Epidemiol Infect. (1996), **117**, 361–365

Evidence of hantavirus in wild rodents in Northern Ireland

C. McCAUGHEY[1],* W. I. MONTGOMERY[2], N. TWOMEY[2], M. ADDLEY[2], H. J. O'NEILL[1] AND P. V. COYLE[1]

[1] *Regional Virus Laboratory, Royal Hospitals Trust, Grosvenor Road. Belfast BT12 6BN, UK*
[2] *School of Biology and Biochemistry, Medical Biology Centre, The Queen's University of Belfast, Belfast BT9 7BL, UK*

(*Accepted 17 May 1996*)

SUMMARY

A survey of evidence of rodent hantavirus infection in County Down, Northern Ireland was carried out by using immunofluorescence to detect virus antigen and antibody. Antibodies to hantavirus (R22 strain of Seoul virus and Hantaan 76–118) were found in 11/51 (21·6%) brown rats (*Rattus norvegicus*), 1/31 (3·2%) field mice (*Apodemus sylvaticus*) and 17/59 (28·8%) house mice (*Mus domesticus*). Seven rodents had evidence of hantavirus antigen in lung tissues. Antibody positive animals were significantly more likely to be adults than juveniles ($P = 0·04$) but and there was no sex difference between antibody positive and negative animals. House mice were more likely to be antibody positive if captured inside farm outbuildings ($P = 0·08$). Attempts to culture virus from the rodent material were unsuccessful. This work demonstrates a substantial rodent reservoir for hantavirus in Northern Ireland.

INTRODUCTION

Hantavirus infection is recognised worldwide as a cause of persistent infection in rodents and a variety of acute clinical presentations in humans who become infected through contact with rodent secretions. The clinically important rodent reservoir of hantavirus varies with locality throughout the world: mainly *Apodemus spp.* in Asia, *Clethrionomys spp.* in Europe, *Microtus spp.* and *Peromyscus spp.* in America and *Rattus spp.* worldwide. The genotype of virus varies with the rodent host, virus phylogeny closely reflecting host phylogeny [1]. *Rattus spp.* are host to Seoul-type viruses, *Apodemus sp.* are host to Hantaan-type viruses and *Clethrionomys spp.* are host to Puumala-type viruses. The clinical features and severity of hantavirus disease in humans varies according to the genotype of infecting virus [2]. The usual manifestation of hantavirus disease is acute renal failure. Hantaan virus is particularly associated with severe disease in humans and with a high rate of haemorrhagic manifestations. Seoul virus infections are most usually moderate in severity, with a low rate of haemorrhagic manifestations and are particularly associated with hepatitis. Puumala virus infections usually have a mild course and haemorrhagic manifestations are normally absent [2].

Previous studies have indicated the presence of human hantavirus infection in Northern Ireland [3–5]. Serological studies have suggested that human hantavirus infection in Northern Ireland may be caused by a virus similar to R22, a Seoul-like hantavirus, thus implicating rats as the rodent reservoir [3, 6]. There are only three rodent species in Northern Ireland, the house mouse (*Mus domesticus*) the field mouse (*Apodemus sylvaticus*) and the brown rat (*Rattus norvegicus*). The bank vole, (*Clethrionomys glareolus*),

* Address for reprints.

present in most of Northern Europe is not found in Northern Ireland. We initiated a study to look for evidence of hantavirus infection in each of the three indigenous rodent species. On the basis of the rodents present and the previous evidence of Seoul reactive human sera [3] we chose to use R22 virus and Hantaan 76–118 virus for serological screening. Because of the absence of bank voles we did not use a Puumala virus antigen.

MATERIALS AND METHODS

Survey methods

Rodent trapping was performed from May 1993 to September 1994 over a wide area of County Down where eight of the patients in our previous study lived [3]. House mice and field mice were captured on eight predominantly pastoral farms and two areas of mixed woodland. Brown rats were captured on four farms, a wild fowl reserve and a municipal land fill rubbish dump. Standard Longworth traps baited with grain were used for house mice and field mice. Bedding material was provided to ensure survival. Traps were set in pairs at 15 m intervals along traplines which followed field boundaries or arbitrary straight lines in the mixed woodland. Traps were also set inside farm buildings. Brown rats were trapped using single entrance, wire cage, traps (360 × 180 × 150 mm) baited with bacon, apple, potato or chocolate. The traps were set in areas where there were rat holes, droppings or sightings of rats.

Traps were left overnight and examined next morning. Rodents were taken to the laboratory in the traps. They were killed by exposure to CO_2 gas and submerged in Virkon (potassium monoperoxysulphate, Antec International Ltd., Suffolk, UK) to kill any ectoparasites. They were examined and species, sex, weight, reproductive condition and physical condition noted. The dead rodents were processed immediately in a class 1 biosafety cabinet.

Antibody tests

A blood sample was removed by intracardiac aspiration and the serum separated. Sera from the rodents at a dilution of 1/20 in phosphate buffered saline (PBS) were tested by the indirect immunofluorescent antibody (IFA) technique [7] using Vero-E6 cells (American Type Culture Collection C 1008) infected with R22, a Seoul type hantavirus [8] or Hantaan 76–118. The serum dilutions were applied to the multiwell slides and incubated in a humidified container for 45 min. The slides were then washed in PBS for 30 min and then FITC conjugated sheep anti-rat IgG or goat anti mouse IgG (Sigma Chemicals, Poole, UK) was applied for 20 min before washing, mounting and examining for characteristic cytoplasmic inclusions using a fluorescence microscope. All positive sera were then titrated to determine the antibody titre and were also tested against uninfected Vero-E6 cells.

Antigen detection

A sample of lung was removed aseptically and stored at −70 °C. A further sample of lung was used to make 10 impression smears on a multiwell slide which was fixed in acetone and stored at −20 °C until testing. Antigen was detected using convalescent sera from local human cases of hantavirus disease and a polyclonal rat anti-Hantaan serum. The human sera diluted 1/20 in PBS and the rat anti-Hantaan diluted 1/32 were applied to the fixed multiwell slides and incubated at 37 °C for 45 min. Slides were washed for 1 h in PBS and FITC conjugated goat anti-human IgG or sheep anti-rat IgG (Sigma Chemicals, Poole, UK) applied for 20 min before washing, mounting and examining for characteristic cytoplasmic inclusions using a fluorescence microscope.

Virus isolation

Virus isolation was attempted by inoculating subconfluent monolayers of Vero-E6 cells at pass 26–28 with 10% suspensions of lung tissues from both seropositive and antigen positive animals. Inoculated cell cultures were maintained in Eagle's minimum essential medium supplemented with 2% fetal calf serum, L-glutamine and antibiotics. Six passages at 14 day intervals were carried out on each sample. Cell cultures were tested at each passage by IFA using human convalescent sera from patients in Northern Ireland and with polyclonal rat anti-Hantaan serum.

RESULTS

Trapping

A total of 141 rodents were trapped of which 51 were brown rats, 31 field mice and 59 house mice. Brown

https://doi.org/10.1017/S0950268800001540 Published online by Cambridge University Press

Table 1. *Prevalence of serum antibody to hantavirus and virus antigen in lung in rodents trapped in Northern Ireland*

Species	No. tested	No. Ab pos (%)	No. Ag pos (%)	Total pos* (%)
Brown rat (*Rattus norvegicus*)	51	11 (21·6)	0 (0)	11 (21·6)
Field mouse (*Apodemus sylvaticus*)	31	1 (3·2)	3 (9·7)	4 (12·9)
House mouse (*Mus domesticus*)	59	17 (28·8)	4 (6·8)	19 (32·2)†
Total	141	29 (20·5)	7 (5·0)	34 (24·1)

* Antigen and/or antibody positive.
† Two animals positive for both antigen and antibody.

Table 2. *Sexual maturity of animals and antibody status*

	Antibody status		
	Positive	Negative	*P* value
House mouse (*M. domesticus*)			
Adult	15	30	
Juvenile	2	12	$P = 0{\cdot}14$ (Fishers exact)
Field mouse (*A. sylvaticus*)			
Adult	1	24	
Juvenile	0	6	$P = 0{\cdot}8$ (Fishers exact)
Brown rat (*R. norvegicus*)			
Adult	11	31	
Juvenile	0	9	$P = 0{\cdot}08$ (Fishers exact)
All rodents			
Adult	85	27	
Juvenile	27	2	$P = 0{\cdot}04$ (χ^2)

rats proved more difficult to trap than the other two species. After one or two rats were captured at a specific site the others became wary of the traps. Brown rats were trapped more efficiently using chocolate and bacon than by using apple or potato.

Antigen and antibody testing

The prevalence of positive animals varied from 0–57% between different sites. Overall 34 (24·1%) of the animals were positive for lung antigen or serum antibody or both (Table 1). All but one of the antibody positive mice were house mice and the titres ranged from 20–160 to Hantaan virus. Only two mice had a titre of 160. The titre to R22 virus was 2- to 4-fold less than the titre to Hantaan virus. The rats had higher titre antibody in the range 40-640. Nine of the 11 positive rats had antibody titres of 160 or greater. The reactivity was similar for Hantaan virus and R22 virus.

Table 3. *Seroprevalence of hantavirus infection in farm-caught house mice in farm outbuildings and outdoors*

Location caught	Total	Antibody positive	Antibody negative
Inside	43	15	28
Outdoors	16	2	14
$P = 0{\cdot}08$ (Fisher's exact test)			

Of the seven animals positive for hantavirus antigen in lung tissue only two were antibody positive and both of these were house mice. One of the antigen positive animals, a house mouse had very pronounced staining, the rest were weak. All animals that were antigen positive were positive with all six human sera used and with the rat anti-Hantaan. Virus isolation attempts on lung samples from animals positive for antigen or antibody were unsuccessful.

Antibody positive animals were significantly more likely to be adults than juveniles when data were combined for all three species ($P = 0{\cdot}04$) (Table 2). Most of the house mice were trapped inside farm outbuildings and these mice were more likely to be antibody positive than those trapped outside (Table 3) although this effect was not quite significant. ($P = 0{\cdot}08$).

Seven rats noted to be very heavily infested with fleas were all antibody negative. One rat exhibited

https://doi.org/10.1017/S0950268800001540 Published online by Cambridge University Press

obvious signs of illness. It was in a state of collapse with a mucopurulent conjunctivitis, mucous discharge from the nose and injected oropharynx. On dissection the lung had the gross appearance of a haemorrhagic pneumonia. It was antibody positive (titre 160 to R22). One rat had multiple old healed injuries consisting of tears to both ears and upper lip and a stump tail. It was antibody positive (titre 320 to R22). There was no significant sex difference in positivity rate for any of the rodent species. Pregnancy and lactation were unrelated to positivity rate.

DISCUSSION

This study confirmed the presence of hantavirus in the rodent population of Northern Ireland. The prevalence of infection in rodents by serology and antigen detection in our population (24·1%) was considerably higher than that reported (1·8%) in Great Britain [9] but is similar to that reported in serosurveys elsewhere [10]. Lack of correlation between antigen and antibody assays may represent early infection before development of antibody. All of the rats were scored as antigen negative. However, the fluorescence with both human sera and with the rat anti-Hantaan reagent was difficult to read due to background staining. This nonspecific staining was not appreciably affected by increased washing and may have obscured detection of virus inclusions in the rat lung specimens.

The finding that antibody positive animals were significantly more likely to be adults than juveniles supports previous suggestions that transmission of this persistent infection in rodents is horizontal and not vertical, and that acquisition of hantavirus in rats is usually at the onset of sexual maturity [10]. The presence of injuries inflicted by other rats has previously been identified as a risk factor in hantavirus infection in rats [10]. Our description of a rat with multiple injuries which was antibody positive may provide supportive evidence for this.

The finding that house mice (*M. domesticus*) were more likely to be antibody positive if captured inside farm outbuildings implies that this environment may favour more efficient transmission of the virus. This may be due to increased crowding and socialization or to enhanced environmental survival of shed virus.

Although it is unlikely that the clinical signs described in the ill rat were due to hantavirus which normally causes no disease in the rodent host, it is of note that the only ill rat we collected was antibody positive. Seven rats were observed to be very heavily infested with fleas were all antibody negative. This is a good indication that fleas are not implicated in the transmission of the hantavirus. Ectoparasites have previously been implicated in hantavirus transmission [11]. Difficulty in virus isolation is well recognized for some hantaviruses. Passage through susceptible laboratory rodents may be necessary for primary isolation of some hantaviruses [12]. Further studies are planned using the polymerase chain reaction and animal inoculation to determine the nature of the hantavirus in the preserved tissue specimens.

ACKNOWLEDGEMENTS

We thank Dr G. Lloyd, CAMR Porton Down, Salisbury for the gift of Hantaan 76–118 infected E-6 cells and also a polyclonal rat anti-Hantaan serum. We are also grateful to the farmers and other landowners who granted access to their properties.

REFERENCES

1. Xiao SY, Leduc JW, Chu YK, Schmaljohn CS. Phylogenetic analyses of virus isolates in the genus hantavirus, family Bunyaviridae. Virology 1994; **198**: 205–17.
2. Lloyd G. Hantavirus. In: Morgan-Capner P, ed. Current topics in clinical virology. London: Public Health Laboratory Service, 1991: 181–204.
3. McKenna P, Clement J, Matthys P, Coyle PV, McCaughey C. Serological evidence of hantavirus disease in Northern Ireland. J Med Virol 1994; **43**: 33–8.
4. Davies EA, Rooney PJ, Coyle PV, Simpson DIH, Montgomery WI, Stanford CF. Hantavirus and leptospira. Lancet 1988; **88**: 460–1.
5. Stanford CF, Connolly JH, Ellis WA, et al. Zoonotic infections in Northern Ireland farmers. Epidemiol Infect 1990; **105**: 565–70.
6. McKenna P, Clement J, McCaughey C, Coyle PV. Wild rats: a vector of hantavirus disease in Northern Ireland? Am J Trop Med Hyg 1993; **49**: 183–4.
7. Lee PW, Gibbs CJ Jr, Gajdusek DC, Yanagihara R. Serotypic classification of hantaviruses by indirect immunofluorescent antibody and plaque reduction neutralization tests. J Clin Microbiol 1985; **22**: 940–4.
8. Song G, Hang C-S, Liao H-X, et al. Isolation of EHF-related agent from *Rattus norvegicus* captured in patient's home in endemic area of the mild type of hemorrhagic fever. Acta Microbiol Sin 1982; **22**: 373–7.
9. Pether JV, Lloyd G. The clinical spectrum of human hantavirus infection in Somerset, UK. Epidemiol Infect 1993; **111**: 171–5.

10. Glass GE, Childs JE, Korch GW, LeDuc JW. Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*). Epidemiol Infect 1988; **101**: 459–72.
11. Zhang Y. Study on natural infection of epidemic haemorrhagic fever virus in *Leptotrombidium scutellare*. [Chinese.] Chung-Hua Yu Fang i Hsueh Tsa Chih [Chinese Journal of Preventive Medicine] 1990; **24**: 261–4.
12. Elliott LH, Ksiazek TG, Rollin PE, et al. Isolation of the causative agent of hantavirus pulmonary syndrome. Am J Trop Med Hyg 1994; **51**: 102–8.