Bulletin of the World Health Organization, 64 (1): 139-144 (1986)

Global survey of antibody to Hantaan-related viruses among peridomestic rodents*

J. W. LEDUC,[1] G. A. SMITH,[1] J. E. CHILDS,[1] F. P. PINHEIRO,[2] J. I. MAIZTEGUI,[3] B. NIKLASSON,[4] A. ANTONIADES,[5] D. M. ROBINSON,[6] M. KHIN,[7] K. F. SHORTRIDGE,[8] M. T. WOOSTER,[9] M. R. ELWELL,[10] P. L. T. ILBERY,[11] D. KOECH,[12] E. S. T. ROSA,[13] & L. ROSEN[14]

A global serological survey of rodents was conducted to determine the distribution and prevalence of antibody to Hantaan-related viruses, which are the causative agents of haemorrhagic fever with renal syndrome (HFRS) in man. Over 1700 rodent sera from more than 20 sites worldwide were examined by immunofluorescent antibody assay. High-titred positive sera were further tested by plaque reduction neutralization tests with prototype Hantaan virus and urban rat-associated Hantaan-like virus. Antibody-positive rodents were found in most, but not all, sites sampled. The highest antibody prevalence rates were found in Baltimore, MD, USA and Bélem, Brazil, and Rattus norvegicus *was the species most often found positive.* Bandicota indica *and* B. bengalensis, *species previously not recognized as hosts of hantaviruses, were also positive. Neutralization tests detected antibody in* Rattus *sera specific for urban rat-associated Hantaan-like virus, but failed to establish the specificity of antibody in* Bandicota *sera. These results indicate that Hantaan-related viruses exist beyond the currently recognized boundaries of HFRS in man and suggest that human HFRS-like disease might be occurring in other areas of the world where rodent-human contact is common.*

Several distinct viruses are now associated with haemorrhagic fever with renal syndrome (HFRS) in man. Hantaan virus is recognized as the etiologic agent of Korean haemorrhagic fever in Korea (*1*) and of epidemic haemorrhagic fever in China (*2*). Puumala virus is thought to cause nephropathia epidemica in Scandinavia and much of western Europe (*3*, *4*) and Hantaan-like viruses have been found in domestic rats, both free-living and laboratory animals, which have been associated with a "mild" form of HFRS in man in Asia and in several laboratory settings (*5-9*). Prospect Hill virus is antigenically related to Hantaan virus, but as yet has not been associated with human disease, although specific antibody has been found among mammalogists in the United States (*10*, *11*). All these viruses share antigenic and genetic similarities and collectively have been proposed to form a new genus, *Hantavirus*, within the virus family Bunyaviridae (*12*).

Recognition that the Hantaan-like virus found in urban rats is distinct from the prototype Hantaan virus is a recent advance (*13*). When infected individuals were first identified among free-living rats in the urban centres of Asia and among laboratory rats in medical research centres, it was feared that Hantaan virus had adapted to growth in this new host species. Speculation arose that international shipping might have disseminated infected rats from their Asian foci

* The views of the authors of this article do not purport to reflect the positions of the United States Department of the Army or the Department of Defense. Requests for reprints should be addressed to Dr J. W. LeDuc, Department of Epidemiology, Disease Assessment Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701-5011, USA.

[1] United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, USA.

[2] Pan American Health Organization, Washington, DC, USA.

[3] Instituto Nacional de Estudios sobre Virosis Hemorrágicas, Pergamino, Argentina.

[4] National Bacteriological Laboratory, Stockholm, Sweden.

[5] Aristotelian University of Thessaloniki, School of Medicine, Thessaloniki, Greece.

[6] United States Naval Medical Research Unit No. 3, Cairo, Egypt.

[7] Department of Medical Research, Ministry of Health, Rangoon, Burma.

[8] University of Hong Kong, Hong Kong.

[9] United States Naval Medical Research Unit No. 2, Manila, Philippines.

[10] Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

[11] Commonwealth Department of Health, Woden, ACT, Australia.

[12] Ministry of Health, Nairobi, Kenya.

[13] Instituto Evandro Chagas, Bélem, Brazil.

[14] University of Hawaii, Honolulu, HI, USA.

throughout the world. As the virus isolates from rats were characterized, however, it became apparent that they were clearly different from the prototype Hantaan virus (*6*, *13*). These results allayed fears that Hantaan virus had adapted to domestic rats, yet the public health significance of the urban rat-associated Hantaan-like virus remains substantial. Strong evidence exists that strains of this virus are capable of causing overt human disease (*2*, *5–9*). Further, the cosmopolitan distribution of domestic rats suggests that HFRS may exist undiagnosed in areas well beyond its traditionally recognized enzootic boundaries. This study was undertaken to determine the global distribution of Hantaan-related viruses among peridomestic rodents as a first step in determining if associated human HFRS is likely to be widespread.

MATERIALS AND METHODS

Serum collections. Rodents were live-trapped by collaborators at various sites around the world from 1981 to 1983. They were captured primarily in domestic and peridomestic habitats in or near urbanized population centres. The captured animals were identified as to species, anaesthetized, and exsanguinated by cardiac puncture. The sera were separated, stored frozen, and transported to the US Army Medical Research Institute of Infectious Diseases where they were examined for antibody to Hantaan and related viruses.

Immunofluorescent antibody assay. The sera from the captured rodents were examined for antibody to Hantaan and related viruses by immunofluorescent antibody (IFA) assays following standard procedures described previously (*13*) and using Vero E-6 cells (American Type Culture Collection number CRL 1586) infected with the 76-118 strain of Hantaan virus. "Spot slides" were prepared by Dr George French (Salk Institute, Swiftwater, PA, USA) and were stored at −20 °C prior to use. The sera were examined at dilutions of 1:8 through 1:2048 or greater in fourfold increments, and were considered positive if the characteristic cytoplasmic fluorescence was present at $\geqslant$1:32 dilutions.[a] The IFA test has previously been shown to be broadly cross-reactive among hantaviruses and is known to serve as a good screening assay.

Plaque reduction neutralization tests. Selected sera from rodents captured in geographically diverse areas, which possessed high IFA antibody titres to Hantaan virus, were further tested by plaque reduction neutralization (PRN) tests. Sera were tested with both prototype Hantaan virus, strain 76-118, and Girard Point virus, an urban rat-associated hantavirus originally isolated from *Rattus norvegicus* captured in Philadelphia, PA, USA (*13*). Girard Point virus is closely related to several other strains of urban rat-associated hantaviruses and antibodies to all these rat strains react to a higher titre with Girard Point virus than to the prototype Hantaan virus in the PRN test (*12*, *13*). Neutralization tests were performed using Vero E-6 cells and the procedures described previously (*13*).

Briefly, approximately 200 plaque-forming units of virus were mixed with an equal volume of diluted serum and allowed to react overnight at 4 °C. The virus–serum mixtures were then inoculated onto drained confluent monolayers of Vero E-6 cells grown in 25-cm^2 flasks. After one hour's incubation at 37 °C, the inoculated cells were covered with nutrient agarose overlay (10% heat-inactivated fetal bovine serum, 2 mmol/l L-glutamine, 1% non-essential amino acids, and 1% agarose in Eagle's minimum essential medium with antibiotics) and incubated at 37 °C for approximately 10 days, then stained with a dilute (1:25 000) neutral red solution, and the plaques counted 24–48 hours later. The neutralization titres were recorded as the reciprocal of the greatest serum dilution reducing 50% (or more) of the plaque dose. Positive and negative serum controls were included in all IFA and PRN tests.

RESULTS

Over 1700 rodent sera were obtained from more than 20 different sites around the world between 1981 and 1983. With the exception of Antarctica, all the major land masses were sampled. Antibody to Hantaan virus was detected by IFA tests in the sera from rodents captured in nearly all the locations sampled. These results are shown in Table 1. Highest antibody prevalence rates were found in Baltimore, MD, USA at 64%, followed by Bélem, Brazil at 56%. Several other locations had antibody prevalence rates in excess of 20%, and the overall prevalence rate for all sites and all species tested was 20.8%. There were, however, some sites with no evidence of infection in the local rodent population; for example, the isolated village of Tsepelovo in Greece (none positive out of 51 *R. rattus* tested) and several sites in California, USA.

Realizing that the IFA test is broadly cross-reactive among all recognized members of the genus *Hantavirus*, we further tested selected positive sera by the more specific PRN test. All sera selected for testing gave a titre of at least 2048 by IFA, with the exception of one sample at 1024 (from Houston, TX,

[a] The anti-rat FITC conjugate was from Cappel Laboratories, Westchester, PA, USA.

Table 1. Prevalence of antibody to Hantaan virus among rodents from various areas of the world as determined by immunofluorescent antibody assays[a]

Location	No. positive/ No. tested	Percentage positive
North America		
Philadelphia	15/122	12
Baltimore	108/170	64
Houston	9/115	8
Various cities in California	0/56	
South America		
Bélem	30/54	56
São Paulo	5/35	14
Recife-Olinda	2/36	6
Buenos Aires	11/101	11
Europe		
Malmö	2/8	25
Tsepelovo (Greece)	0/51	
Africa		
Port Said	2/43	5
Alexandria	4/51	8
Suez	2/50	4
Mombasa	3/30	10
Asia		
Rangoon	14/63[b]	22
Hong Kong	41/134	31
Manila	70/219	32
Thailand	21/311[c]	7
Taiwan	6/27	22
Australia		
Queensland	8/19	42
Victoria	3/8	38
Northern Territory	2/16	13
South Australia	2/12	17
Western Australia	0/7	
New South Wales	2/2	100
Total	362/1740	20.8

[a] Specimens were positive if the titre was $\geqslant 32$.
[b] Includes 11/59 (19%) *Bandicota bengalensis*.
[c] Includes 10/65 (15%) *Bandicota indica*.

USA). Sera from rats captured in North America, South America, Africa and Asia were included. Results of these tests are presented in Table 2. In all instances, the highest PRN titres were to Girard Point virus and not to prototype Hantaan virus. These results suggest that the infecting virus was not the latter, but rather one of the closely related strains of urban rat-associated Hantaan-like virus.

Sera from several different species of rodents were examined in this study. As shown in Table 3, IFA antibody was detected most frequently in sera from *R. norvegicus*, with an overall antibody prevalence rate of 26.7%. Antibody was not, however, restricted to this species. *Bandicota indica* and *B. bengalensis* sera from Thailand and Burma, respectively, were also frequently positive. While many sera from these species had relatively low titres (32–128) and may represent false positive reactions, sera from some individuals of both species had high antibody titres by IFA ($\geqslant 512$) and were clearly true positive reactions. When *Bandicota* sera with high IFA titres were tested by PRN, equivocal and inconclusive results were found. Consequently, we are at present unable to determine whether the prototype Hantaan virus, an urban rat-associated virus, or another as yet unidentified virus was the infecting agent. Studies on this topic are continuing.

DISCUSSION

The results of this study clearly show that the Hantaan-related viruses are widely distributed around the world. While not ubiquitous, these viruses are well established in their rodent hosts and are abundant in regions beyond those traditionally associated with HFRS in man. Previous studies indicated a focal distribution of urban rat-associated Hantaan-like viruses (*13*) and this was observed in the present study as well. Antibody-positive individuals seemed to cluster at certain sites and were generally not uniformly distributed throughout all the populations sampled.

The specificity of the antibody detected in the IFA test to the virus appears clear for the *Rattus* sera tested, but remains unresolved for the *Bandicota* sera. The PRN test results showed convincingly that the *Rattus* sera possessed specific antibody for the urban rat-associated Hantaan-like virus. These results are consistent with the growing number of isolations of Hantaan-like viruses from rats captured from various areas of the world. They also serve to reaffirm our earlier observations regarding the value of the PRN test to differentiate the antibodies to these two groups of viruses (*13*). We conclude from these results that urban rat-associated Hantaan-like viruses are likely to be encountered wherever large populations of *Rattus* rodents exist.

The finding of antibody to Hantaan virus by IFA in the sera of *Bandicota* rodents extends the known host range of Hantaan-related viruses. The species found positive generally live in relatively close association with man in much of Asia and could conceivably be the source of human infection if the infecting virus is shown to be pathogenic for man. Although the identity of that virus is at present unknown and additional studies are needed, a Hantaan-like virus has been isolated from lung tissues of *B. indica* captured in Thailand (LeDuc et

Table 2. Comparison of immunofluorescent antibody (IFA) assay and plaque reduction neutralization (PRN) test results with prototype Hantaan (HTN) and urban rat-associated Girard Point (GP) viruses and selected *Rattus norvegicus* sera from various areas of the world

	IFA	PRN	
Location	Hantaan virus	Hantaan virus	GP virus
North America			
Philadelphia	8192[a]	160[b]	2560
Houston	1024	40	1280
South America			
Brazil	2048	320	5120
	2048	<8	2048
Argentina	2048	128	1024
Africa			
Egypt	2048	<8	2048
	>2048	128	2048
Asia			
Hong Kong	>2048	32	>2048
	>2048	8	>2048
Philippines	2048	8	>2048
	>2048	32	>2048
Thailand	2048	32	2048
Taiwan	2048	128	>2048
Reference control sera			
HTN mouse hyperimmune ascitic fluid	*>2048*[c]	*>2048*	1280
Serum from patient with Korean haemorrhagic fever	*512*	*>2048*	320
GP rat serum #1	>2048	160	*>2048*
GP rat serum #2	2048	40	*1280*

[a] Reciprocal of highest serum dilution showing characteristic cytoplasmic fluorescence.

[b] Reciprocal of highest serum dilution reducing ⩾50% of plaque dose.

[c] Homologous reactions are in italics.

al., unpublished observations). Preliminary results suggest that this virus is more closely related to the urban rat-associated Hantaan-like virus than to the prototype Hantaan virus; however, further studies are required to confirm these initial results.

Table 3. Antibody prevalence rates to Hantaan virus among different rodent species as determined by immunofluorescent antibody assay[a]

Species	No. positive/ No. tested	Percentage positive
Rattus norvegicus	243/910	26.7
R. rattus	36/370	9.7
R. exulans	1/18	5.5
Bandicota indica	10/65	15.4
B. bengalensis	11/59	18.6

[a] The specimen was positive if the titre by immunofluorescent antibody assay was ⩾32, using Hantaan virus, strain 76-118 infected Vero E-6 cells. A total of 318 sera tested from rodents identified to genus only are not included in this Table.

Antibody prevalence rates presented in this study were based on the IFA test, using titres of ⩾32 as positive. This test is known to exhibit false positive reactions, although most false positive reactions occur below a titre of 32. None the less, some false positive results may have contributed to the rates presented. Their impact should be minimal, however, since the objectives of this study were qualitative rather than quantitative. Nearly all the sites reported as positive had some sera of high titre (⩾512), and many were confirmed by PRN tests. It is not known whether false negative IFA reactions occur.

The widespread distribution of Hantaan-related viruses in domestic and peridomestic rodents raises the possibility that haemorrhagic fever with renal syndrome may well exist undiagnosed in regions of the world beyond its traditionally recognized enzootic boundaries. Several reports have documented HFRS after human exposure to rat-associated Hantaan-like virus from infected laboratory and free-

living domestic rats (*2*, *5–9*). Conceivably this disease in man could occur in any area of the world where infected rat–human contact is common. A search for HFRS in man in such areas is now in progress in several locations to examine this possibility.

ACKNOWLEDGEMENTS

The authors extend special thanks to the many people who helped collect the rodents and process the sera. Special recognition is extended to Dr Hla Naing and Dr May La Linn, Burma; Dr J. L. Becker, Argentina; Dr A. C. Linhares, Brazil; Dr Markapol Tingpalapong, Thailand; Dr H. Hoogstraal, Egypt; Dr M. Reardon, Kenya; Dr C. Hayes, Philippines; and Mr Chau Gar-Wai, Hong Kong. The direction and guidance of Dr K. M. Johnson is gratefully acknowledged.

RÉSUMÉ

ENQUÊTE MONDIALE SUR LA PRÉSENCE D'ANTICORPS DIRIGÉS CONTRE LES VIRUS DU TYPE HANTAAN PARMI LES RONGEURS PÉRIDOMESTIQUES

Une enquête sérologique a été menée à l'échelle mondiale en vue de déterminer la distribution et la prévalence des anticorps dirigés contre les virus du type Hantaan qui sont les agents étiologiques de la fièvre hémorragique avec syndrome rénal chez l'homme. Plus de 1700 sérums de rongeurs provenant de plus de 20 endroits différents ont été examinés en immunofluorescence. Presque partout, on a constaté la présence de rongeurs positifs. C'est à Baltimore, Maryland, Etats-Unis, que le taux de prévalence était le plus élevé (64%), venait ensuite Bélem, au Brésil, avec 56%. Dans plusieurs autres lieux, les taux dépassaient 20%, le taux global pour tous les endroits et l'ensemble des espèces étant de 20,8%. C'est chez *Rattus norvegicus* que les sérums étaient le plus souvent positifs. *Bandicota indica* et *B. bengalensis* qui, jusqu'ici, ne passaient pas pour des hôtes d'hantavirus, se sont également révélés positifs. Les sérums positifs présentant un titre élevé d'anticorps ont été également soumis à des épreuves de neutralisation par réduction des plages au moyen du virus prototype de Hantaan et du virus de type Hantaan associé au rat de ville. Ces épreuves de neutralisation ont permis de déceler dans les sérums des rongeurs du genre *Rattus*, la présence d'anticorps spécifiques des virus de type Hantaan associés au rat de ville, mais il n'a pas été possible d'établir la spécificité des anticorps présents dans les sérums de *Bandicota* au moyen de cette technique. Les résultats obtenus montrent que des virus du type Hantaan existent au-delà des limites actuelles d'extension de la fièvre hémorragique humaine avec syndrome rénal et laissent craindre la présence d'une maladie de ce genre dans d'autres régions du monde où l'homme est fréquemment en contact avec des rongeurs. Des recherches sont en cours en vue de dépister les cas éventuels.

REFERENCES

1. LEE, H. W. ET AL. Isolation of the etiological agent of Korean hemorrhagic fever. *Journal of infectious diseases*, **137**: 298–308 (1978).
2. SONG GAN ET AL. Etiologic studies of epidemic hemorrhagic fever (hemorrhagic fever with renal syndrome). *Journal of infectious diseases*, **147**: 654–659 (1983).
3. BRUMMER-KORVENKONTIO, M. ET AL. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *Journal of infectious diseases*, **141**: 131–134 (1980).
4. NIKLASSON, B. & LEDUC, J. Isolation of the nephropathia epidemica agent in Sweden. *Lancet*, **1**: 1012–1013 (1984).
5. LEE, H. W. ET AL. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. *Journal of infectious diseases*, **146**: 638–644 (1982).
6. SONG, G. ET AL. Antigenic difference between viral strains causing classical and mild types of epidemic hemorrhagic fever with renal syndrome in China. *Journal of infectious diseases*, **150**: 889–894 (1984).

7. UMENAI, T. ET AL. Korean hemorrhagic fever in staff in an animal laboratory. *Lancet*, **1**: 1314–1316 (1979).
8. LEE, H. W. & JOHNSON, K. M. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. *Journal of infectious diseases*, **146**: 645–651 (1982).
9. DESMYTER, J. ET AL. Laboratory rat-associated outbreak of hemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. *Lancet*, **2**: 1445–1448 (1983).
10. LEE, P. W. ET AL. New haemorrhagic fever with renal syndrome-related virus in indigenous wild rodents in the United States. *Lancet*, **2**: 1405 (1982).
11. YANAGIHARA, R. ET AL. Prospect Hill virus: serologic evidence for infection in mammalogists. *New England journal of medicine*, **310**: 1325–1326 (1984).
12. SCHMALJOHN, C. S. ET AL. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science*, **227**: 1041–1044 (1985).
13. LEDUC, J. W. ET AL. Hantaan-like viruses from domestic rats captured in the United States. *American journal of tropical medicine and hygiene*, **33**: 992–998 (1984).