Nigeria	Adults — 1966	115	0.0	0.0	0.0	2.6	0.0	0.0
6	Northern Kenya	Mixed — 1972	24	0.0	0.0	0.0	0.0	0.0	—
7 A	Giohar District, Somalia	Adults — 1966	50	12.0	0.0	0.0	0.0	—	—
7 B	Giohar District, Somalia	Mixed — 1960–63	48	2.1	0.0	2.1	0.0	0.0	0.0
8	Territory of the Afars and Issas [c]	Mixed — 1974	127	0.0	3.1	0.0	0.0	0.0	—
9 A	Omo Valley, Ethiopia [c]	Mixed — 1972	60	0.0	3.3	0.0	0.0	0.0	—
9 B	Omo Valley, Ethiopia [c]	Mixed — 1974	89	0.0	0.0	0.0	0.0	—	—
10	Khartoum Province, Sudan	Adults — 1975	207	13.0	14.0	1.4	27.5	4.3	—
11	El Gezira Province, Sudan	Adults — 1975	80	13.8	16.3	0.0	26.3	1.3	—
12	Equatoria Province, Sudan	Adults — 1975	5	20.0	0.0	80.0	60.0	0.0	—
13	Northern Province, Sudan	Adults — 1975	6	16.7	33.3	16.7	16.7	0.0	—
14	White Nile Province, Sudan	Adults — 1975	7	14.3	14.3	0.0	0.0	0.0	—
15	Upper Nile Province, Sudan	Mixed — 1960–63	91	6.6	0.0	72.5	34.1	11.0	0.0
16	Qalyub, Egypt	Mixed — 1952–54	252	22.6	31.0	0.0	0.0	0.0	0.0
17	Cairo, Egypt	Children — 1975	181	5.0	6.1	0.0	0.0	0.0	—
18	Giza, Egypt	Adults — 1960–63	16	43.8	43.8	0.0	0.0	0.0	0.0
19	Alexandria, Egypt	Adults — 1960–63	37	27.0	21.6	0.0	0.0	0.0	0.0
20	Baltim-Borg Burulus, Egypt	Mixed — 1960–63	45	8.9	6.7	0.0	0.0	0.0	0.0
21	Luxor, Egypt	Adults — 1960–63	64	59.4	56.3	1.6	3.1	0.0	0.0
22	Siwa, Egypt	Mixed — 1960–63	51	2.0	3.9	0.0	0.0	0.0	0.0
23	Sidi Barrâni, Egypt	Mixed — 1960–63	21	4.8	9.5	0.0	9.5	0.0	0.0
24	Bahig, Egypt	Mixed — 1960–63	64	6.3	25.0	1.6	0.0	0.0	0.0
25	El Daba, Egypt	Adults — 1960–63	36	8.3	13.9	8.3	0.0	0.0	0.0
26	Aswan, Egypt	Adults — 1960–63	55	43.6	47.3	0.0	7.3	1.8	0.0
27	Tunisia	Mixed — 1975	155	1.3	0.0	0.0	0.0	0.0	—
28	Tamanrasset, Algeria	Mixed — 1975	46	0.0	0.0	0.0	0.0	0.0	—
29	Djanet, Algeria	Mixed — 1975	37	0.0	0.0	0.0	0.0	0.0	—
30	Marrakech Province, Morocco	Mixed — 1976	20	0.0	0.0	0.0	0.0	—	—
31	Beni-Mellal Province, Morocco	Mixed — 1976	30	0.0	0.0	0.0	0.0	—	—
32	d'Oujada Province, Morocco	Mixed — 1976	27	0.0	0.0	0.0	0.0	—	—
33	Midelt-Itzer, Morocco	Mixed — 1976	35	5.7	2.9	0.0	8.6	—	—
34	Crete, Greece	Adults — 1976	38	0.0	13.1	—	—	—	—
35	Athens, Greece	Mixed — 1973–75	632	8.5	24.7	—	—	—	—
36	Arachova, Greece	Children — 1973	96	0.0	0.0	0.0	0.0	0.0	—
37	Brač, Dalmatia Province, Yugoslavia	Adults — 1975	212	15.6	57.6	—	—	—	—
38	Kosovo Province, Yugoslavia	Mixed — 1975	104	9.6	27.9	—	—	—	—
39	Antalya, Turkey	Adults — 1955	50	22.0	62.0	—	—	0.0	—
40	Massayeb-Al-Kabir, Iraq [c]	Mixed — 1972–73	40	2.5	7.5	—	—	0.0	—
41	Saudi Arabia	Adults — 1967	34	20.6	5.9	0.0	0.0	0.0	—
42	Tabriz, East Azerbaijan Province, Iran	Adults — 1971	100	12.0	26.0	—	—	1.0	0.0
43	Rasht, Gilan Province, Iran	Adults — 1971	93	12.9	21.5	—	—	0.0	0.0
44	Khorasan Province, Iran [c]	Mixed — 1971–75	336	9.5	17.9	—	—	11.0	0.0
45	Tehran Province, Iran [c]	Mixed — 1971–75	257	21.4	30.4	—	—	8.2	0.0
46	Kermanshah Province, Iran	Adults — 1975	32	9.4	28.1	—	—	0.0	0.0
47	Isfahan Province, Iran [c]	Mixed — 1974–75	620	21.8	13.5	—	—	62.1	0.0
48	Khuzestan Province, Iran [c]	Mixed — 1973–75	486	20.4	13.2	—	—	0.2	0.0
49	Moldavian SSR, USSR	Adults — 1976	163	3.7	2.5	—	—	0.0	—
50	Azerbajdžan SSR, USSR	Adults — 1976	98	3.1	3.1	—	—	1.0	—
51	Uzbek SSR, USSR	Adults — 1976	197	7.6	4.1	—	—	1.0	—
52	Tadžik SSR, USSR	Adults — 1976	158	6.3	2.5	—	—	10.1	—
53	Turkmen SSR, USSR	Adults — 1976	100	2.0	6.0	—	—	53.0	—
54	Karachi, Pakistan	Mixed — 1965	75	2.7	9.3	—	—	0.0	0.0
55	Dacca, Bangladesh	Mixed — 1965	75	2.7	12.0	—	—	0.0	0.0
56	Rangoon, Burma	Mixed —	51	0.0	0.0	—	—	—	—
57	Southern Viet Nam	Mixed — 1972	95	0.0	0.0	—	—	0.0	—
58	Northern Peninsular Malaysia	Adults — 1973	103	0.0	0.0	—	—	0.0	0.0
59	Northern China	Adults — 1974–75	379	0.0	0.0	—	—	0.0	0.0

[a] Number refers to locality identification shown in Fig. 1.

[b] No. positive sera/total no. sera tested. Sera producing ⩾80 % plaque inhibition were recorded as positive. Dash = not tested.

[c] Sera tested at 1 : 20 dilution: the remainder were tested at 1 : 10 serum dilution.

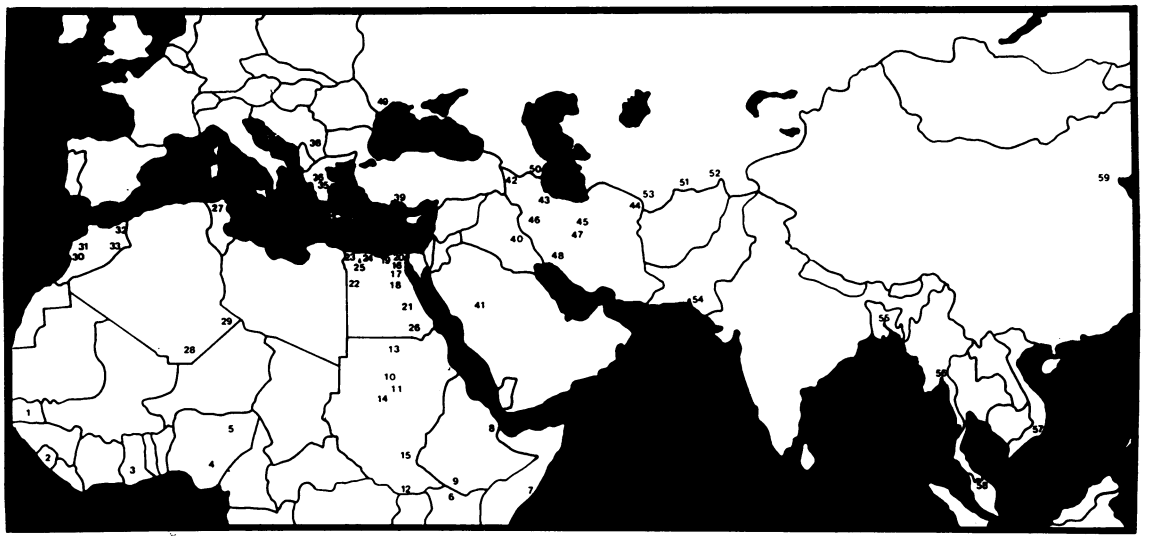

Fig. 1. Map showing the approximate location of the 59 populations sampled. The numbers on the map are identified in Table 1.

in the Old World (*1-4*). The virus strains used were as follows: the prototype Sicilian and Naples strains; Arumowot, strain Ar 1284-64; an undescribed serotype, strain SudAn 754-61; Karimabad, strain 1-58; Salehabad, strain 1-81; Gordil, strain DakAnB 496d; and Saint Floris, strain DakAnB 512. The Sicilian, Naples, Arumowot, Karimabad, and Salehabad strains were obtained from the American Type Culture Collection, Rockville, MD. SudAn 754-61 virus was received from the Center for Disease Control, Atlanta, GA. Gordil and Saint Floris viruses were provided by Dr R. E. Shope, Arbovirus Research Unit, Yale University, School of Medicine, New Haven, CT. Pools of each of the viruses were prepared from infected Vero cell cultures for use in neutralization tests.

Neutralization tests

All human serum specimens were screened at a single dilution (1:10 or 1:20) as noted in Table 1. Serum or blood specimens received on filter paper discs (Schleicher and Schuell, No. 740-E) were eluted overnight at 5°C in phosphate-buffered saline (PBS-G), pH 7.2, containing 0.5% gelatin, penicillin (400 IU/ml) and streptomycin (400 μg/ml) as described in an earlier publication (*7*). Hyperimmune rodent sera were tested in serial twofold dilutions from 1:10 to 1:20 480. All serum dilutions were made in PBS-G. Prior to testing, the sera were inactivated at 56°C for 30 min.

All specimens were tested by the plaque reduction neutralization method (*2, 6*) in 24-well microtitration plate cultures of Vero cells against a fixed virus dose of 40–100 plaque forming units. A single well was used for each human serum; duplicate wells were used for titrations of rodent antisera. The serum–virus mixture was incubated overnight at 5°C prior to inoculation. A double overlay system, employing 1.6% gum tragacanth in the initial overlay, was used and has been described previously (*2, 6*). Magnesium chloride (25 mmol/litre) was added to both overlays as this salt was found to increase the size and improve readability of the virus plaques (*8*). Cultures were read 6–10 days after inoculation. Those sera producing 80% plaque inhibition or more were recorded as positive, indicating specific neutralizing antibodies. All serological tests were performed at the Pacific Research Section, Honolulu.

RESULTS

The results of cross-neutralization tests between the eight Old World Phlebotomus fever virus serotypes and their specific immune sera are summarized in Table 2. With the exception of Saint Floris, each of the virus serotypes was distinct by

Table 2. Results of cross-neutralization tests with 8 Phlebotomus fever virus serotypes [a]

Immune serum	Virus							
	Naples	Sicilian	Arumowot	SudAn 754-61	Gordil	Saint Floris	Karimabad	Salehabad
Naples	*2560*	<10	<10	<10	<10	40	<10	<10
Sicilian	<10	*10240*	<10	<10	<10	<10	<10	<10
Arumowot	10	<10	*≥5120*	<10	<10	40	<10	<10
SudAn 754-61	<10	<10	<10	*2560*	<10	<10	20	<10
Gordil	20	<10	<10	<10	*2560*	1280	<10	<10
Saint Floris	<10	<10	<10	<10	<10	*10240*	<10	<10
Karimabad	<10	<10	<10	40	<10	<10	*1280*	<10
Salehabad	<10	<10	<10	<10	<10	10	10	*5120*

[a] The figures given indicate the highest serum dilution producing ≥80 % plaque inhibition.

the plaque reduction neutralization test and little heterologous crossing occurred. A one-way cross between Saint Floris virus and Gordil antiserum was noted, however, indicating that these two virus serotypes are antigenically more closely related.

Table 1 lists the neutralization test results obtained with Sicilian, Naples, Arumowot, SudAn 754-61, Karimabad, and Salehabad viruses on the human sera from each of the 59 localities sampled. Fig. 1 shows the approximate location of the populations sampled: Fig. 2 the known distribution of *Phlebotomus papatasi.*

Sicilian and Naples viruses

Sicilian and Naples virus neutralizing antibodies usually occurred together, thus their geographical distribution was very similar (Table 1 and Fig. 1). Antibodies against Sicilian virus were observed

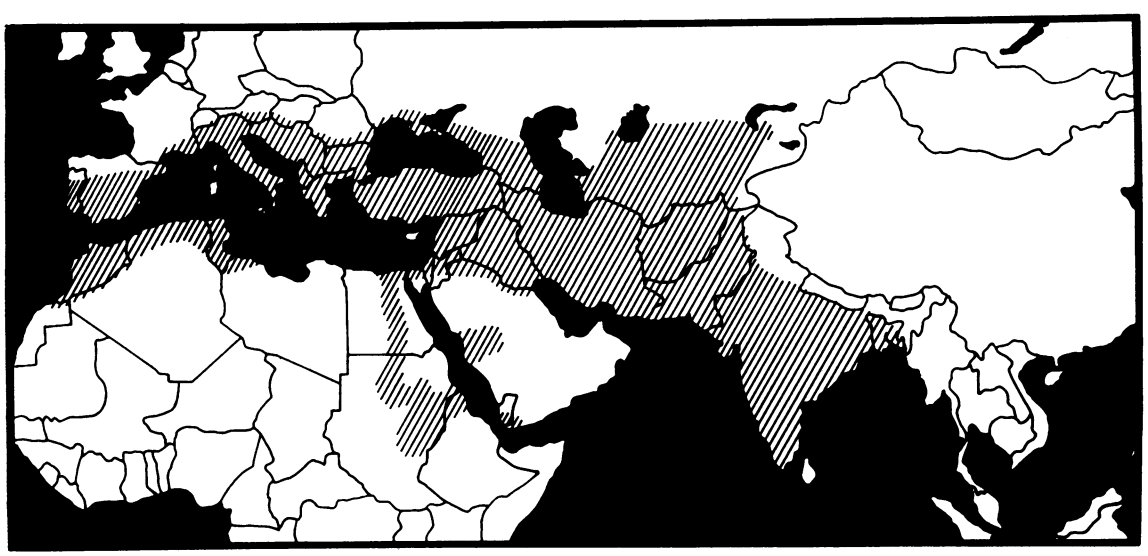

Fig. 2. A partial map of the Old World. The hatched areas represent the known distribution of *Phlebotomus papatasi.* (This map was prepared by Dr D. J. Lewis, Natural History Section, British Museum, London.)

Table 3. Prevalence of Sicilian and Naples virus neutralizing antibodies in 3 representative populations (percentage positive)

Age group (years)	Qalyub district, Egypt			Isfahan Province, Iran			Khuzestan Province, Iran		
	Total no. tested	Sicilian	Naples	Total no. tested	Sicilian	Naples	Total no. tested	Sicilian	Naples
1–5	52	13.5 [a]	17.3 [a]	98	0.0	2.0	110	13.6	0.9
6–10	51	21.6	31.4	161	3.1	2.5	169	19.5	7.1
11–20	89	20.2	38.2	76	10.5	15.8	121	23.1	24.8
21–30	14	42.9	21.4	48	41.7	22.9	43	25.6	23.3
31–40	14	42.9	28.6	41	36.6	34.1	17	17.6	23.5
41–50	11	36.4	27.3	34	32.4	44.1	13	15.4	30.8
51–60	11	9.1	33.3	20	40.0	50.0	4	50.0	0.0
> 60	10	40.0	50.0	8	12.5	50.0	9	55.5	33.3

[a] Percentage positive.

among human populations living in Bangladesh, Egypt, Greece, Iraq, Iran, Morocco, Pakistan, Saudi Arabia, Somalia, Sudan, Tunisia, Turkey, the USSR, and Yugoslavia. Naples antibodies were also found in all of the above countries except Somalia and Tunisia. In addition, Naples antibodies alone were present in sera from Ethiopia and the adjoining Territory of the Afars and Issas. No antibodies against Sicilian or Naples virus were found among populations sampled in West Africa, South-East Asia, and China. Interestingly, antibodies to these 2 agents were detected only in areas where *Phlebotomus papatasi* occurs (Fig. 1 and 2).

Table 3 shows the prevalence of Sicilian and Naples virus neutralizing antibodies by age group in 3 different communities in Egypt and Iran. In general, the prevalence of antibodies to both agents appears to increase with age. Results obtained in Qalyub with Naples and Sicilian, and in Khuzestan with Sicilian indicate that these viruses are endemic in both areas and that many of the inhabitants are infected during childhood. Unfortunately, in most of the communities tested, the sample size and age distribution of the population were inadequate to determine the age-specific antibody rates.

Arumowot and SudAn 754-61 viruses

Since Arumowot and SudAn 754-61 viruses have been isolated only in Africa (*1*, *2*), neutralization studies with these two agents were restricted mainly to human sera from that region. Arumowot antibodies were found among populations in Egypt, Somalia, and Sudan (Table 1). Arumowot infection rates in most communities were low, except for two provinces in southern Sudan (Equatoria and West Nile) where the antibody prevalences were 80.0 and 72.5%, respectively.

Neutralizing antibodies against SudAn 754-61 virus were observed among persons living in Egypt, Morocco, Nigeria, and Sudan. In Sudan the prevalence of SudAn 754-61 antibodies varied from 0 to 60.0%; however, human infection rates in the other three countries were generally low.

Karimabad and Salehabad viruses

Karimabad virus neutralizing antibodies were recorded in 2 geographically isolated regions of Central Asia and Africa (Table 1 and Fig. 1). The highest prevalence was recorded among residents of central and north-eastern Iran (Isfahan, Teheran and Khorasan Provinces) and neighbouring regions of the USSR (Tadžik and Turkmen Soviet Socialist Republics). A second focus of Karimabad antibodies was observed in southern Egypt (Aswan) and Sudan.

Table 4 compares the prevalence of Karimabad virus neutralizing antibodies among residents of Isfahan and Khorasan Provinces in Iran. In both populations antibody rates increase with age. Results from Isfahan Province indicate that most of the residents are infected during childhood.

No antibodies against Salehabad virus were detected in any of the human population tested (Table 1).

Table 4. Prevalence of Karimabad virus neutralizing antibodies among residents of Isfahan and Khorassan Provinces, Iran

Age group (years)	Isfahan Province		Khorasan Province [a]	
	No. positive/ total no. tested	Percentage positive	No. positive/ total no. tested	Percentage positive
1–5	38/98	38.8	0/17	0.0
6–10	101/161	62.7	0/31	0.0
11–15	36/51	70.6	9/46	19.6
16–20	20/25	80.0	2/8	25.0
21–30	33/48	68.8	7/16	43.8
31–40	37/41	90.2	7/11	63.6
41–50	26/34	76.5	7/11	63.6
51–60	16/20	80.0	0/1	—
>60	7/8	87.5	3/5	60.0

[a] Ali-Abad and Esmaiil-Abad villages only.

Gordil and Saint Floris viruses

Since these two agents were received near the end of our study, only a subsample of the African sera were tested against them. The results of these studies are summarized in Table 5. Neutralizing antibodies against Gordil virus were detected in one serum each from Somalia and Sudan. Saint Floris antibodies were found in residents of southern Egypt, Somalia, and Sudan. Despite the close antigenic relationship between Saint Floris and Gordil viruses noted previously with rodent hyperimmune sera (Table 2), none of the human sera neutralizing Saint Floris virus were positive with Gordil or vice versa.

Table 5. Prevalence of Gordil and Saint Floris virus neutralizing antibodies in selected African populations

	Locality [a]	Total no. sera tested	Percentage positive [b]	
			Gordil	Saint Floris
5	Northern Nigeria	51	0.0	0.0
6	Northern Kenya	24	0.0	0.0
7 A	Giohar District, Somalia	24	4.2	—
7 B	Giohar District, Somalia	47	0.0	2.1
9 A	Omo Valley, Ethiopia	57	0.0	0.0
15	Upper Nile Province, Sudan	91	1.1	13.2
26	Aswan, Egypt	55	0.0	1.8
28	Tamanrasset, Algeria	21	0.0	—
29	Djanet, Algeria	37	0.0	—

[a] Number refers to locality identification in Table 2 and Fig. 1.
[b] No. positive sera/total no. sera tested. Sera producing ⩾ 80 % plaque inhibition were recorded as positive.

DISCUSSION

The results of this study provide new information on the geographical distribution and prevalence of human infection with the eight known Phlebotomus fever virus serotypes of the Old World. Previous knowledge of their occurrence has been obtained mainly from field isolations. Although important, these recoveries often reflect the location of virus laboratories and do not necessarily indicate the complete geographical distribution of the viruses. Furthermore, most of the field isolations have been made from animals or insects, consequently little is known about the ability of many of these viruses to infect man.

Sicilian and Naples viruses

The prototype strain of Sicilian virus was isolated by Sabin & Sweet (9) from pooled sera collected from two sick soldiers in Italy in 1943. Subsequent recoveries of this agent been made from humans and/or sandflies in Egypt, India, Iran, and Pakistan (1, 10, 11). Results of the present study (Table 1) indicate that Sicilian virus is also present in Bangladesh, Greece, Iraq, Morocco, Saudi Arabia, Somalia, Sudan, Tunisia, Turkey, the southern European and central Asian republics of the USSR, and Yugoslavia. Previous serological studies have found a low prevalence of Sicilian haemagglutinating antibodies among human residents of southern France (C. Hannoun, personal communication, 1976) and Portugal (*12*), suggesting that the virus may also be present in these two countries.

Naples virus was first isolated from a febrile patient in Italy in 1944 (*9*): additional recoveries have been made in Egypt, India, Iran, Pakistan and the Soviet Union (*1*, *11*, *13*). Our serological results (Table 1) extend the known distribution of this agent to include Bangladesh, Ethiopia, Greece, Iraq, Morocco, Saudi Arabia, Sudan, Territory of the Afars and Issas, Turkey, and Yugoslavia. The absence of Naples and Sicilian virus neutralizing

Table 6. Expected and observed frequencies of persons with Naples and Sicilian virus neutralizing antibodies in 3 different communities

	Qalyub, Egypt	Isfahan Province, Iran (person >10 years)	Khuzestan Province, Iran
Naples positive	0.310 (78/252[a])	0.300 (68/227)	0.132 (64/486)
Sicilian positive	0.226 (57/252)	0.278 (63/227)	0.204 (99/486)
Expected frequency of double reactors	0.070 (0.310×0.226)	0.083 (0.300×0.278)	0.027 (0.132×0.204)
Observed frequency of double reactors	0.087 (22/252)	0.106 (24/227)	0.047 (23/486)

[a] No. positive/Total no. tested (see Table 3).

antibodies among populations in central Africa and eastern Asia suggests that these agents do not occur in these two regions.

The presence of Naples and Sicilian virus neutralizing antibodies among residents of Dalmatia, Yugoslavia (Table 1) is of some historical interest, since this is the region where the original studies on the epidemiology of sandfly fever were carried out by Doerr et al. (14). Although the virus serotype studied by Doerr and his coworkers is unknown, the predominance of Naples antibodies among the present adult residents of Dalmatia (ages 33 to 80 years) suggests that it may have been the Naples serotype.

Several characteristics of the human antibody patterns observed with Naples and Sicilian viruses imply that both agents have a similar ecology. First, their geographical distribution is quite similar and seems to parallel that of *P. papatasi*. Reported isolations (*1*, *10*, *11*, *13*) as well as the results of our serological studies indicate that Naples and Sicilian viruses are present in the Mediterranean coastal regions of Europe and North Africa, the Nile valley, most of South-West Asia, areas adjacent to the Black and Caspian Seas, and central Asia as far east as Bangladesh (Table 1 and Fig. 1). As shown in Fig. 2, this closely approximates the known distribution of *P. papatasi*. Available evidence indicates that this sandfly species is the principal vector of both viruses (*10*, *15*, *16*). Interestingly, both cutaneous and visceral leishmaniasis are also endemic in most of the regions where Naples and Sicilian viruses occur (*17*).

Secondly, in many of the populations sampled, the prevalence of Naples and Sicilian neutralizing antibodies was similar (Tables 1 and 3). In order to confirm this observation and to check the specificity of our neutralization test results, we compared the expected and observed frequencies of persons with antibodies to both Naples and Sicilian viruses in three different communities. The results of these calculations are shown in Table 6. In each community the observed frequency of double positives was slightly higher than the expected, perhaps indicating some cross-reactivity. However, the higher observed frequency is also compatible with the hypothesis that persons with high sandfly exposure and infected with one Phlebotomus fever virus serotype are at greater risk of being infected with a second. The failure to demonstrate heterologous neutralizing antibodies in man (*6*) or in animals (Table 2) experimentally infected with Naples and Sicilian viruses also support the latter hypothesis and the specificity of our neutralization test results.

A third pattern that is noted with the Naples and Sicilian (Table 3) as well as with the Karimabad (Table 4) test results is that the respective antibody rates increase with age during the first 20 to 30 years of life, but do not continue to rise in the older age groups. There appear to be two possible explanations for this phenomenon. The most obvious is that neutralizing antibodies to Phlebotomus fever group viruses may have a relatively short life, their titres falling to nondetectable levels 20–30 years after infection. If the neutralization test detects only those persons with infections of relatively recent origin (i.e., within the past 20 years), then this might account for the plateau effect observed among the older age groups. However, this hypothesis is not supported by results of serological studies among Athens residents (*18*), which indicate that detectable levels of Naples and Sicilian neutra-

lizing antibodies persist for at least 30 years after infection.

Another explanation for the static antibody rates observed among the older population groups (Tables 3 and 4) might be that not all persons in a community have equal exposure to sandflies and that some people apparently escape infection. This latter hypothesis is compatible with field observations that the distribution of sandflies within a community is usually patchy (*7*, *19*). These insects often show a marked preference for certain quarters of a city or even for certain houses within a village. In view of the short flight range of these insects (*20-22*), the above data may indicate that some individuals or families within a community are at greater risk of virus infection than others. Such focal patterns of human infection and clustering of cases within a community have been described previously with cutaneous (*23*, *24*) and visceral leishmaniasis (kala azar) (*25*), two other sandfly-borne diseases.

Arumowot virus

Arumowot virus was first isolated in 1963 from mosquitos (*Culex antennatus*) collected in Sudan (*1*). Subsequent recoveries of this agent have been made in the Central African Republic,[a] Ethiopia, Nigeria, South Africa, and Southern Rhodesia all from animals or insects (*1*, *4*, *33*). Our results (Table 1) indicate that the virus is also present in Egypt and Somalia. Collectively, these data suggest that Arumowot virus is widely distributed in Africa. While the disease potential of Arumowot virus for man is unknown, the serological data show that man is infected with this agent. Furthermore, the high prevalence of Arumowot neutralizing antibodies in man in the Upper Nile and neighbouring Equatoria Provinces in Sudan suggest that the virus is endemic in this region. Interestingly, the six arthropod isolations of Arumowot virus have been from mosquitos (*1*, *33*). Experimental studies also have demonstrated that Arumowot virus multiplies in *Aedes albopictus* and *Culex fatigans* following intrathoracic inoculation (*26*), suggesting that this agent may actually be mosquito-borne.

SudAn 754-61 virus

SudAn 754-61 virus, an undescribed member of the Phlebotomus fever group, was originally isolated in 1961 from animals in Sudan (*4*). Additional recoveries of this agent have been made in Benin, Nigeria, and Senegal (*1*, *4*, *27*). Our neutralization test results (Table 1) add Egypt and Morocco to the known geographical distribution of the virus. Although these serological data indicate that human infection with SudAn 754-61 virus is fairly common in several African countries, its disease potential for man is unknown.

During arbovirus studies in West Africa between 1966 and 1970 (*4*), a total of 53 isolations of SudAn 754-61 was made from wild animals. Most of the recoveries were from rodents, suggesting that these animals are involved in the ecology of the virus. Interestingly, this agent has never been recovered from an arthropod.

As noted previously with Naples and Sicilian, the geographical distribution of Arumowot and SudAn 754-61 viruses also appears to overlap. Because of the high prevalence of antibodies to the latter two agents observed among residents of Upper Nile and Equatoria Provinces in Sudan (Table 1), we considered the posibility that some of these positives might be due to nonspecific cross-neutralization. In Upper Nile Province, neutralizing antibody rates for Arumowot and SudAn 754-61 viruses were 72.5 (66/91) and 34.1% (31/91), respectively. Using these values, the expected percentage of double positives would be 24.7% ($72.5 \times 34.1/100$). The observed percentage of double reactors to Arumowot and SudAn 754-61 among this population was 26.4% (24/91), suggesting that the neutralization test results were specific. This hypothesis is further supported by the results from Khartoum and El Gezira Provinces (Table 1) where 27.2% (78/287) of the combined population samples had neutralizing antibodies to SudAn 754-61 virus but only 1.0% (3/287) were positive to Arumowot.

Karimabad virus

The prototype strain of Karimabad virus was isolated from an unidentified pool of Iranian sandflies in 1959 (*1*). A total of 11 subsequent isolations of this agent have been made in Iran from *P. papatasi*, the presumed vector (*10*). Although the disease potential of Karimabad virus for man is unknown, our results (Table 1) indicate that there is a significant amount of human infection. The high Karimabad infection rates observed in Iran among residents of Isfahan and Khorasan Provinces (Table 4) suggest that the virus is highly endemic in this region. Interestingly, while Karimabad virus is present in some of the areas where

[a] Renamed Central African Empire in 1976.

the Naples and Sicilian serotypes occur (Table 1), it appears to have a much more limited geographical distribution than the latter two agents. Our serological data indicate that Karimabad virus is present in central and north-east Iran as well as in several of the neighbouring republics of the Soviet Union (Fig. 1). The presence of Karimabad neutralizing antibodies in residents of southern Egypt and Sudan was unexpected, suggesting that this virus or an antigenically related agent also occurs in the Nile Valley.

Salehabad virus

Salehabad virus was originally isolated from a single pool of Iranian sandflies in 1959 (*1*). No subsequent recoveries of this agent have been made. The absence of Salehabad virus neutralizing antibodies in any of the sera tested (Table 1) suggests that human infection with this agent is rare or does not occur.

Gordil and Saint Floris viruses

The two remaining Phlebotomus fever virus serotypes, Gordil and Saint Floris, were both isolated from rodents by French workers in the Central African Republic [a] in 1971 (*1*, *3*). No other recoveries of these viruses have been reported nor have they been associated with human illness. Since these two agents were received midway through our study, only a subsample of sera from selected African populations was tested against them. The results (Table 5) indicate that Gordil virus also occurs in Somalia and Sudan and that Saint Floris is present in these two countries as well as in Egypt. With the exception of Upper Nile Province, Sudan, where 13.2% (12/91) of the sample population had antibodies against Saint Floris virus, the prevalence of antibodies to these two agents was generally low, suggesting that they infrequently cause human infection.

The absence of Sicilian, Naples, Karimabad or Salehabad neutralizing antibodies in sera of residents of Burma, Viet Nam and Peninsular Malaysia (Table 1) suggests that these viruses do not occur in South-East Asia. Both *P. papatasi* (Fig. 2) and leishmaniasis (*17*) are also absent from this region.

The absence of Phlebotomus fever virus antibodies among former residents of North China is rather surprising, since an earlier report in the literature suggested that sandfly fever was present in this region. In 1915, R. A. Bolt, an American physician working in Peking, described a disease suggestive of classical sandfly fever that occurred each summer among foreign missionary personnel and students from South China (*28*). The disease affected only newcomers to the region and followed closely upon exposure to sandfly bites. Although *P. papatasi* does not occur in China (Fig. 2). *P. chinensis*, an important man-biting species and vector of visceral leishmaniasis, is widely distributed in the central and northern provinces of the country (*29*). In view of this, our data suggest that if sandfly fever occurs in China, it is probably due to an as yet unknown Phlebotomus fever virus serotype(s). This would not be surprising, since there are already at least 25 different serotypes recognized from various regions of the world (*1-3*).

Unfortunately, our serological studies do not answer the essential question—what is the real public health importance of Phlebotomus fever group viruses? The demonstration of specific neutralizing antibodies in human serum indicates previous virus infection, but it does not necessarily mean that a clinical illness occurred. While the pathogenicity of Naples and Sicilian viruses for man is well documented (*5*, *6*), the human disease potential of the other six Old World Phlebotomus fever virus serotypes is unknown. Additional studies on their pathogenicity are necessary before their public health importance can be fully evaluated.

Furthermore, there is some evidence that the clinical manifestations of sandfly fever may vary with age. In susceptible adults, inoculation of the Naples or Sicilian viruses produces classical sandfly fever, an acute self-limited illness of 2–4 days duration, characterized by fever, headache, anorexia, myalgia, photophobia, low-back, and retro-orbital pain (*5*, *6*). Several authors have suggested, however, that the disease in children is somewhat milder (*30-32*). This implies that the age at which infection occurs may influence the severity of the resulting illness. In areas where sandfly fever is endemic, most of the population is probably infected during childhood (*7*, and Tables 3 and 4) and suffers only a relatively mild illness. In contrast, when nonimmune adults (tourists, settlers, soldiers, etc.) enter an endemic area, classical sandfly fever usually results. Thus the Phlebotomus fever group viruses may be of potentially greater importance to outsiders than they are to persons residing within the endemic areas.

[a] Renamed Central African Empire in 1976.

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RÉSUMÉ

ÉTUDES SÉROLOGIQUES SUR L'ÉPIDÉMIOLOGIE DE LA FIÈVRE À PHLÉBOTOMES DANS L'ANCIEN MONDE

On a étudié des sérums humains provenant de 59 localités différentes de l'Ancien Monde; on y a recherché, par la neutralisation au moyen de la méthode de réduction des plages, des anticorps contre 8 sérotypes de virus responsables de fièvre à phlébotomes, à savoir les sérotypes Sicile, Naples, Arumowot, SudAn 754-61, Karimabad, Salehabad, Gordil et Saint Floris). La distribution géographique et la prévalence de l'infection humaine due à ces divers virus sont exposées, d'après les résultats de ces études sérologiques. Les anticorps neutralisants contre les virus Naples et Sicile ont été décelés dans des populations humaines vivant sur le littoral méditerranéen, dans la Vallée du Nil, les régions voisines de la Mer Noire et de la Mer Caspienne ainsi qu'en Asie centrale et, vers l'est, aussi loin que le Bangladesh. En général, les anticorps à l'égard de ces deux virus étaient trouvés ensemble, et exclusivement dans les localités où *Phlebotomus papatasi* était présent.

Au contraire, les anticorps contre les virus Arumowot et SudAn 754-61 ont été observés surtout en Afrique centrale. Ainsi, des anticorps contre l'un de ces agents, ou contre l'un et l'autre, ont été signalés en Somalie, au Soudan, en Egypte, au Nigéria et au Maroc. Des anticorps neutralisants contre le virus Karimabad ont été découverts en Iran et dans les régions limitrophes de l'URSS, aussi bien qu'au Soudan et dans le sud de l'Egypte. Il n'a été trouvé aucun indice d'infection humaine par le virus Salehabad. Les taux d'infection par les virus Gordil et Saint-Floris étaient généralement faibles; des anticorps contre l'un de ces agents ou contre les deux ont été observés dans des populations en Somalie, au Soudan et en Egypte. On trouvera représentés les résultats des épreuves de neutralisation; les conséquences de ces résultats, en matière d'épidémiologie de la fièvre à phlébotomes dans l'Ancien Monde, sont examinées.

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Annex 1

SOURCES OF SERA

Sera from Ghana, Liberia, Senegal, Somalia (1966), and Saudi Arabia were obtained from Dr K. M. Johnson and Mr C. F. Peters, Center for Disease Control, US Public Health Service, Atlanta, GA, USA. Specimens from Nigeria (1970–74) were collected during arbovirus studies by the Virus Research Laboratory, University College Hospital, Ibadan, and were received through the courtesy of Dr A. Fabiyi, Dr O. Tomori, Dr A. Fagbami, and Dr T. Monath. Human sera from

northern Nigeria (1966) were collected during a World Health Organization survey for yaws and were provided by Dr R. E. Shope, Arbovirus Research Unit, Yale University, School of Medicine, New Haven, CT, USA. Specimens from Kenya, Territory of the Afars and Issas, Ethiopia (1972), and Algeria were obtained during arbovirus studies by the Institut Pasteur, Paris, France. Additional blood specimens from Ethiopia (1974) were collected by Dr G. Fuller and were kindly supplied by Dr O. Wood, US Naval Medical Research Unit No. 5, Addis Ababa. Sudanese sera (1975) were provided by Dr O. Marcus-Jones, Virology Department, National Health Laboratories, Khartoum. Specimens from Somalia (1960–63), Sudan (1960–63) and Egypt (1952–54 and 1960–63) were obtained from the WHO Reference Serum Bank, Yale University, through the courtesy of Dr A. S. Evans. Sera from Cairo were sent by Dr I. Z. Iman, Egyptian Organization for Biological and Vaccine Production, Agouza, Egypt. Tunisian sera were kindly provided by Dr B. Nabli, Centre Ophtalmologique, Laboratoire de Recherches Virologiques, Tunis. Sera from Morocco were furnished by Dr M. A. Chabaud, Institut Pasteur du Maroc, Casablanca. Turkish serum specimens were supplied by Dr D. C. Gadjusek, National Institute of Neurologic Diseases and Stroke, National Institutes of Health, Bethesda, MD, USA. Greek sera were provided by Dr G. Papaevangelou, Department of Hygiene and Epidemiology, University of Athens Medical School and by Dr Leon Rosen, Pacific Research Section, Honolulu. Specimens from Yugoslavia were obtained by the Andrija Štampar School of Public Health, Medical Faculty, University of Zagreb. Blood specimens from Iraq were collected during investigations of an outbreak of mercury poisoning and were provided by Dr T. W. Clarkson, Department of Radiation Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, NY, USA. Specimens from Iran were obtained during arbovirus studies by the School of Public Health and Institute of Public Health Research, University of Teheran. Sera from the Soviet Union were provided by the Arbovirus Department, Ivanovsky Institute of Virology, USSR Academy of Medical Sciences, Moscow. Human sera from Pakistan and Bangladesh were supplied by the WHO Serum Reference Bank, Institute of Hygiene and Epidemiology, Center of Epidemiology and Microbiology, Prague, Czechoslovakia through the kindness of Dr L. Syrůček. Burmese sera were collected from patients hospitalized in Rangoon during a chikungunya virus outbreak and were obtained from Dr S. B. Halstead, Department of Tropical Medicine and Medical Microbiology, School of Medicine, University of Hawaii, Honolulu. The samples from southern Viet Nam were received from Dr N. T. Thanh, Department of Microbiology, Faculty of Medicine, Saigon. These specimens originated from many areas of the country and were submitted for serological confirmation of suspected cases of dengue. Malaysian sera were obtained from persons living in several different states in the north-east portion of the country by Dr R. Donaldson, US Army Medical Research Unit, Institute for Medical Research, Kuala Lumpur, during a survey for melioidosis. Sera from former residents of northern China were provided by Dr J. H. Cross, US Naval Medical Research Unit No. 2, Taipei, Province of Taiwan. These specimens were collected from retired Chinese servicemen born in North China but now living in the Province of Taiwan.