

THE DISTRIBUTION AND PREVALENCE OF HUMAN INFECTION
WITH PHLEBOTOMUS FEVER GROUP VIRUSES IN IRAN

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ABSTRACT

A total of 1,497 serum and blood specimens from residents of 18 different Iranian communities was examined by plaque reduction neutralization test for antibodies against four (Naples, Sicilian, Karimabad and Salehabad) Phlebotomus fever virus serotypes. Neutralizing antibodies against the Naples and Sicilian serotypes were found in every community sampled, indicating that these viruses are widely distributed in the country. In contrast, Karimabad antibodies were restricted mainly to residents of Isfahan, Tehran and Khorassan Provinces. No evidence of human infection with Salehabad virus was found. *Results of this study indicate that the frequency of human infection with Phlebotomus fever viruses is high in Iran.* The symptoms of sandfly fever are also reviewed.

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A. Introduction

Previous serologic studies (1,2) have demonstrated the presence of antibodies against several Phlebotomus fever group arboviruses in Iranian residents. Four different Phlebotomus fever virus (PFV) serotypes (Naples, Sicilian, Karimabad and Salehabad) have been recovered from humans or sandflies in Iran (2), although little is known about their geographic distribution or frequency of human infection within the country. Because of the non-specific symptoms of sandfly fever, which are difficult to differentiate from other mild virus infections, most cases of the disease are probably unrecognized. However, in view of the wide-spread distribution of *Phlebotomus papatasi* (3-9) and the frequency of cutaneous leishmaniasis (9-11) in many rural areas of Iran, one might suspect that human infection with sandfly-transmitted viruses is common.

In order to determine the prevalence of PFV infection and its public health importance in Iran, we recently tested 1,497 sera from residents of 18 different communities for antibodies against the four PFV serotypes known to occur within the country. The present paper reports the results of this serologic survey.

B. Materials and Methods

Sera and populations sampled: Figure 1 shows the approximate location of the 18 communities sampled. Blood specimens from residents of Ali-Abad Esmail-Abad, Neishabor, Varamin, Yelengi, Gholounabad, Deigi, Malieheh, Salur-Abad and Khorousi were obtained by finger prick on filter paper discs (Schleicher & Schnell, No. 740-E) between June and August 1975. The blood soaked disc were dried and frozen (-20°C) and were subsequently reconstituted in 1.0 ml of phosphate-buffered saline, pH 7.2, containing 0.5% gelatin, to prepare an approximate serum dilution of 1:20. Persons of all ages were bled in these communities, although the majority of donors were children.

Specimens from the remaining cities were collected between 1971 and 1975 by venipuncture from persons attending government health and family planning centers. Consequently, most of these specimens were from adults, many of whom reside in nearby villages and come to the cities for medical care. Thus the samples tested are not truly representative of the urban population of these communities. The latter sera were stored at -20°C until tested and were examined at a 1:10 dilution, using the same diluent noted above.

Viruses tested: All sera were tested against four Phlebotomus fever virus serotypes known to occur in Iran. Virus strains used were Sandfly fever, Naples strain; Sandfly fever, Sicilian strain; Karimabad, strain I-58; and Salehabad, strain I-81, originally obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. One hundred sera from Isfahan were also tested against Arumowot and SudAn 754-61 viruses, two additional Phlebotomus fever group agents isolated in Africa (12). The virus pools used in

neutralization tests were prepared from infected cultures of Vero cells.

Neutralization test : Prior to testing, all specimens were heat-inactivated at 56°C for 30 minutes. Neutralization tests were performed in 24-well, microplate cultures of Vero cells, by a previously described (12-13) plaque reduction neutralization method, utilizing a double overlay system with 1.6% tragacanth gum in the initial overlay. Blood and serum specimens were examined at the dilutions noted above against a fixed virus dose (40 to 80 plaque forming units), using a single microplate well per sample. Those specimens producing $\geq 80\%$ plaque inhibition were recorded as positive, indicating specific neutralizing antibodies. All serologic tests were performed at the Pacific Research Section, Honolulu, Hawaii, U.S.A

C. Results

Table 1 lists the Sicilian, Naples and Karimabad neutralization test results obtained in each of the 18 communities sampled. Antibodies to both the Sicilian and Naples virus serotypes were found in every community, although the infection rates varied considerably. The prevalence of Sicilian virus neutralizing antibodies ranged from 4.1% (Ali-Abad) to 34.2% (Dezful), while the Naples virus infection rates varied from 3.0% (Deigi) to 36.6% (Tehran). These differences may be due in part to the age composition of the populations sampled, since in some communities (i.e. Tehran) mostly adults were tested, while in others (i.e. Deigi) our sample consisted mainly of children. In general, however, there appeared to be no marked geographic differences in the occurrence of antibodies against these two viruses.

In contrast, Karimabad neutralizing antibodies were found in only 11 of the 18 communities sampled. With the exception of one positive individual each (both adults) from Tabriz and Deigi, Karimabad infection was localized to Isfahan, Tehran and Khorassan Provinces. Since detailed histories of previous travel or residency were not obtained from the serum donors, it is possible that these formentioned two individuals were infected elsewhere.

None of the specimens had antibodies to Salehabad virus. Likewise, all of the 100 Isfahan sera tested against the two African PFV serotypes (Arumowot and SudAn 754-61) were also negative.

D. Discussion

Results of this study (Table 1) indicate that the Sicilian and Naples virus serotypes are widely distributed in Iran and infect a significant number of persons. Neutralizing antibodies against these two agents were found in every community sampled. Both viruses have been previously isolated in Iran from humans or sandflies (2). Their presumed vector is *P. papatasi*.

In susceptible adults, both of these viruses produce classical sandfly fever, an acute, self-limited illness of two to four days duration, characterized

by fever, headache, myalgia, malaise, retro-orbital pain and occasionally nausea and vomiting (13,14). The disease is accompanied by a marked leukopenia (13,14). Little information is available on the symptoms of sandfly fever in children, but some authors (15,16) have suggested that the illness is milder in this age group. Cases of the disease typically occur in the summer months (May to September) during the period of sandfly activity. Although our serologic results indicate that human infection is common, no recorded clinical data are available on the frequency of sandfly fever in Iran. Because of the non-specific nature of the disease and its sporadic occurrence in endemic areas, most cases of sandfly fever are probably unrecognized. However, physicians should consider this disease in their differential diagnosis of summer febrile illnesses, particularly in persons travelling or working in rural areas where sandflies are abundant.

In contrast to the ubiquity of the Naples and Sicilian serotypes, our data (Table 1) indicate that Karimabad virus has a more restricted geographic distribution in Iran. Antibodies against the latter agent were found mainly in residents of Isfahan, Tehran and Khorassan Provinces. The highest Karimabad infection rates were found in Isfahan Province, suggesting that the virus is endemic in that region. The virus was first isolated from sandflies collected in the Varamin area (2,17), although the clinical manifestations of Karimabad infection in man are unknown.

The failure to detect antibodies against Salehabad virus in any of the sera tested suggests that this agent is of little public health importance in Iran. A single isolate of this virus was obtained from sandflies collected in the Varamin area in 1959 (2,17).

Likewise, no evidence of Arumowot or SudAn 754-61 virus infection was found in 100 human sera tested from Isfahan. This is not surprising since these viruses are of African origin (12) and probably do not occur in Iran.

Because of the similar Naples, Sicilian and Karimabad virus infection rates observed in several communities (Table 1), we attempted to check the specificity of our results by calculating the expected and observed frequencies of dual infections with these agents. In the Tehran sample, for example, 27.4% (45/164) of the sera were positive to Sicilian virus and 36.6% (60/164) were positive to Naples. The expected frequency of double reactors should be 10% (.274x.366). The observed frequency of double positives in this sample was 12.8% (21/164). In Ali-Abad, 33.8% (25/74) of the population had Naples antibodies and 31.1% (23/74) reacted with Karimabad. The expected frequency of Naples — Karimabad double positives should be 10.5% (.338x.311). The observed frequency was 13.5% (10/74). In both communities, the observed frequency 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 PFV serotype are at greater risk of being infected with a second. The

absence of Karimabad neutralizing antibodies in Khuzestan Province where the Naples and Sicilian infection rates were relatively high as well as the dissimilar Naples and Sicilian infection rates observed in other communities (Ali-Abad, Kermanshah and Salur-Abad) tend to support this hypothesis. The failure to demonstrate heterologous neutralizing antibodies in volunteers infected with a single PFV serotype (Naples or Sicilian) also support the specificity of our neutralization test results (13).

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TABLE 1
PREVALENCE OF PHLEBOTOMUS FEVER GROUP VIRUS
NEUTRALIZING ANTIBODIES IN VARIOUS IRANIAN
HUMAN POPULATIONS

Community-Provinve	Number of Persons tested	Percentage positive*		
		Sicilian	Naples	Karimabad
Tabriz (U)**, East Azerbaijan	100	12.0	26.0	1.0
Rasht (U), Gilan	93	12.9	21.5	0.0
Ali-Abad (R)**, Khorassan	74	4.1	33.8	31.1
Esmail-Abad (R), Khorassan	72	5.5	4.2	16.7
Neishabor (U), Khorassan	90	6.7	11.1	4.4
Mashad (U), Khorassan	100	19.0	22.0	1.0
Tehran (U), Tehran	164	27.4	36.6	5.9
Varamin (R), Tehran	93	10.8	19.4	11.8
Isfahan (U), Isfahan	134	14.9	6.7	53.0
Yelengi (R), Isfahan	20	20.0	10.0	75.0
Gholounabad (R), Isfahan	64	14.1	6.3	50.0
Kermanshah (U), Kermanshah	32	9.4	28.1	0.0
Dezful (U), Khuzestan	38	34.2	26.3	0.0
Deigi (R), Khuzestan	133	8.3	3.0	0.8
Malieheh (R), Khuzestan	36	13.9	5.6	0.0
Salur-Abad (R), Khuzestan	143	29.4	6.3	0.0
Khorousi (R), Khuzestan	55	9.1	16.4	0.0
Abadan (U), Khuzestan	56	26.8	42.9	0.0

* Sera producing $\geq 80\%$ plaque inhibition/total tested

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Figure 1 Map of Iran showing the approximate location of the 18 communities sampled.

