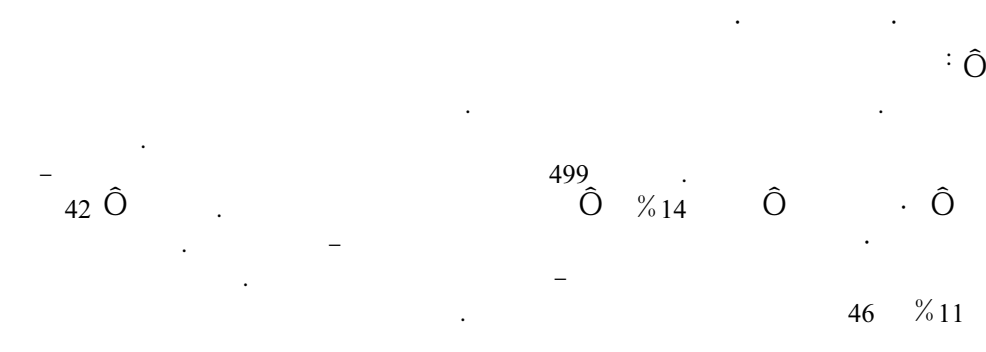


Seroprevalence of three emerging arboviral infections in Kuwaiti nationals

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ABSTRACT Diseases caused by dengue, sandfly fever and hanta viruses pose a major health risk in many countries. We determined the threat of these arboviral infections through a serological survey for antibodies using enzyme linked immunosorbent assay (ELISA) based tests. Hantavirus-specific antibodies were also detected using immunofluorescence. Of 499 samples tested for dengue virus IgG antibodies 14% were positive by all the ELISA tests. Among the 42 showing strong IgG reactivity, only 1 was positive for dengue virus IgM antibodies. All samples tested for IgG antibodies to sandfly fever virus were negative. Hantavirus antibodies were detected in 11% of the 46 samples from high-risk individuals. The low prevalences suggest that at present these infections are not a serious problem in Kuwait.

Séroprévalence de trois infections arbovirales émergentes chez des ressortissants koweïtiens

RESUME Les maladies causées par les virus de la dengue, de la fièvre à pappataci et les hantavirus présentent un risque sanitaire majeur dans de nombreux pays. Nous avons déterminé la menace que posent ces infections arbovirales par une enquête sérologique à la recherche d'anticorps en utilisant des tests ELISA (méthode immuno-enzymatique). Des anticorps spécifiques dirigés contre les hantavirus ont également été détectés par immunofluorescence. Sur les 499 échantillons testés à la recherche d'anticorps IgG contre le virus de la dengue, 14 % étaient positifs dans tous les tests ELISA. Parmi les 42 échantillons qui montraient une forte réaction aux IgG, un seul était positif pour les anticorps IgM contre le virus de la dengue. Tous les échantillons testés à la recherche d'anticorps IgG contre le virus de la fièvre à pappataci étaient négatifs. Des anticorps anti-hantavirus ont été détectés dans 11 % des 46 échantillons provenant de sujets à haut risque. Les faibles taux de prévalence donnent à penser que ces infections ne constituent pas actuellement un grave problème au Koweït.

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Introduction

Kuwait is a desert country with a high percentage of foreigners from neighbouring Arab and Asian countries where both vector-borne (dengue and sandfly fever) and rodent-borne (hanta) viral infections are endemic.

Dengue viruses produce a mild, self-limiting disease, dengue fever (DF), and a severe form, dengue haemorrhagic fever (DHF). The disease is reported in over 100 tropical and subtropical countries with about 2 billion people at risk [1]. The frequency of dengue epidemics has markedly increased with hyperendemic transmission and expansion to newer geographical areas involving South, Central and North America, Africa, China and Australia. The factors responsible for the worldwide increase in dengue and other viral infections are closely linked to changes in human ecology and behaviour [2]. Although, the common vector of dengue virus (*Aedes aegypti*) is not found in Kuwait, another potential vector (*A. caspius*) is present [3]. A study carried out during 1979–1982 in this country showed that about 20% of human sera tested had dengue virus-specific antibodies [4]. The resurgence of DF in the past few years in a number of countries from where a large work force comes to Kuwait underlines the importance of investigating the problem in the country.

Sandfly fever or 3-day fever is known to be endemic to the Middle East [5,6]. This is an acute febrile disease caused by a virus transmitted by the sandfly (*Phlebotomus papatasi*). The disease is characterized by a sudden onset and intense symptoms; biphasic fever with a peak of 40 °C, frontal headache, retro-orbital pain, severe myalgia, nausea, abdominal pain and

diarrhoea may all be associated with sandfly fever. Convalescence is occasionally prolonged for weeks [7]. The virus has two major types: the Sicilian and the Naples. Most sandfly fever cases have been reported from Saudi Arabia. A survey conducted in that country showed that 21% of adults were positive for Sicilian virus and 6% for Naples virus [8]. Since Kuwait and Saudi Arabia are neighbours, and the vector for sandfly fever virus is present in Kuwait [9], it was considered appropriate to include this virus in the serological screening for antibodies.

Hantaviruses are causative agents of at least two different syndromes; haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) [10–12]. Hantavirus infections have a worldwide distribution. Annual incidence of HFRS alone is about 200 000 cases, with an approximate mortality rate of 2%–10% [13]. In recent years, hantaviruses have been spreading from one geographical location to another [14,15]. Hantavirus causes persistent infection in rodents, and although the reservoir varies with location, the virus is closely associated with members of the Muridae family. Rodents shed the virus and the main source of infection is aerosolized excreta [16,17]. Since these rodents are present in Kuwait, hantavirus infection may pose a potential hazard to the local population.

As stated before, the vectors and carriers for the three arboviral infections are present in Kuwait, and therefore it is possible that local transmission may occur leading to human disease. In this study, evidence for infection in Kuwaiti nationals was sought by testing sera for the presence of antibodies against these viruses.

Methods

Study population

The prevalence of antibodies to dengue and sandfly fever viruses in Kuwaiti nationals was determined by using serum samples from patients admitted to the Mubarak Al-Kabir Hospital with non-febrile ailments, or referred from other hospitals in Kuwait during 1997–99. For dengue virus seroprevalence, a total of 499 serum samples were collected, representing four age groups: 0–5 years, 6–15 years, 16–30 years and ≥ 31 years (Group I). In addition, serum samples from 47 Kuwaitis, aged between 18 and 55 years, who had never left the country, were also tested for dengue virus specific antibodies (Group II). For sandfly fever viruses, 350 serum samples obtained from adult Kuwaitis (age 18–55 years) were tested (Group III). In addition, sera collected at random from 46 Kuwaiti males (adults 18–55 years) living in farming areas, where 2.5% of rodents were found to have anti-hantavirus antibodies [18], were tested for the presence of hantavirus-specific (both Puumala and Hantaan) IgG antibodies (Group IV).

Dengue-IgG plate ELISA

The test was done by using an in-house enzyme linked immunosorbent assay (ELISA) for which the dengue virus antigen, control antigen and appropriate serum controls were kindly provided by the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America. The procedure described by Gentry et al. was followed [19]. In brief, wells of 96-well flat-bottom microtitre plates (Nunc, Denmark) were coated with the antigen at 4 °C overnight. Plates were washed three times followed by blocking with 5% skimmed milk. Serum samples were diluted 1:100 and added to the wells in duplicate

(100 μ L/well). After incubation at 37 °C for 1 hour, the wells were washed three times and the conjugate (anti-human-IgG coupled with horseradish peroxidase) was added. After further incubation for 1 hour and washing, the reaction was developed with the substrate. The optical density (OD) at 450 nm was read with an ELISA plate reader (Dynatech, Denmark). The OD values of the test serum wells minus the OD values of the background well (control antigen) were recorded. For determining the cut-off value, 20 sera known to be negative for flaviviruses were tested. The cut-off value was the mean OD value of the negative samples plus 3 standard deviations. The samples with OD values above the cut-off were considered positive.

Dengue IgG and IgM dot ELISA

The test kits for dengue IgG and IgM dot blot ELISA were purchased from Genelab Diagnostics, Singapore and used according to the manufacturer's instructions.

Dengue IgG and IgM immunochromatographic ELISA

This test was performed as recommended by the manufacturer, using kits from PanBio, Australia. The test is a capture type ELISA on a strip with immobilized anti-human IgM and IgG antibodies. Colloidal gold-labelled anti-dengue monoclonal antibody reacts with the dengue antigen stabilized on the device. The antigen-monoclonal antibody-gold complex reacts with captured IgM or IgG. In a positive sample, a purple line or lines are formed.

Dengue IgM capture ELISA

This test was done by using anti-human-IgM coated ELISA plates according to the manufacturer's instructions (PanBio, Australia).

Sandfly fever virus IgG-ELISA

To detect sandfly fever virus antibodies in the serum specimens, an in-house ELISA test was used. Wells of 96-well plates were coated with antigens prepared from Sicilian and Naples strains of the sandfly fever virus. Both the antigens and control sera were kindly provided by US Naval Medical Research Unit No. 3 (Cairo, Egypt). The test was performed as recommended by the Research Unit. In principle, the procedure was similar to the dengue IgG-ELISA.

Hantavirus IgG-ELISA and immunofluorescence

Hantavirus-specific antibodies in human sera were detected to both Puumala (European) and Hantaan (Asian) strains separately. ELISA kits were purchased from Progen (Heidelberg, Germany). The method recommended by the manufacturer was followed. Sera showing positive reactions were retested by an immunofluorescence test using kits from Progen according to the manufacturer's instructions.

Results

Dengue virus antibodies in Kuwaitis

To determine the seroprevalence of dengue virus IgG antibodies, the sera from 499 Kuwaiti nationals were tested for IgG antibodies by plate and dot blot ELISA tests. The sera showing positivity in both the tests were considered positive. The results showed that, depending on the age of subjects tested, the prevalence of dengue virus IgG antibodies in Kuwaiti nationals ranged between 10% and 20% (Table 1). However, the overall seroprevalence of anti-dengue virus IgG antibodies was 14%, i.e. 70 of the 499 sera tested were positive (Table 1). The serum samples classified as high positives by both tests ($n = 42$) were also tested for the presence of dengue virus IgM antibodies. Four proved to be positive by IgM dot blot test. However, when these samples were retested by IgM capture, ELISA and immunochromatographic tests, only 1 sample showed IgM reactivity in all the tests. All 47 serum samples collected from Kuwaitis who had never left the

Table 1 Prevalence of IgG antibodies to dengue-, sandfly- and hantaviruses in Kuwait

Viruses	Group	Age group (years)	No. positive/ no. tested	Positive (%)
Dengue	I	0-5	11/98	11
		6-15	11/102	11
		16-30	11/112	10
		> 31	37/187	20
		Total	70/499	14
Dengue	II	Adults ^a	0/47	0
Sandfly (Naples and Sicilian strains)	III	Adults	0/350	0
Hantavirus, Puumala	IV	Adults	4/46	9
Hantavirus, Hantaan		Adults	1/46	2

^aAdult Kuwaiti nationals who had never left the country.

country were negative for dengue virus IgG antibodies.

Sandfly fever virus antibodies in Kuwaitis

A total of 350 randomly collected serum samples from adult Kuwaiti nationals were tested for the presence of IgG antibodies against Sicilian and Naples strains of sandfly fever virus. None of the sera showed positive antibody titre.

Hantavirus-specific antibodies in Kuwaitis

Serum samples from 46 Kuwaiti nationals who lived in the areas with the possibility of frequent exposure to hantavirus infected rodents were tested for IgG antibodies against both Hantaan and Puumala virus strains. Four (9%) samples had IgG antibodies to Hantaan and 1 (2%) to Puumala virus strains.

Discussion

The study was designed with the presumptions that, a) the residents of Kuwait who have never left the country should not have antibodies against dengue viruses as there is no evidence of the known vectors (*A. aegypti/albopictus*) in this country, b) since the sandfly *Ph. papatasi*, the vector of sandfly fever virus, is present in Kuwait, antibodies to this virus may be present in Kuwaiti nationals and c) the residents of Kuwait who have never travelled abroad but who live in rodent infested areas may have hantavirus antibodies.

Indeed, none of the never-travelled residents were positive for dengue virus IgG antibodies. In addition, the overall antibody prevalence in Kuwaiti nationals was only 14%, which is comparable to the level (20%) reported by Al-Nakib et al. in a

study conducted during 1979–82 [4]. These results suggest that there has been no increase in dengue virus activity in Kuwait for the past 20 years. Furthermore, the level of antibody prevalence in Kuwaiti nationals is far lower than the level found in expatriates coming from dengue-endemic countries, e.g. India, where the prevalence of dengue-specific antibodies is between 65% and 90% [20,21]. It seems that, despite the large proportion of the work force coming to Kuwait from dengue-endemic countries, the virus has not been transmitted within Kuwait. This may be explained by the fact that the mosquito *A. caspius* present in the country is not an efficient vector for transmitting the virus.

Another factor which may prevent the human-to-human transmission may be geographical. The desert climate with low rainfall (< 60 mm/year) does not support the natural breeding of the potential vector. However, a recent surveillance study in Saudi Arabia revealed that in the Jeddah area there were 665 confirmed dengue infections [22]. This was explained by the expansion of the city, which resulted in a tremendous increase in freshwater containers and these proved to be breeding sites for mosquitoes. Since there is a continuous expansion of residential areas in Kuwait city, the risk of introducing dengue virus into the country is real, a risk further enhanced by the large farming areas where freshwater reservoirs are open to mosquito breeding.

Our study did not demonstrate the presence of antibodies against sandfly virus in Kuwaiti nationals. Although the principal vector for sandfly fever virus, *Ph. papatasi*, has been found in Kuwait, attempts by Cope et al. in 1996 to demonstrate the presence of the virus using virus isolation in cell culture from a large number

of flies were unsuccessful [9]. Thus, their study together with our results shows that the sandfly fever virus is not yet present in Kuwait. On the other hand, Ibrahim et al. in 1974 reported rather high antibody prevalence for both Naples and Sicilian strains of sandfly fever virus in Kuwait [23]. The discrepancy in the results may partly be attributed to the fact that Ibrahim et al. had tested people belonging to different nationalities living in Kuwait, whereas our study is restricted to Kuwaiti nationals only. Thus, the individuals coming from areas endemic for sandfly fever virus, e.g. Egypt, might be responsible for the high antibody prevalence reported. Furthermore, the assays used by Ibrahim et al. showed cross-reactivity among arboviruses, which might also have contributed to their findings [24]. The tests that we used in our study were highly specific for the detection of antibodies to each group of arbovirus tested, i.e. dengue, sandfly and hantaviruses.

There are rats (*Rattus norvegicus*) and mice (*Mus. musculus*) in Kuwait infected with hantaviruses [18]. However, antibody prevalence of 2.3% and 3.6% in the rats and mice respectively is far lower than what has been reported from Wisconsin and Minnesota [14] (20% of rats and 8% of mice had hantavirus antibodies) and from Northern Ireland [25] (22% of rats, 29% of mice) but is close to the level (1.1%) found in Nagoya city, Japan for rats [26].

Despite the relatively low level of hantavirus activity in rodents in Kuwait, there is a definite chance of humans acquiring infections. Of the 46 individuals who were at high risk of contact with rodent excreta, 4 (11%) had antibodies to hantaviruses. This prevalence in humans is of the same order as reported from Egypt [27], Sweden

[28] and the Netherlands [29]. However, the mere presence of antibodies does not have a direct correlation with disease manifestation in humans. In Wisconsin and Minnesota, antibodies to hantaviruses were found in humans as early as 1984 [30] but the serious disease HPS associated with hantavirus was reported only in 1993 [15]. We are not aware of any report of human hantavirus-associated disease in Kuwait. However, since laboratory facilities for the specific diagnosis of suspected cases were not available in the past, it is impossible to know the impact of hantavirus infection in the country.

In conclusion, our study suggests that, despite the presence of a potential vector (*A. caspius*), dengue virus activity is low in Kuwait and local transmission is not occurring. In addition, despite the abundant presence of sandflies during the mild winter months, there is no sandfly fever virus activity in Kuwait. Moreover, the low prevalence of hantavirus antibodies in high-risk people indicates that at present the Kuwaiti population has a limited risk of acquiring hantavirus disease. However, considering the rapid spread of these emerging arboviral infections in neighbouring countries and those from which some of the Kuwait work force comes, constant vigil and surveillance is needed in Kuwait.

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