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Hantavirus-specific antibodies in rodents and humans living in Kuwait

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Abstract

Hantaviruses are found in widely scattered areas of the world and are transmitted by inhalation of virus-contaminated aerosols of rodent excreta. The present study was undertaken in Kuwait to investigate the serological evidence for hantavirus infection in rodents and humans. Sera were collected from 283 wild rodents and 183 human subjects (46 Kuwaitis and 137 non-Kuwaitis). The rodent sera were investigated for the presence of antibodies against the Seoul and Puumala strains of the hantaviruses by enzyme-linked immunosorbent assay and immunofluorescence technique using the virus-infected Vero E6 cells. The findings showed the presence of anti-hantavirus antibodies in seven out of the 283 (2.8%) rodents. Antibodies against the Seoul strain were present in six (2.1%) and against the Puumala strain in three (1%) rodents. Further, it was observed that three out of 84 (3.6%) of the *Rattus norvegicus* and four out of 174 (2.3%) *Mus musculus* had anti-hantavirus antibodies. Two rodents belonging to species *Mus musculus* had antibodies against both strains of the hantaviruses. Out of 183 human sera, 13 (7%) were positive for hantavirus antibodies. Among the Kuwaitis 5/46 (11%) and among the non-Kuwaitis 8/137 (6%) were positive for the hantavirus antibodies. Antibodies to both Puumala and Hantaan strains were detected in Kuwaitis as well as in non-Kuwaitis. Although no human case of hantavirus illness has yet been reported in Kuwait, the serological evidence of infection suggests a constant vigil. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Hantaviruses are enveloped, trisegmented RNA viruses belonging to the Bunyaviridae family. At least 30 hantaviruses are now recognized throughout the world. They are causative agents of at least two different syndromes: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) [1,2,3]. Hantavirus infections have a worldwide importance. Annual incidence of HFRS alone is about 200 000 cases with an approxi-

mate mortality rate of 2–10%. The disease occurs in Europe, Asia and the Americas [4]. During recent years hantaviruses have been spreading from one geographical location to the other. For example, the first hantavirus epidemic (HPS) occurred in southwest USA in 1993 [5] and later it reached to other geographical locations [6]. Hantaviruses are primarily rodent-borne and cause persistent infection in rodents. Though the reservoir varies with location, the hantaviruses are primarily associated with the rodents of the Muridae family. Rodents shed the virus and the main source of infection is aerosolized excreta [7–9]. The rodents of the Muridae family are present in Kuwait, and therefore, hantavirus infections pose a potential hazard to the population. However, no data are available on this viral infection in Kuwait. Moreover, the disease is prevalent in other parts of the world but not reported in Kuwait. The present study was therefore conducted to determine the serological evidence of infection with hantaviruses both in rodents and humans living in Kuwait in close proximity with each other.

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2. Materials and methods

2.1. Study population

A total of 283 rodents (*Rattus norvegicus*, $n = 84$; *Mus musculus*, $n = 174$; *Nesokia indica*, $n = 7$; and *Tattera indica*, $n = 18$) were trapped in 1997–1998 in different areas of Kuwait by the Insect and Rodent Control Division, Community Health Department, Kuwait. The trapped rodents were examined for species, sex, weight, physical and reproductive conditions. They were anesthetized and exsanguinated by cardiac puncture to collect blood for serum separation. In addition, serum samples were collected from 46 Kuwaiti and 137 non-Kuwaiti residents living in the same areas as the rodents with the possibility of frequent exposures to rodents.

2.2. Hantavirus antibodies in rodent sera

The rodents' sera were tested for detection of antibodies against the Puumala and Seoul strains of hantaviruses using the immunofluorescence antibody (IFA) technique described previously [10]. In brief, rodent sera were diluted $20\times$ in phosphate-buffered saline (PBS, pH 7.4) and applied to the multi-well slides with virus-infected Vero E6 cells (American Type Culture Collection C 1008). The slides were incubated in a humidified container for 45 min and washed with PBS for 30 min. The slides were further incubated with fluorescein isothiocyanate-conjugated anti-rat IgG antibodies for 20 min. The slides were washed with PBS and examined for characteristic cytoplasmic inclusions using a fluorescence microscope. All positive sera were then titrated to determine the antibody titer.

2.3. Hantavirus antibodies in human sera

Hantavirus-specific IgG antibodies in human sera were detected to Puumala (European) and Hantaan (Asian) strains by using enzyme-linked immunosorbent assay (ELISA). The ELISA kits were purchased from Progen (Heidelberg, Germany) and used according to the manufacturer's instructions. The sera positive by ELISA were also tested by an IFA kit (Progen) according to the manufacturer's instructions.

Table 1
Distribution of hantavirus antibodies in different species of rodents

Rodent species	Number of tested rodents	Rodents positive for anti-hantavirus antibodies	
		Number	Percent
<i>R. norvegicus</i>	84	3	3.6
<i>M. musculus</i>	174	4	2.3
<i>N. indica</i>	7	0	0
<i>T. indica</i>	18	0	0
Total	283	7	2.5

Table 2
Hantavirus strain-specific antibodies in rodent sera

Virus strain	Number of tested rodents	Rodents positive for anti-hantavirus antibodies	
		Number	Percent
Seoul	283	6	2.1
Puumala	283	3	1.1
Seoul+Puumala	283	2	0.7

3. Results

3.1. Hantavirus-specific antibodies in rodent sera

Hantavirus-specific antibodies were detected in four out of 174 (2.3%) and three out of 84 (3.6%) sera of *M. musculus* and *R. norvegicus*, respectively (Table 1). Wild rats belonging to *Tattera* and *Nesokia* genera did not have detectable level of antibodies to hantaviruses (Table 1). With respect to the presence of virus strain-specific antibodies in sera of 283 rodents, anti-Seoul and anti-Puumala antibodies were detected in six (2.1%) and three (1.1%) sera, respectively (Table 2). Moreover, sera from two (0.7%) rodents showed the presence of anti-Seoul as well as anti-Puumala antibodies (Table 2). These results suggested mixed infection with both of the virus strains.

3.2. Hantavirus-specific antibodies in human sera

The results with human sera for anti-hantavirus antibodies with respect to age and virus strain in Kuwaitis and non-Kuwaitis are summarized in Tables 3 and 4. In addition, anti-hantavirus strain-specific antibodies with respect to the nationalities of the tested subjects are also given (Table 5). The results showed that 5/46 (11%) Kuwaitis and 8/137 (6%) non-Kuwaitis were positive for anti-hantavirus antibodies (Table 3). Among the positive Kuwaitis, one (2%) had antibodies to Puumala and four (9%) to Hantaan virus strains. Among the sera from non-Kuwaitis, four (3%) had antibodies to Puumala and four (3%) to Hantaan virus strains (Table 4). With respect to the nationalities of the tested subjects, the results did not show any significant difference in the positivity of sera to anti-hantavirus antibodies (Table 5)

Table 3
Hantavirus antibodies in human sera from Kuwaitis and non-Kuwaitis

Age group (years)	Number positive/tested (% positive)		
	Kuwaitis	Non-Kuwaitis	Kuwaitis+non-Kuwaitis
21–30	3/13 (23%)	3/37 (8%)	6/50 (12%)
31–40	2/15 (13%)	2/50 (4%)	4/65 (6%)
41–50	0/18 (0%)	3/38 (8%)	3/56 (5%)
51–60	0/0 (0%)	0/12 (0%)	0/12 (0%)
Total	5/46 (11%)	8/137 (6%)	13/183 (7%)

Table 4

Hantavirus strain (Puumala and Hantaan)-specific antibodies in human sera from Kuwaitis and non-Kuwaitis

Age group (years)	Number positive/tested Kuwaitis		Number positive/tested non-Kuwaitis	
	Puumala	Hantaan	Puumala	Hantaan
21–30	1/13	2/13	1/37	2/37
31–40	0/15	2/15	2/50	0/50
41–50	0/18	0/18	1/38	2/38
51–60	0/0	0/0	0/12	0/12
Total	1/46 (2%)	4/46 (9%)	4/137 (3%)	4/137 (3%)

4. Discussion

The results of this study show for the first time that rats (*R. norvegicus*), mice (*M. musculus*) and humans in Kuwait are infected with hantaviruses. However, the 2–3% antibody prevalence in rodents of Kuwait is lower than that found in Wisconsin and Minnesota [4], where 20% of rats and 8% of mice had hantavirus antibodies, and in Northern Ireland (22% for rats, 29% for mice) [11], but it is close to the seropositivity level of 1.1% in rats of Nagoya, Japan (1.1% for rats) [12] and 2–4% seroprevalence in the rodent population of the Nakhon Pathom and Nakhon Ratchasima provinces of Thailand [13].

Among the hantavirus-infected rodents, the presence of antibodies against the Puumala and Seoul strains in two rodents is interesting because it suggests dual infection. Kuwait has a very active seaport where ships come from all over the world. Therefore, the mixing of rodents from east and west is very likely in Kuwait and might have resulted in cross-infection. This also points to active transmission of hantavirus infection in Kuwait.

Thus, despite the relatively low level of hantavirus activity in rodents in Kuwait, there is a chance for humans to acquire infections. Of the 46 Kuwaiti individuals who were at high risk to contact rodent excreta, five (11%) had antibodies to hantaviruses. This prevalence level is the same as reported in studies conducted in Egypt [14], Sweden [15] and The Netherlands [16]. However, the mere presence of antibodies does not have a direct correlation with the manifestation of the disease in humans. In Wisconsin and Minnesota, antibodies to hantaviruses were

detected in humans as early as 1984 [3], but serious diseases (HPS) associated with hantaviruses were reported only in 1993 [5].

We are not aware of any report on human hantavirus-associated disease in Kuwait. However, the diagnostic facilities were not available in the past, and without those it is practically impossible to determine the impact of hantavirus infection in the country. The establishment of serological methods to detect anti-hantavirus antibodies will now facilitate the diagnosis of clinically suspected cases of HFRS and HPS.

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Table 5

Nationality-wise distribution of hantavirus strain (Puumala and Hantaan)-specific antibodies in the human sera

Nationality	Number of sera	Number of sera positive for antibodies to	
		Puumala virus	Hantaan virus
Kuwaiti	46	1	4
Egyptian	51	2	1
Afghani	36	1	1
Pakistani	35	0	1
Other nationalities ^a	15	1	1

^aOther nationalities include Iranians ($n=9$), Bangladeshis ($n=3$) and Sri Lankans ($n=3$).

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