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A minority of seropositive wild bank voles (*Clethrionomys glareolus*) show evidence of current Puumala virus infection

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SUMMARY

Bank voles (*Clethrionomys glareolus*) serve as the reservoir for Puumala (PUU) virus, the aetiologic agent of nephropathia epidemica. The animals are believed to be persistently infected and the occurrence of serum antibodies is usually taken as an evidence of active infection. We found serum antibodies to PUU virus in 42 of 299 wild bank voles captured in a PUU virus endemic area. PUU virus RNA was demonstrated in lung specimens of 11 of these 42 animals and in 2 of them antigen was also found. Thus in the lungs of 31 of 42 seropositive animals neither PUU virus RNA nor antigen was detected. In 2 of 257 seronegative animals, lung specimens showed presence of PUU virus antigen and RNA. Isolation of PUU virus from lung tissue was successful in all 4 antigen-positive bank voles but in none of 16 tested antigen-negative animals. In conclusion, only a minority of bank voles with serum antibodies to PUU virus showed evidence of current infection.

INTRODUCTION

Hantavirus disease encompasses 2 distinct clinical entities: haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) [1–4]. HFRS and HPS are caused by related but clearly distinct viruses, and have different clinical manifestations. HFRS is associated with Puumala (PUU), Seoul (SEO), Hantaan (HTN) and Dobrava (DOB) viruses and is characterized by fever, haemorrhages and acute renal insufficiency, while HPS is caused by Sin Nombre (SN) virus and presents with fever and noncardiac pulmonary edema. Each hantavirus is believed to have its own rodent reservoir. PUU virus is associated with *Clethrionomys glareolus*, HTN virus with *Apodemus agrarius*, SEO virus with *Rattus spp*, DOB virus with *Apodemus flavicollis* and SN virus with *Peromyscus maniculatus*.

The interaction of hantaviruses with their reservoir hosts is poorly understood. Isolation attempts have been performed successfully on lung tissue from rodents shown to have specific serum antibodies to the agent. This experience, together with the demonstration of infectious virus in excretions from bank voles, is held to imply that the rodents may become persistently infected in spite of the presence of specific serum antibodies [5–8]. The evidence of persistent infection is, however, scant and particularly in wild-living bank voles, the reliability of the antibody response as an indicator of current infection remains to be studied.

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Due to progress of methodology, including assays for antigen detection in tissue by ELISA [9] and PCR for detection of PUU virus RNA [10], the occurrence of PUU virus among seropositive bank voles can now be more reliably investigated. In the present study on 299 bank voles captured in their normal habitat in Northern Sweden, evidence was obtained suggesting that presence of PUU virus antibodies does not necessarily imply infectiousity.

MATERIALS AND METHODS

Rodent collection

Four areas were chosen in the county of Västerbotten in Northern Sweden, in the vicinities of patients who had presented with NE 3, 9, 10 and 38 weeks before onset of the study. For each case area, a control area was randomly selected at a distance of 10 km from the case area [11]. Trapping was performed from 5 October to 3 November 1995, using snap traps (Etutuote Ky, Vaasa, Finland) baited with dried apples. Totally, 6000 trap nights were included. Lungs from the rodents were excised and kept at -70 °C until tested. From each animal, one lung was used for antigen-detection by ELISA and the other lung for PCR and isolation of virus. Blood from the chest cavity of the rodents was collected using Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd, Tokyo, Japan). The filters were dried and subsequently eluted according to the manufacturer's instructions.

Assay of hantavirus antibody in blood samples

Blood samples from *C. glareolus* were investigated by an ELISA for antibodies to SEO, SN and PUU virus recombinant truncated hantavirus nucleocapsid proteins (rNΔ) (aa 1–117; [12]) expressed in *E. coli*. The SEO rNΔ served as a representative of HTN-like hantaviruses. ELISA was performed as previously described, with minor modifications [13]. Briefly, microtitre plates (MaxiSorp, NUNC, Roskilde, Denmark) were coated overnight at room temperature with 0·2 μg/well of hantavirus rNΔ. One hundred μl of rodent blood at an estimated dilution of 1/200 in PBS-Tween® 20, supplemented with 4% (wt/vol) defatted milk powder, was incubated at 37 °C for 2 h. Wells were thereafter incubated at 37 °C for 1 h with horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma, St Louis, MO), diluted 1/2000. The reaction was developed using tetra-methylbenzidine (K-blue, ELISA Technologies, Division of Neogen Corporation, Lexington, KY) and the absorbance was measured in a spectrophotometer at 450 nm. Control wells contained no antigen. Serum from one and the same NE patient was used as a positive control. A net absorbance of $\geqslant 0{\cdot}2$ units between antigen and control wells was defined to show the presence of antibodies.

Blood samples shown by ELISA to contain anti-PUU virus antibodies were also tested by an immunofluorescence assay (IFA) using PUU virus (strain Sotkamo) propagated in Vero E6 cells. The infected cells were fixed on glass slides by air drying and acetone treatment. Blood (dilution 1/25) was incubated for 30 min at 37 °C followed by rabbit anti-mouse immunoglobulin FITC-labelled conjugate (dilution 1/20) (DAKO). The slides were read in an Olympus fluorescence microscope at 400× magnification.

Antigen-detecting ELISA (Ag-ELISA)

Vole lungs were homogenized using an ULTRA-TURRAX® T 25 device (IKA-Labortechnik, Janke & Kunkel Gmbh & Co, Germany). The assay was performed as described elsewhere [9]. Wells of microplates were coated with goat anti-human IgM antibody (Sigma, St. Louis, MO) and human IgM antibodies were subsequently bound to the wells. Sera from two HFRS patients infected with HTN/DOB virus or PUU virus were used as sources of IgM. A 10% suspension of homogenized bank vole lung at a dilution of 1/4 in PBS-Tween 20 supplemented with 0·5% of BSA was incubated in the wells overnight at 4 °C. A polyclonal rabbit antiserum, produced against recombinant PUU nucleocapsid protein, was used to detect bound antigen. After incubation with swine anti-rabbit antiserum coupled to horseradish peroxidase conjugate (DAKO, Copenhagen, Denmark), tetra-methylbenzidine was added and the reaction was read at 450 nm. The cut-off was calculated as the mean plus two standard deviations, based on testing of 20 PCR and antibody negative bank voles. This corresponded to an absorbance value of 0·2 OD and values $> 0{\cdot}2$ OD were regarded as positive.

Nested polymerase chain reaction (nPCR)

RNA was extracted from homogenized vole lung using the RNeasy Total RNA kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. First-strand cDNA was synthesized using a

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Table 1. *Primers used in reverse transcription and nPCR for the detection of PUU virus S segment of RNA in lung tissue of* C. glareolus

Name	Specification	Position	DNA sequence
Universal	End-primer*	1–13	TAGTAGTAGACN(C/T)C
FE1	PUU† S‡ outer 5′	1–25	TAGTAGTAGACTCCTTGAAAAGCTA
PUU5	PUU S outer 3′	509–481	CGTGTCCCCTTATTTTCCTTTACAGTCTG
ΔPUU1	PUU S inner 5′	43–67	ATGAGTGACTTGACAGATATCCAAG
ΔPUU2	PUU S inner 3′	393–370	TGCTGTTTGGCCACTTGGTTCTTC

* Schmaljohn CS, et al. [14].
† PUU virus, strain Sotkamo.
‡ Small genome segment.

hantavirus specific oligonucleotide primer [14]. In this reaction, 3 μl of total RNA and 2 μl of the primer were heated at 90 °C for 5 min in a total volume of 10 μl. This material was reverse transcribed into cDNA in a total volume of 20 μl containing 200 U of Superscript reverse transcriptase in the buffer supplied by the manufacturer (Life Technologies) in the presence of 39 U of RNase inhibitor (Promega), 5 μl of 0·1 mM dithiothreitol (DTT) and 2·5 μl of each dNTP (UltraPure; Pharmacia Biotech). The mixture was incubated at 37 °C for 1 h followed by heating at 99 °C for 5 min. Thereafter, PCR was performed in a reaction volume of 50 μl containing 5 μl of cDNA, 2·5 mM of $MgCL_2$, 0·1 mM of each dNTP, 25 units of Taq polymerase, and 1 μl of each primer in Taq polymerase buffer (500 mM HCl, 100 mM Tris-HCl, 1% Triton X-100). Primers derived from the 5′-region of the PUU (strain Sotkamo) virus S segment were used [15] (Table 1). Twenty-five cycles were run, each consisting of 45 s at 94 °C, 45 s at 56 °C (except for initial three cycles at 45 °C) and 45 s at 72 °C. The amplified product was used in a nested PCR (nPCR). Ten μl of the sample were diluted in 90 μl of the reaction mixture (see above) and the reaction was run for an additional 35 cycles (each consisting of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C). The amplified product (351 bp) was subsequently subjected to electrophoresis through a 1·5% agarose gel and visualized by staining with ethidium bromide. Negative controls and PCR reagent controls were included in all experiments. Extraction of RNA, PCR, and electrophoresis were all performed in separate laboratories.

In order to determine the sensitivity of the nPCR, Vero E6 cells infected with PUU virus, strain Sotkamo were tested. According to IFA 95% of cells were infected. One ml of cell suspension (800000 cells/ml) was centrifuged at 3000 r.p.m. for 10 min. Total RNA was extracted from the cell pellet. When RNA was serially diluted in 2-fold steps, the detection limit by nPCR was 1:3200, approximately corresponding to 250 infected cells.

Isolation of virus

Two hundred microliters of a 10% suspension of homogenized lung tissue were adsorbed onto Vero E6 cells in 2 glass tubes for 2 h. After washing, the cultures were incubated at 37 °C in a rolling drummer for 2 weeks in Dulbecco's modified medium supplemented with 5% foetal calf serum. Then the cells were passaged into a 25 cm^2 plastic flask and incubated for another 4 weeks. After a total of 6 weeks of incubation, the cells were tested by IFA for the presence of viral antigen. For detection of hantaviral antigen, the inoculated Vero E6 cells were dried on glass slides, treated with cold acetone and then incubated with a polyclonal rabbit antiserum (anti-PUU virus IgG titre > 640, anti-HTN titre 320, as determined by IFA) and separately with a serum from a patient in the convalescence phase of NE (anti-PUU virus IgG titre > 640) at a dilution of 1/40 for 30 min at 37 °C. After subsequent incubation for 30 min at 37 °C with swine anti-rabbit or rabbit anti-human IgG (DAKO) FITC-labelled conjugates, both at 1/20 dilution, the slides were examined in a fluorescence microscope. Cultures positive by IFA were confirmed by nPCR.

RESULTS

Presence of antibodies

Out of 299 *C. glareolus* captured, blood samples from 42 contained anti-PUU virus antibodies detectable by ELISA (Table 2*a*, *b*). In 40 of these 42 animals IFA was confirmatory.

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Table 2. *Detection of PUU virus antigen and RNA and isolation of PUU virus in wild-living* C. glareolus (n = *299*) *with or without detectable serum anti-PUU virus antibodies*

Test	No. of positive animals/ no. of animals tested
(*a*) Seronegative animals (*n* = 257)	
PUU virus antigen	3/257
PUU virus RNA*	2/29
PUU virus isolation†	2/2
(*b*) Seropositive animals (*n* = 42)	
PUU virus antigen	2/42
PUU virus RNA	11‡/42
PUU virus isolation†	2/18

* PCR for PUU virus RNA was performed on the 3 antigen-positive and 26 other randomly selected animals. Two of the 3 antigen positive animals were RNA-positive as well.

† Isolation attempt was done only on lung specimens from the 2 antigen/RNA positive animals.

‡ Including the 2 antigen-positive animals.

§ Isolation of PUU virus was attempted on lung tissue from the 2 antigen and RNA positive animals, on all 9 antigen-negative and RNA-positive animals, and on 7 randomly selected sheer antibody-positive animals. Isolation attempts were successful only in the 2 antigen and RNA positive animals.

Presence of PUU virus antigen

In 5 out of 299 rodents PUU virus antigen was detected in lung tissue (median OD value: 0·27, range 0·22–0·65). Three antigen-positive animals were seronegative.

Presence of PUU virus RNA

nPCR was performed on lung tissue from the 3 seronegative animals which showed presence of antigen and from all 42 seropositive animals. In 2 of the former 3 animals PUU virus S segment RNA was successfully amplified (Table 2*a*). In 11 of the 42 seropositive animals, including the two antigen positive ones, viral RNA was detected (Table 2*a*). Thus, in 31 of the 42 seropositive animals (74%), neither PUU antigen nor RNA was found.

Presence of infectious virus

PUU virus was successfully isolated from all 4 antigen and RNA positive animals. Isolation attempts were unsuccessful in all 9 RNA and seropositive and in 7 out of 31 randomly selected sheer antibody-positive animals. One cell culture inoculated with lung tissue from a RNA and antibody positive rodent was found to be contaminated with bacteria at week 5 and was tested by nPCR only. The sample proved negative. The identity of all PUU virus isolates was verified by nPCR with primers specific to the S segments of PUU, HTN, and DOB viruses (data not shown). Two of the culture and RNA positive animals lacked antibodies as analysed by ELISA (Table 1*a*). These animals were seronegative also when tested by IFA against their own viral strains.

The present study was performed concomitantly with an epidemiological investigation showing a higher rodent density and higher prevalence of antibodies to hantavirus in the vicinities of households afflicted with NE (case areas) than in control areas [11]. All 13 PCR-positive rodents, among them the 4 culture positive animals, originated from case areas.

DISCUSSION

When bank voles are experimentally infected with PUU virus, they respond with induction of antibodies which are demonstrable along with infectious virus for up to 9 months [16]. This response may readily be taken as evidence of persistent infection, and the occurrence of serum antibodies thus to indicate the presence of virus [5–8, 17]. Such a view is, however, not supported by the present data. On the contrary, the majority of bank voles positive for anti-PUU virus antibodies showed no evidence of current infection. In lung tissue of the bank voles, an organ held to be consistently and densely infected with PUU virus, the nPCR detected PUU virus RNA in only 11 of 42 seropositive animals and by ELISA, two of these animals disclosed the presence of antigen (Table 2*b*).

Altogether, the assay of anti-PUU virus antibodies, PUU virus antigen, and PUU virus RNA yielded four patterns of findings, indicating a more dynamic course of the natural hantavirus infection in *C. glareolus* than has been previously recognized. In such a scenario, 2 of 257 seronegative animals exhibiting lung deposits of viral antigen, RNA, and infectious virus but no detectable humoral immune response, were at an early phase of infection. Experimental data indicate that the antibody response develops 2 weeks after inoculation of bank voles [16]. A second phase of the infection might be represented by the two animals containing PUU virus antigen, RNA, and infectious virus along

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with a specific antibody response. Nine animals with RNA and serum antibodies, but no antigen, might belong to a third phase. In these animals, a surplus of antibodies might have blocked the antigen and neutralized the virus, since attempts to isolate the virus were unsuccessful in all these animals. Finally, a fourth phase of the course of infection might be represented by the majority of seropositive animals (31/42). These animals lacked detectable antigen as well as RNA and isolation attempts in seven randomly selected subjects were all negative. These animals might have had a low grade infection, below the detection limit of the present assays, or they might even have eradicated the infection.

When interpreting the present results, it should be recalled that the study was performed during a short period of time and did not cover various phases of the 4-year cycle of population density of *C. glareolus* seen in Northern Scandinavia [18]. Even though less than 10% of the animals examined showed evidence of current infection, the results do not exclude that, during some other phase of the growth cycle, a burst of infection might have occurred. Evidently the population was in decline, since the density of animals was dramatically reduced the following year in Northern Sweden (unpublished observation). A burst of infection with a high density of newly infected animals has been reported in studies of SN virus [19, 20]. During an outbreak of HPS in the US, SN virus RNA and specific antibodies were concomitantly detected in more than 90% of *P. maniculatus* [19, 20]. More in similarity with our data were those on a Prospect Hill-like virus [21]. In that study only about 20% of seropositive wild *M. pennsylvanicus* had granular fluorescence in their lungs consistent with the presence of virus. In another study, nearly 40% of seropositive *A. agrarius* lacked HTN virus antigen detectable by IFA [22].

In the present study, 2 of 257 seronegative animals were found to contain infectious virus. These animals may have been infected close in time before sampling and before the development of a detectable antibody response. Experimental data have indicated that an antibody response occurs about 2 weeks after inoculation of bank voles with PUU virus [16]. In studies on deer mice, Childs and colleagues reported that 55% of seronegative animals collected during a HPS outbreak were PCR positive [19]. This high percentage may reflect sampling during a burst of spread of virus at an early stage of an outbreak, while the low percentage in our material might implicate that sampling was performed after a burst of viral spread.

The present data afforded some information relevant to the question of why in vitro isolation of PUU virus from seropositive animals is not easily performed [23]. Obviously, the failure to isolate PUU virus may be due to a neutralizing effect of anti-PUU virus antibodies. In those two bank voles which showed demonstrable PUU virus antigen and RNA but no detectable antibodies, virus was successfully isolated. Attempts to isolate virus from 18 antibody-positive animals were successful only in those two who had detectable antigen. The importance of neutralization seems to be compatible with experience of *in vitro* isolation of PUU, SN and Tula viruses from their reservoir animals. In these experiments, isolation was facilitated by a preceding passage of virus from wild animals to non-infected laboratory animals [24–26]. Thus sampling early in the course of infection seems to be important for successful isolation of hantaviruses.

Assuming that the presence of PUU virus antigen and successful viral isolation are indicators of infectiousity, the present data suggest that contagious animals were infrequent in the areas studied. It is unknown to what extent individual members of a rodent population may contribute to a reservoir of hantavirus. This should depend to a large extent on how long time an individual animal may retain the infection. In one study, viral antigen was detectable in the lungs of weanling bank voles by IFA as long as 9 months after intramuscular inoculation [16]. In another study, HTN virus was successfully isolated from several organs 6 months after inoculation of newborn laboratory rats [17]. On the contrary, HTN virus RNA was not detected in lungs by PCR on day 28 after inoculation of adult ICR mice [27]. An extrapolation of these data to wild animals is somewhat hampered by the use of parenteral inoculation to infect laboratory animals [5–8, 16, 17], a route which may not necessarily lead to a natural course of infection. In wild-living bank voles, little is known of the longevity of PUU virus after infection. A reasonable interpretation of our results would be that the contagiousity of most seropositive bank voles was low at the time of investigation. Similarly, a low degree of contagiousity of hantavirus seropositive rodents was implicated from studies under laboratory conditions on colonies of deer mice [20, 28]. In spite of the occurrence of animals positive for anti-SN virus antibodies in the colonies, most cage-mate animals

remained seronegative indicating that viral spread did not occur within the colony.

In the present study, all 4 antigen positive bank voles, and also all other 9 RNA positive animals examined were captured in the vicinities of households recently afflicted with NE. The present observation may thus reflect viral spread to humans in the areas studied and if this holds true, only a minority of the bank voles of the areas seemed to be capable of viral spread. In line with that, a study from the US showed a low prevalence of human disease in spite of exceedingly high hantavirus seroprevalence (up to 48%) among rodents [29].

In conclusion, current PUU virus infection was demonstrated in only a minority of seropositive bank voles in the areas studied. It remains to be determined whether this is true only within a given phase of an endemic cycle. If consistent over prolonged periods, this would imply that spread of PUU virus to humans may depend upon the presence of a few contagious animals. Our data also indicate that the occurrence of anti-PUU virus antibodies in bank voles cannot simply be taken as proof of current infection.

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REFERENCES

1. Gajdusek DC. Muroid virus nephropathies and muroid viruses of the Hantaan virus group. Scand J Infect Dis 1982; **36**: 96–108.
2. Lee HW, Lee PW, Baek LJ, Chu JK. Geographical distribution of hemorrhagic fever with renal syndrome and hantaviruses. Arch Virol 1990; **111** (Suppl. 1): 5–18.
3. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 1993; **262**: 914–7.
4. Duchin JS, Koster FT, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. New Engl J Med 1994; **330**: 949–55.
5. Lee PW, Yanagihara R, Gibbs CJ, Gajdusek DC. Pathogenesis of experimental Hantaan virus infection in laboratory rats. Arch Virol 1986; **88**: 57–66.
6. Yamanouchi T, Domae K, Tanishita O, et al. Experimental infection in newborn mice and rats by hemorrhagic fever with renal syndrome (HFRS) virus. Microbiol Immunol 1984; **28**: 1345–53.
7. Dohmae K, Okabe M, Nishimune Y. Experimental transmission of hantavirus infection in laboratory rats. J Infect Dis 1994; **170**: 1589–92.
8. Nazum EO, Rossi CA, Stephenson EH, LeDuc JW. Aerosol transmission of Hantaan and related viruses to laboratory rats. Am J Trop Med Hyg 1988; **38**: 636–40.
9. Alexeyev OA, Elgh F, Ahlm C, et al. Hantavirus antigen detection using serum immunoglobulin M as the capturing antibody in an enzyme-linked immunosorbent assay. Am J Trop Med Hyg 1996; **54**: 367–71.
10. Xiao SY, Yanagihara R, Godec MS, et al. Detection of hantavirus RNA in tissues of experimentally infected mice using reverse transcriptase-directed polymerase chain reaction. J Med Virol 1991; **33**: 277–82.
11. Ahlm C, Alexeyev OA, Elgh F, et al. High prevalence of hantavirus antibodies in bank voles (*Clethrionomys glareolus*) captured in the vicinity of households afflicted with nephropathia epidemica. Am J Trop Med Hyg 1997; **56**: 674–8.
12. Elgh F, Lundkvist Å, Alexeyev OA, et al. Serological diagnosis of hantavirus infection by enzyme-linked immunosorbent assay based on detection of immunoglobulin M and G responses to recombinant nucleocapsid protein of five viral serotypes. J Clin Microbiol 1997; **35**: 1122–30.
13. Elgh F, Wadell G, Juto P. Comparison of the kinetics of Puumala virus specific IgM and IgG antibody responses in nephropathia epidemica as measured by a recombinant antigen-based enzyme-linked immunosorbent assay and an immunofluorescence test. J Med Virol 1995; **45**: 146–50.
14. Schmaljohn CS, Hasty SE, Dalrymple JM, et al. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. Science 1985; **227**: 1041–4.
15. Vapalahti O, Kallio Kokko H, Salonen EM, Brummer Korvenkontio M, Vaheri A. Cloning and sequencing of Puumala virus Sotkamo strain S and M RNA segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. J Gen Virol 1992; **73**: 829–38.
16. Yanagihara R, Amyx HL, Gajdusek DC. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (*Clethrionomys glareolus*). J Virol 1985; **55**: 34–8.
17. Tanishita O, Takahashi Y, Okuno Y, et al. Persistent infection of rats with haemorrhagic fever with renal syndrome virus and their antibody responses. J Gen Virol 1986; **67**: 2819–24.

18. Nyström K. Incidence and prevalence of endemic benign (epidemic) nephropathy in AC county, Sweden, in relation to population density and prevalence of small rodents. Acta Med Scand (Suppl) 1977; **609**: 1–92.
19. Childs JE, Ksiazek TG, Spiropoulou CF, et al. Serologic and genetic identification of Peromyscus maniculatus as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis 1994; **169**: 1271–80.
20. Otteson EW, Riolo J, Rowe JE, et al. Occurrence of hantavirus within the rodent population of northeastern California and Nevada. Am J Trop Med Hyg 1996; **54**: 127–33.
21. Burek KA, Rossi CA, LeDuc JW, Yuill TM. Serologic and virologic evidence of a Prospect Hill-like hantavirus in Wisconsin and Minnesota. Am J Trop Med Hyg 1994; **51**: 284–94.
22. Ruo SL, Li YL, Tong Z, et al. Retrospective and prospective studies of hemorrhagic fever with renal syndrome in rural China. J Infect Dis 1994; **170**: 527–34.
23. McKee KT, LeDuc JW, Peters CJ. Hantaviruses. In: Belshe RB, ed. Textbook of human virology, 2nd ed. St. Louis: Mosby-Year Book, Inc, 1991: 615–32.
24. Yanagihara R, Svedmyr A, Amyx HL, et al. Isolation and propagation of nephropathia epidemica virus in bank voles. Scand J Infect Dis 1984; **16**: 225–8.
25. 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.
26. Vapalahti O, Lundkvist Å, Kukkonen SKJ, et al. Isolation and characterization of Tula virus, a distinct serotype in the genus *Hantavirus*, family *Bunyaviridae*. J Gen Virol 1996; **77**: 3063–7.
27. Kariwa H, Kamimura M, Arikawa J, Yoshimatsu K, Takashima I, Hashimoto N. Characterization of the mode of Hantaan virus infection in adult mice using a nested reverse transcriptase polymerase chain reaction: transient virus replication in adult mice. Microbiol Immunol 1995; **39**: 35–41.
28. Hjelle B, Jenison SA, Goade DE, Green WB, Feddersen RM, Scott AA. Hantaviruses: clinical, microbiologic and epidemiologic aspects. Crit Rev Clin Lab Sci 1995; **32**: 469–508.
29. White DJ, Means RG, Birkhead GS, et al. Human and rodent hantavirus infection in New York state: public significance of an emerging infectious disease. Arch Intern Med 1996; **156**: 722–6.