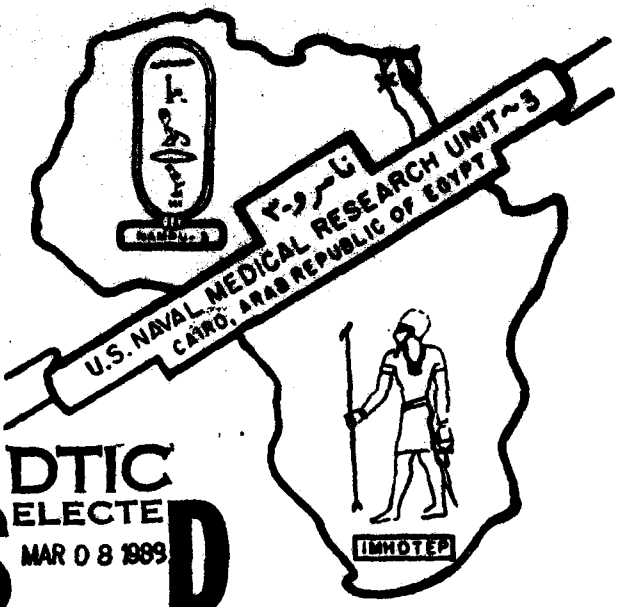

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SAND FLY FEVER-NAPLES INFECTION IN EGYPT

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Abstract. Two Egyptian male patients with sand fly fever-Naples virus infection are presented. The virus was isolated from one patient while both patients had diagnostic rises in indirect fluorescent antibody titers to the virus. The viral isolate, SFN 85055, grows to much higher titers and plaques more efficiently than the prototype sand fly fever-Naples virus and should facilitate work with this virus.

In Egypt our knowledge of sand fly fever-Naples (SFN)[1] infection is based upon a limited number of investigations over the past 35 years. Taylor[2] obtained 2 isolates in 1952–1953 from patients with fever and myalgia, and Schmidt et al.[3] obtained SFN virus isolates from sand flies in 1959. In sera collected in Egypt from 1960–1963, Tesh et al.[4] found 23% prevalence of SFN virus antibodies detected by plaque reduction neutralization test (PRNT). Schmidt et al.[5] recorded an 11% neutralizing antibody prevalence in adult residents of metropolitan Cairo; Darwish and Hoogstraal,[6] applying the complement fixation (CF) test to human sera collected from Sharqiya in 1976, found a frequency of 6%.

In two other countries in the Mediterranean basin, Greece and Italy, SFN infection prevalence has decreased coincidentally with the post-World War II antimalarial campaign which utilized extensive amounts of insecticide.[7,8] Since similar antimalarial measures were also widely utilized in Egypt in the 1950s and 1960s,[6] a similar decrease of SFN infection may have occurred.

We diagnosed SFN infection in 2 male Egyptians with nonspecific fever and myalgia. A strain of SFN virus was isolated, demonstrating the persistence of SFN infection in Egypt. The isolate is technically easier to work with than the prototype SFN virus and may simplify further investigations of phlebotomus or sand fly fevers.

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MATERIALS AND METHODS

Virus isolation

Acute sera were inoculated onto Vero (*Cercopithecus aethiops* kidney) clone E-6 and BHK (baby hamster kidney: *Mesocricetus auratus*) clone-15 and C6/36 *Aedes albopictus* cell lines. Mammalian cell lines were blind-passed once after 10 days. All cultures of C6/36 cells and those of mammalian cell lines with CPE were screened by an indirect fluorescent antibody (IFA) method against a panel of antisera to viruses known to occur in the Mediterranean and North African region, including sand fly fever-Sicilian virus, SFN virus, Rift Valley fever (RVF) virus, Toscana (TOS) virus, and West Nile virus (WNV).

Serology

IFA test. IFA tests were performed on acute and convalescent sera.[9] Sera were examined for IgM and IgG by a modification of the method of Wulff and Johnson.[10] Sera containing IgM antibodies were screened for rheumatoid factor by tube dilution.[11]

Plaque reduction neutralization test. Plaque reduction neutralization tests were carried out in Vero cells according to Earley et al.[12] and Webb et al.[13] except that sera were not heat inactivated and the incubation of sera and virus was carried

TABLE 1

Cross-neutralization of sand fly fever-Naples (SFN) virus, Toscana (TOS) virus, and Rift Valley fever (RVF) virus

Antisera to virus	Virus			
	SFN 85055	SFN prototype	TOS ISS.Phl.3	RVF ZH 501
SFN 85055	1,280*	320	160	< 10
SFN prototype	5,120	320	160	< 10
TOS ISS.Phl.3	5,120	80	≥ 10,240	< 10
RVF ZH 501	< 10	< 10	< 10	5,120

* Titer at which > 80% of plaques were neutralized.

out overnight at 4°C. SFN (prototype) and TOS (ISS.Phl.3) viruses were obtained from R. B. Tesh, Yale Arbovirus Research Unit (YARU), New Haven, Connecticut. RVF (ZH 501) virus was obtained from J. M. Meegan, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Ft. Detrick, Frederick, Maryland.[14]

Patients

The patients described in this report are part of a larger study reported by Darwish et al.[15] in which 55 patients with fever and myalgia admitted to the Imbaba Fever Hospital (IFH), Giza (near Cairo), Egypt, were screened for arboviral disease. Patients were of either sex and older than 10 years.

RESULTS

Case 1

A 29-year-old male laborer with no previous travel from his town, Abu Alghet, in Qalubyia Governorate (25 km north of Cairo) experienced a sudden onset of fever, chills, and myalgia accompanied by headache, back pain, malaise, and muscle weakness. Within 1 day of the onset of symptoms, he was admitted to the IFH where an acute blood sample was taken for viral isolation and serology. The patient had an oral temperature of 39°C. Fever resolved over 3 to 4 days.

Case 2

A 28-year-old male farmer residing in Azbat Abu Alters, Menufyia Governorate (50 km northeast of Cairo) experienced fever, chills, and myalgia accompanied by malaise, weakness, and headache. Three days later, he was admitted to the IFH with a low grade fever which resolved after 2 days.

Virus isolation and serology

SFN (85055) virus was isolated in Vero E-6 cells from the acute blood sample from Case 1. The isolate was identified serologically by IFA at the U.S. Naval Medical Research Unit No. 3 and was neutralized by reference Naples virus antisera at YARU (R. B. Tesh, personal communication). In addition, cross-neutralization of the isolate performed at USAMRIID demonstrated its identity with the prototype SFN virus (Table 1). Also in Case 1, there was a diagnostic rise in antibody titer between the acute serum obtained one day following the onset of fever when there was no detectable IFA and a convalescent serum obtained 12 days later (IgG/A/M = 64, IgG = 32, IgM = 8). Eight months following infection, neither IgG nor IgM antibodies to SFN virus were detected by IFA test.

In Case 2, no virus was isolated from serum collected 4 days following the onset of fever; however, there was a diagnostic rise in IFA between acute serum obtained 4 days following fever (IgG/A/M = 32, IgG = 16, IgM = 32) and convalescent serum obtained 11 days later (IgG/A/M = 256, IgG = 64, IgM = 32). Eight months later, IgG to SFN virus was still present (IgG = 16), but IgM was not detected. Rheumatoid factor was not detected in either case.

Virus characteristics

SFN (85055) virus grows readily in Vero cells and plaques more readily and reproducibly than with the prototype SFN virus. In our hands, SFN (85055) virus produced clear and distinct 2–4 mm diameter plaques in Vero cells within 4–5 days as compared to pinpoint plaques by SFN

(prototype) virus. After 4 days, SFN (85055) virus produced 10^7 PFU/ml in Vero cell cultures while the prototype virus produced only 10^4 PFU/ml.

Both homologous and heterologous neutralization tests gave higher titers with SFN (85055) virus than with the prototype (Table 1). Indeed, endpoint titers for antisera to SFN (85055) virus and prototype SFN virus were 1,280 and 5,120, respectively, when SFN (85055) virus is used as the challenge virus, while it was only 320 for SFN (prototype) virus.

DISCUSSION

This report confirms that SFN virus continues to be transmitted in the general Cairo region despite insecticide use. This is the same area from which Taylor[2] isolated SFN virus from humans 30 years ago. SFN virus induces a transient, nonspecific fever with myalgia. SFN viremia is transient and is not detectable after the second or third day of fever.[16] Indeed, we were unable to isolate the virus from blood taken 4 days after the onset of fever. Patients with similar clinical presentations in Egypt are often diagnosed as having an "influenza-like" viral syndrome. In accordance with the findings of this report, physicians practicing in Egypt or attending travelers returning from Egypt should consider infections with arboviruses including SFN virus in patients presenting with nonspecific symptoms such as fever and myalgia.

We were surprised by the absence of detectable IFA to SFN virus in serum collected 8 months after the infection in one of the patients. Similarly, both Taylor[2] and Sabin[16] remarked on the rapid waning of CF antibodies to this virus.

Identity of the isolate 85055 virus with SFN prototype virus is demonstrated by the cross-neutralization reaction in Table 1. The isolate is more efficiently neutralized by antisera to 3 of the tested members of the phlebotomus serogroup (85055, prototype SFN virus, and TOS ISS.Phl.3). However, there does seem to be a dissimilarity of the reaction pattern between 85055 and SFN (prototype) virus in the one-way cross with TOS antiserum. One possible explanation is that SFN (85055) and TOS viruses may share epitopes associated with neutralization that SFN (prototype) virus lacks.

The technical ease in culturing SFN (85055) virus greatly facilitates serological testing for SFN virus infection. We suggest that with additional experience, SFN (85055) virus could be used in diagnostic virology laboratories in order to technically simplify testing and possibly improve diagnostic sensitivity.

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