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High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples and Toscana) among Cypriots

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SUMMARY

Neutralizing antibodies to sandfly fever Naples, sandfly fever Sicilian and Toscana viruses were investigated among 479 sera collected from a normal human population in Cyprus. Antibody prevalence rates of 57%, 32% and 20% were found to Naples, Sicilian and Toscana viruses, respectively. The observed frequency of dual and triple infections was higher than would be expected with a random chance of infection. Antibody prevalence rates were similar for men and women for all three viruses tested, but one of two study sites had significantly higher antibody prevalence to Naples and Sicilian viruses than the other. Individuals with antibodies to both Naples and Toscana viruses had higher antibody levels to Naples virus than those with antibodies to Naples virus only. If the antibody prevalence rates found in this study reflect a history of clinical disease as described in the literature, sandfly fever poses a significant public health problem in Cyprus.

INTRODUCTION

Sandfly fever Naples virus (SFN) and sandfly fever Sicilian virus (SFS) infections are endemic in humans in several countries in the Middle East, central Asia, as well as in the Mediterranean countries. The distribution of human infection is the same as its vector *Phlebotomus papatasi* [1]. Toscana virus (TOS) infections have been described from Italy, where the vector is *Phlebotomus perniciosus*, and Portugal where the vector is unknown [2, 3]. Two other phleboviruses, Corfou and Arbia viruses, have recently been isolated from phlebotomine sandflies in the Mediterranean region [4, 5]. There is some cross-reaction between different sandfly fever (SF) viruses using serological assays such as complement fixation and indirect immunofluorescence [4, 6]. However, with the exception of weak cross-reactions between SFN and TOS viruses there are no

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cross-reactions between the SFS, SFN, TOS, Arbia and Corfou viruses by neutralization test [6, 7]. Antibody prevalence rates for SFN and SFS viruses have been found to exceed 50% in areas around the Mediterranean [1, 8]. Although antibody prevalence is high in many parts of the Mediterranean region, there are few reports of clinical cases among the native population. In the older literature, SF in Cyprus has been mentioned only by Sabin in 1944 [9]. In 1984, seven cases of SFS infection were diagnosed serologically among clinically ill Swedish soldiers of the UN force stationed in Cyprus [10]. In 1985 the incidence of SF virus infections caused by SFS, SFN and TOS in a UN contingent in Cyprus during one summer season was 4%; 7 cases of SFS, 3 cases of SFN and 1 case of TOS infection occurred among 298 soldiers observed for 6 months. SFS and SFN viruses were also isolated from two of these soldiers [11]. A study among short-stay tourists visiting a certain hotel in Cyprus showed a much higher infection rate, 63% [12]. The present study was undertaken to estimate the antibody prevalence rates to SFS, SFN and TOS among the native population in Cyprus.

METHODS

Serum samples

Serum samples were collected from 396 in- and out-patients who visited the medical, surgical, paediatric and orthopaedic clinics at Larnaca ($n = 261$) and Paralimni ($n = 135$) hospitals in the autumn of 1985 and the spring of 1986. The hospitals of Larnaca and Paralimni cover Larnaca and Famagusta districts, respectively. Sera were randomly collected at the biochemical laboratories from patients regardless of suspected diagnosis, history of previous infectious diseases, or present symptoms. To obtain sera from younger adults samples were also collected from volunteer blood donors from Larnaca ($n = 32$) and from Paralimni ($n = 51$). Altogether 223 sera from men and 256 from women were collected. All sera were transported from Cyprus to the National Bacteriological Laboratory (NBL), Stockholm, Sweden on dry ice and stored at -20 °C until tested.

Sera were identified as to the individual's age and sex and geographic area of residence. Crude age–specific (10-year age-groups) antibody prevalence rates were determined separately for the two areas sampled and calculation of standardized antibody prevalence rates for each area was based on the 1986 national Cypriot census.

Virus strains

The following strains of Sandfly fever viruses (genus Phlebovirus, family Bunyaviridae) were used: SF Sicilian (Sabin), SF Naples (Sabin) and Toscana (ISS.Phl.3) [6]. They were kindly provided by Dr R. Tesh, Yale Arbovirus Research Unit, New Haven, Connecticut, USA.

Serological tests

Neutralizing antibodies against SFS, SFN and TOS were determined by a plaque reduction neutralization test (PRNT) as described by Earley and colleagues [13] but modified according to McCown and colleagues [14] by adding DEAE-

Table 1. *Observed and expected frequency of dual or triple infections caused by sandfly fever Sicilian virus (SFS), sandfly fever Naples virus (SFN) and Toscana virus (TOS) among 479 Cypriots*

Antibodies to	Observed	Expected	Significance
SFN and SFS	23% (110/479)	18% (57% × 32%)	$P < 0.0005$
SFN and TOS	15% (72/479)	11% (57% × 20%)	$P < 0.0005$
SFS and TOS	8·1% (39/479)	6·4% (32% × 20%)	$P < 0.05$
SFN, SFS and TOS	6·9% (33/479)	3·6% (57% × 32% × 20%)	$P < 0.005$

dextran, dimethyl sulfoxide (1%) and heparin (20 units/ml) to the agar overlay. The second overlay containing neutral red was added on day 4 for SFS and TOS and on day 7 for SFN. Plaques were counted the following day. All sera were tested at dilutions of 1:10, 1:40 and 1:160. Sera which reduced plaque counts by $\geqslant$ 80% at a dilution 1:10 were considered positive. Those found positive at a 1:10 dilution only, were retested for confirmation.

Statistical tests

The chi-square test was used to compare the frequency of different titres among positive sera and for comparison of the age adjusted antibody prevalence rates in the two districts studied. The chi-square test was also used to determine if the chance of dual and triple infection was greater than could be expected by random risk. Comparison of geometric mean titres for different groups of infected individuals were analysed by applying a *t*-test to the logarithm of the titre. The *t*-test was considered as a convenient approximation to a permutation test.

RESULTS

Specific neutralizing antibodies were found in 333/479 (70%) sera to at least one of the three viruses tested with a distribution of 271/479 (57%) for SFN, 155/479 (32%) for SFS and 95/479 (20%) for TOS. The observed frequencies of dual (all possible combinations), or triple infections were significantly higher than expected (Table 1). The antibody prevalence data for the three different viruses in the respective age groups are shown in Figs. 1A–C. Antibody prevalence rates increased with age for SFN only. The frequencies of sera with titres of 10, 40, and $\geqslant$ 160, respectively, for the three different viruses are shown in Figs. 1A–C. The frequency of positive sera with a titre of $\geqslant$ 40 was higher for SFN (71%) compared with both SFS (51%) and TOS (53%) ($P < 0.001$ and $P < 0.01$, respectively). The geometric mean titre (GMT) to SFN was significantly higher ($P < 0.01$) in the group of individuals with antibodies to both SFN and TOS (GMT 58) as compared with those with SFN antibodies only (GMT 34). Such differences were not seen when other antibody combinations were compared. The median age for those who had experienced three, two, one or none of the different infections were 60, 52, 45 and 27 years of age, respectively. Age adjusted antibody prevalences are seen in Table 2. Similar antibody prevalences in men and women were found for all three viruses studied. The number of individuals seropositive for SFS and SFN was significantly higher in the Larnaca district ($P < 0.001$ and $P < 0.001$, respectively).

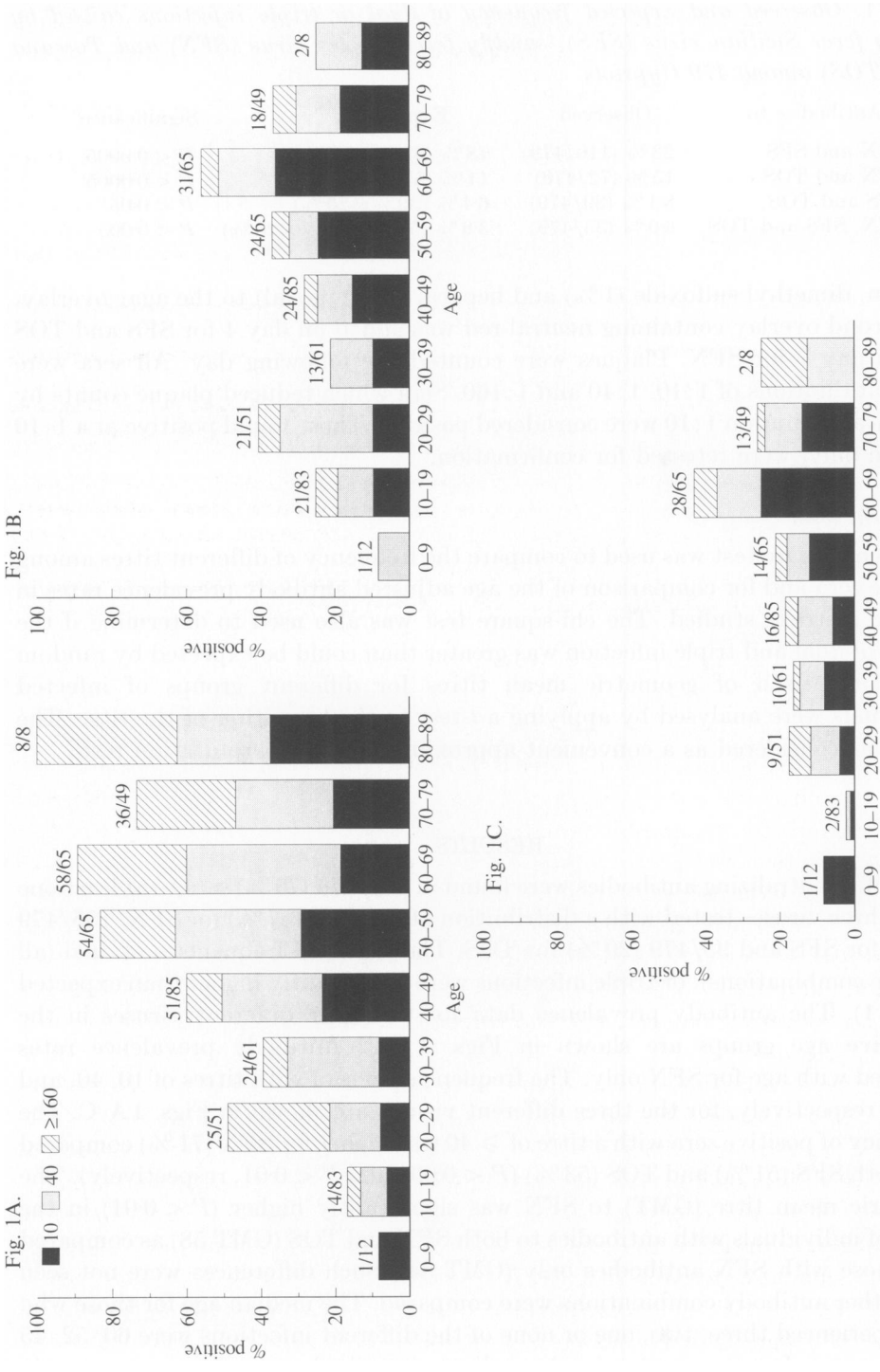

Fig. 1. Age-related antibody prevalence and titres to sandfly fever Naples virus (Fig. 1A), sandfly fever Sicilian virus (Fig. 1B) and Toscana virus (Fig. 1C) in 479 Cypriots. Filled bars = sera tested at 1:10, dotted bars = 1:40, striped bars: 1:160. No. positive/no. tested is indicated for each age-group.

Table 2. *Age adjusted antibody prevalence rates (%) for Larnaca and Famagusta districts*

Virus	Women		Men	
	Larnaca	Famagusta	Larnaca	Famagusta
SF Naples	56·5	40·1	66·5	42·9
SF Sicilian	43·0	22·9	38·3	21·0
Toscana	19·3	13·4	23·4	19·9

DISCUSSION

Since SF viruses are significant human pathogens among non-immune visitors to Cyprus [10–12] we performed a seroepidemiological study among Cypriots to evaluate the potential problem in the local population. High antibody prevalence rates were found to all three viruses investigated (Sicilian, Naples and Toscana), with 70% of the study population antibody positive to at least one of the viruses.

Other central and eastern Mediterranean countries have previously been studied thoroughly for antibody prevalence to SFN, SFS and TOS viruses [1, 2, 8]. SFS and SFN infections are common in most areas investigated with antibody prevalence figures in the same range of magnitude as in the present study in some geographical regions [1]. However, no direct comparison of results in different studies can be made because methods for antibody detection and age distribution in the populations investigated were not standardized. Toscana virus has been isolated relatively recently [7] and earlier seroepidemiological studies have therefore often not included this virus. The previous serologically verified case of Toscana virus infection in Cyprus as well as the present seroepidemiological survey expands the geographic range where this virus is known to circulate. The frequency of two and three different SF infections in one person was more common than expected with random risk. This is in concordance with earlier findings for SF in other regions [1, 2].

A sero-survey for neutralizing antibodies in neighbouring countries (Egypt, Yugoslavia and Turkey) by Tesh also found SFN to be more common than SFS [1]. A similar ratio between SFN and SFS antibodies was found in the present study. This is in contrast to the studies among Swedish UN troops on Cyprus and among Swedish tourists. SFS infection was diagnosed serologically in 64/69 (93%) verified cases among UN soldiers and tourists during 1984–8 [10–12]. This difference may be explained by a focal distribution of different SF viruses in Cyprus. It is also possible that the number of different SF virus infections change over time. Since the results in the present sero-survey reflects a very long time period (possibly the entire life-time of the person investigated) this could affect the outcome. It is also possible that SFN neutralizing antibodies persist for longer time periods than SFS neutralizing antibodies. In the present study antibodies to TOS and SFS were similar in all age groups above 30 years of age while antibodies to SFN showed an increase by age. The frequency of antibody titres of $\geqslant 40$ were significantly higher among the SFN antibody positive individuals as compared with SFS or TOS antibody positive persons. It is possible that SFN has the capability to cause a higher antibody response *per se*, or that higher titre levels are

a result of continuous re-exposure. It should be noted, however, that individuals with antibodies to the two serologically closely related SFN and TOS had higher antibody titres to SFN than those with antibodies to SFN alone. A booster effect caused by infection with a related phlebovirus has been described earlier [15]. It is also possible that other phleboviruses circulate in Cyprus. This would complicate the seroepidemiological interpretation even when the PRNT is used. If the relative difference in incidence and antibody prevalence found on Cyprus depends on a difference in persistence of antibodies post infection it greatly affects the usefulness of seroepidemiology as a tool with which to estimate endemicity.

The very high prevalence of antibody to these viruses is in contrast to the absence of reports of clinical disease among the local population. It remains to be determined whether this is due to the occurrence of mild or asymptomatic infections (e.g. infections occurring in childhood with milder symptoms) or whether sandfly fever actually causes significant morbidity among the native population in Cyprus.

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