JOURNAL OF VIROLOGY, Oct. 1994, p. 6751–6754
0022-538X/94/$04.00+0

Vol. 68, No. 10

Genetic Identification of a Novel Hantavirus of the Harvest Mouse *Reithrodontomys megalotis*

BRIAN HJELLE,[1,2,3]* FRANCESCA CHAVEZ-GILES,[1] NORAH TORREZ-MARTINEZ,[1] TERRY YATES,[4] JOHN SARISKY,[5] JAMES WEBB,[6] AND MICHAEL ASCHER[7]

Department of Pathology, University of New Mexico School of Medicine,[1] University of New Mexico Cancer Research and Treatment Center,[2] United Blood Services,[3] and Museum of Southwestern Biology, Department of Biology, University of New Mexico,[4] Albuquerque, New Mexico; Navajo Area Indian Health Service, Window Rock, Arizona[5]; and Orange County Vector Control District, Santa Ana,[6] and Viral and Rickettsial Disease Laboratory, California State Department of Health Services, Berkeley,[7] California

Received 18 May 1994/Accepted 29 June 1994

We have cloned the S genomic segment of a novel hantavirus of the harvest mouse *Reithrodontomys megalotis*. The virus is phylogenetically distinct from other hantaviruses. The new hantavirus was identified in harvest mice separated by ~1,000 km. A wood rat (*Neotoma mexicana*) was found to be infected with the harvest mouse hantavirus.

Hantaviruses have an RNA genome consisting of the segments L, M, and S. The L segment may encode the transcriptase, the S segment encodes the nucleocapsid protein N, and the M segment encodes the envelope glycoproteins G1 and G2 (7). The hantavirus genus includes etiologic agents for hemorrhagic fever with renal syndrome (Hantaan virus [HTN], Seoul virus [SEO], and Puumala virus [PUU]) and hantavirus pulmonary syndrome (HPS) (2, 3, 11, 17, 29). The Four Corners virus (FCV) has been implicated in >70 cases of HPS in 15 western states (3, 10). Other cases of HPS were caused by novel agents in Florida (4) and in Louisiana (3). The prototypic New World hantavirus is the Prospect Hill virus (PHV) of the meadow vole, *Microtus pennsylvanicus* (14).

Serologic evidence of FCV infection has been obtained for seven rodent genera (5). Reverse transcription PCR (RT-PCR) confirmed the presence of FCV RNA in three genera, suggesting that FCV can "spill over" from its major reservoir species, *Peromyscus maniculatus* (5, 16), into other rodent hosts. Harvest mice (*Reithrodontomys megalotis*) had serologic evidence of infection but lacked detectable FCV RNA (5). We show that *R. megalotis* is host to a novel species of hantavirus.

Serologic screening for FCV antibodies. Blood samples were obtained from 45 rodents (24 *P. maniculatus*, 17 *Peromyscus eremicus*, and 4 *R. megalotis* animals) trapped for hantavirus surveillance in Orange County, Calif. Sera (1:400 dilution) were screened for FCV antibodies by Western immunoblotting using a TrpE-FCV nucleocapsid fusion protein (12, 13). Immune complexes were detected with 1:1,000 alkaline phosphatase-conjugated anti-*Peromyscus leucopus* immunoglobulin G (Kirkegaard & Perry, Gaithersburg, Md.) followed by nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (12).

RT-PCR. Numerous consensus primers with sequence similarity to the M and S segments of FCV and related hantaviruses (PHV and PUU) were used in nested PCR (11, 17, 22), as described previously (11). Only S-segment cDNAs were successfully amplified. Table 1 lists primers that amplified cDNA of the prototype Orange County *R. megalotis* (RM-97) hantavirus. Portions of the S segment of other *R. megalotis* rodents from Orange County (RM-45, RM-92, and RM-98) and from Apache County, Ariz. (RMNK-51), were also amplified, as were cDNAs from *Peromyscus* animals trapped in Orange County (PM-222), Apache County (PMNK-55, PTNK-43, and PTNK-56), or Sante Fe County, N. Mex. (PL-1153).

Sequencing and phylogenetic analysis. The cDNAs were isolated, cloned, and sequenced as described previously (11). Internal sequencing primers were synthesized as necessary to allow double-stranded sequencing of the inserts of at least two clones of each amplimer. Weighted phylogenetic analysis was conducted as described in the legend to Fig. 2 (9, 15, 23). The groupings were significantly supported by heuristic bootstrap analysis (8, 23).

The viral sequences used and their GenBank accession numbers were as follows: HTN 76-118 (21), M14626; SEO SR-11 (1), M34881; PUU Sotkamo (24), X61035; PUU P360 (28), L11347; PHV-1 (18), X55128; FCV Case H (22), L25784; FCV 3H226 (NM-1) and NM-H (11, 22), U02474. Remaining tissues are archived in the Division of Biological Materials at the Museum of Southwestern Biology, University of New Mexico.

A hantavirus enzootic in *R. megalotis* in Orange County and Apache County. Two of the 45 rodent serum samples from Orange County (a *P. maniculatus* sample [PM-222] and an *R. megalotis* sample [RM-97]) had antibodies reactive with FCV. The identification of a seropositive *R. megalotis* animal prompted study of 21 additional archived *R. megalotis* samples. Four more seropositive *R. megalotis* animals were identified.

Sixty-three rodents trapped near the home of an Apache County HPS patient were tested for hantavirus antibodies. Thirty-six were *P. maniculatus* or *Peromyscus truei*, one was *Neotoma mexicana*, and fifteen were *R. megalotis*. Of those 52 animals, 27 were seroreactive to FCV, including 4 *R. megalotis* animals and the *N. mexicana* animal.

The sequence of the gene for the nucleocapsid protein of the RM-97 hantavirus showed that the *R. megalotis* hantavirus is distinct from its closest relative, FCV. A nucleotide sequence distance of 23.7% between the RM-97 hantavirus nucleocapsid gene and that of FCV was measured. The amino acid distance

* Corresponding author. Mailing address: Department of Pathology 337-BRF, University of New Mexico School of Medicine, Albuquerque, NM 87131. Phone: (505) 277-5872. Fax: (505) 277-1950. Electronic mail address: bhjelle@medusa.unm.edu.

TABLE 1. Primers used in amplification of hantavirus S-segment cDNA from various sources

Source[a]	Primers[b]		GenBank accession no. of product
	Outer	Inner	
H-AZ-A	1, 980	20, 476	U11433
RM-97	1, 980	20, 476	U11427
NMNK-164	1, 980	20, 476	U11429
RM-45	1, 980	20, 476	U11425
RMNK-51	1, 980	20, 476	U11430
PM-222	1, 980	20, 476	U11436
PMNK-55	1, 980	20, 476	U11431
PTNK-43	1, 980	20, 476	U11432
PTNK-56	1, 980	20, 476	U11434
PL-1153	1, 980	20, 476	U11435
RM-97	167, 2059	190, 1326	U11427
RM-97	1147,[c] 2059	1234,[c] 2059	U11427
NMNK-164	167, 2059	190, 1257	U11429
RM-92	167, 476	190, 423[c]	U11426
RM-98	167, 476	190, 423	U11428

[a] Prefixes: H, human patient; RM, *R. megalotis*; NM, *N. mexicana*; PM, *P. maniculatus*; PT, *P. truei*; PL, *P. leucopus*. NK, animal from the Arizona site.

[b] Numbers are coordinates of the 5′-most residue of each primer. Sequences are as follows: 1, 5′ TAGTAGTAGACTTCGT(AG)AA(GA)AGCTACTA; 20, 5′ AAGCTACTACGACTAAAGCT; 167, 5′ AGCACATTACAGAGCAGAC GGGC; 190, 5′ AGCTGTGTCTGCATTGGAGA; 423, 5′ AGACAATCG GCAGGGTAAAGCT; 476, 5′ CCTCTAGTTGACAACATAT; 980, 5′ CGAT CTGGAGCACA(TC)GCAAA(GT)ACCCA; 1147, 5′ CAATCAATGGGAAT TCAACTGGA; 1234, 5′ GGTGATGATATGGATCCTG; 1257, 5′ TAGCT CGGGATCCAT(AG)TCATCACC; 1326, 5′ GGCTCTTGGTT(AT)GATAT CTCTTT; and 2059, 5′ TAGTAGTATACTCCTTGAAAAGCAA.

[c] Primer specific for the *R. megalotis* hantavirus sequence.

was 15% (Table 2), much larger than the 0 to 2% observed among FCVs from the western United States (19, 22).

Orange County *R. megalotis* hantavirus sequences differed from each other by 0 to 1.3%. No *R. megalotis* hantavirus sequence was less than 16.6% different from FCV. *R. megalotis* and *Peromyscus* nucleocapsid genes from Orange County and Apache County were compared (Fig. 1 and 2). *R. megalotis* nucleocapsid sequences (as well as that of an extremely similar virus sequence obtained from the seropositive *N. mexicana*) were easily distinguished from those of *Peromyscus* spp.

Phylogenetic analyses using the first 415 nucleotides (nt) of coding sequence of the S segment verified that the *R. megalotis* hantavirus is novel (Fig. 2). A bootstrap probability of 100% was measured for the node from which *R. megalotis* hantaviruses and FCVs diverge. The same branch order was observed

TABLE 2. Distance matrix for the complete nucleocapsid gene and protein sequences of the prototypic (RM-97) *R. megalotis* hantavirus in comparison with those of various other viruses[a]

Virus	Distance (%)[b] from:						
	RM97	NM-1	NM-H	PHV-1	PU-So	SR11	76-118
RM-97		23.7	23.5	30.4	31.2	38.2	38.3
NM-1	15.0		3.6	29.8	30.1	38.4	37.2
NM-H	15.0	0.0		29.8	30.8	37.7	37.1
PHV-1	25.9	24.1	24.1		27.2	37.3	36.5
PU-So	28.0	28.7	28.7	13.3		36.7	38.9
SR-11	37.8	38.5	38.5	37.1	37.8		26.7
76-118	37.6	37.1	37.1	37.8	38.7	17.9	

[a] Virus isolates are as follows: NM-1 and NM-H, FCV (11, 22); PHV-1, PHV (18); PU-So, PUU Sotkamo (24); 76-118, HTN (21); and SR-11, SEO (1).

[b] Numbers above the diagonal represent nucleotide distances, whereas those below the diagonal represent amino acid distances.

```
          1        10        20        30        40        50
RM-97     MSNLKELQDNITAHEQQLVTARQKLKDAEKAVEVDPDDVNKSTLQSRRAA
RM-45     --------------------------------------------------
RMNK-51   --------------------------------------------------
NMNK-164  --------------------------------------------------
RM-92     -
PM-222    --T---V-----L----------------R---L----------------
H-NM-1    --T---V-----L----------------R---L----------------
H-NM-H    --T---V-----L----------------R---L----------------

                  60        70        80        90        100
RM-97     VSALETKLGELKRQLADFVTSQKLASKPVDPTGLEPDDHLKEKSSLRYGN
RM-45     --------------------------------------------------
RMNK-51   --------------------------------------------------
NMNK-164  --------------------------------------------------
RM-92     --------------------------------------------------
PM-222    -------------E---LIAA------------I----------------
H-NM-1    -------------E---LIAA------------I----------------
H-NM-H    -------------E---LIAA------------I----------------
H-ID-1          -------E---LIAA------------I----------------

                  110       120       130       140       150
RM-97     VLDVNSIDLEEPSGQTADWKSIGLYILSFTLPIVLKALYMLSTRGRQTVQ
RM-45     ----------------------------------
RMNK-51   -------------------R----------------
NMNK-164  --------------------------------------------------K
RM-92     --------------------------
PM-222    ----------------------------A---I----
H-NM-1    ----------------------------A---I--------------IK
H-NM-H    ----------------------------A---I--------------IK
H-ID-1    ------------T------

                  160       170       180       190       200
RM-97     ENKGTRIRFKDDTSYEEVNGIRKPKHLYVSLPTAQSTMKADEITPGRFRT
NMNK-164  --------------------------------------------------
H-NM-1    ----------S----------------M------------------
H-NM-H    ----------S----------------M------------------

                  210       220       230       240       250
RM-97     ITCGLFPAQVKARNIISPVMGVIGFSFFVKDWTEKIDGFLNSDCPFLPKV
NMNK-164  --------------------------------------------------
H-NM-1    -A-----------------------------M-R--D--AAR-----EQ
H-NM-H    -A-----------------------------M-R--D--AAR-----EQ

                  260       270       280       290       300
RM-97     QGAGEKLLQTIRAYFITRQDQVMQSMLPDITDLMADAQAQGATLFSDITS
NMNK-164  --------------------------------------------------
H-NM-1    KDPRDAA-A-N--------L--DE-KVS--E--I---R-ES--I-A--AT
H-NM-H    KDPRDAA-A-N--------L--DE-KVS--E--I---R-ES--I-A--AT

                  310       320       330       340       350
RM-97     PHSVWVFSCAPDRCPPTALYVAGLPELGAFFSILQDMRNTIMASKAVGTA
NMNK-164  -------A--------------------------------------------
H-NM-1    -------A---------------M-------A-------------S---S
H-NM-H    -------A---------------M-------A-------------S---S

                  360       370       380       390       400
RM-97     EEKMKKKSAFYQSYLRRTQSMGIQLDQRIIIMYMSHWGKEIVNHFHLGDD
NMNK-164  --------------------------------------------------
H-NM-1    ---L----------------------K---L--------A---------
H-NM-H    ---L----------------------K---L--------A---------

                  410       420     428
RM-97     MDPELRQLAQALVDTKVKEISNQELLKL
H-NM-1    ------E---T---I--R------P---
H-NM-H    ------E---T---I--R------P---
```

FIG. 1. Amino acid alignment of the nucleocapsid proteins of the *R. megalotis* hantaviruses RM-97 (prototype), RM-45, and RM-92 of Orange County with the *R. megalotis* hantavirus RMNK-51 and the *N. mexicana* virus NMNK-164 of far eastern Arizona, the *P. maniculatus* hantavirus PM-222 of Orange County, FCV 3H226 (NM-1) and Case H (22) (NM-H) of western New Mexico, and ID-1 of northern Idaho. Hyphens indicate identity with the RM-97 sequence.

when the entire nucleocapsid gene was analyzed (data not shown).

Structure of the S segment and the gene for nucleocapsid protein. The 1,896-nt S segment of the RM-97 hantavirus is smaller than that of FCV (2,059 nt) but larger than those of other hantaviruses. As with FCV, a methionine encoded at position 43 initiates a predicted 428-amino-acid nucleocapsid protein. There is a 569-nt 3′ untranslated region. A reading frame for a putative NSx protein is conserved in the *R. megalotis* and *N. mexicana* S segments (18, 22, 24).

The genera *Peromyscus* and *Reithrodontomys* are considered members of the family Cricetidae by some authors but have been more recently referred to the subfamily Sigmodontinae, family Muridae (20, 26, 30). There is a consistent parallel between the phylogenetic relationships of the predominant

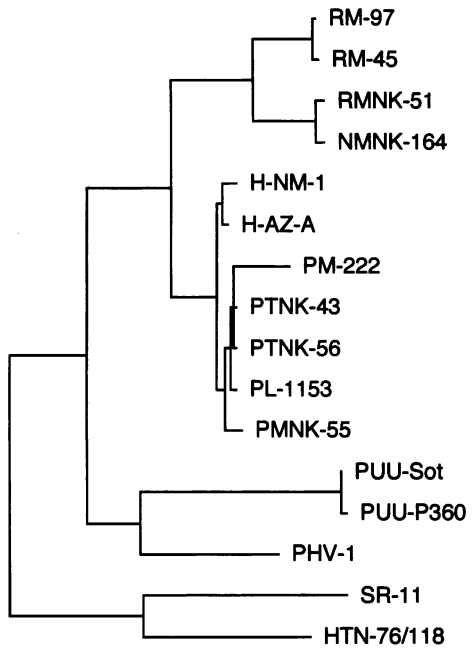

FIG. 2. Parsimony tree analysis comparing nucleocapsid gene sequences of the prototype *R. megalotis* hantavirus (RM-97) with another *R. megalotis* hantavirus from Orange County (RM-45) and the *R. megalotis* and *N. mexicana* hantaviruses from eastern Arizona (RMNK-51 and NMNK-164, respectively). FCV sequences were derived from western New Mexico patient 3H226 (H-NM-1), from an April 1994 eastern Arizona HPS patient (H-AZ-A), from a *P. leucopus* animal trapped in Santa Fe County (PL-1153), and from *P. maniculatus* (PM) and *P. truei* (PT) animals trapped in Orange County (PM-222) or Apache County (PMNK-55, PTNK-43, and PTNK-56). Other sequences were obtained by using the GenBank accession numbers given in the text. The tree was prepared by comparing a portion of the nucleocapsid protein coding sequence (corresponding to FCV S-segment coordinates 43 through 458). Trees were prepared after weighting for infrequent state changes by using the MacClade 3.0 program (9, 15). The MULPARS option of PAUP 3.1.1 (23) was used to generate this minimal-length tree from 415 total sites, of which 255 sites displayed variation. The tree shown was generated by "branch-and-bound" analysis. Horizontal lengths are proportional to the number of state changes, but the lengths are in arbitrary units because of the weighting for character state changes. Vertical distances are for clarity only. The tree was rooted at the midpoint of the greatest patristic distance. Sot, Sotkamo.

rodent hosts of hantaviruses (20, 30) and the phylogenetic relationships among the viruses themselves (27, 29). Thus, the Old World murid hosts of SEO and HTN differ substantially from the sigmodontine and arvicolid hosts of PUU, FCV, and PHV.

The *R. megalotis* hantavirus is more closely related to FCV than to any other well-characterized hantavirus. Thus, the concordance between hantavirus and rodent phylogeny is striking even when closely related species are compared. The close temporal and geographic relationship between distinctive *Peromyscus* and *Reithrodontomys* hantaviruses suggests that hantaviruses do not readily host-switch among different rodent genera (6, 27).

A previous study identified seropositive *R. megalotis* and *Neotoma albigula* animals that lacked detectable FCV RNA (5). Our data suggest that seropositivity among *R. megalotis* and some *Neotoma* species is explained by the new virus. Infection of *N. mexicana* by the harvest mouse hantavirus is presumably another example of "spillover" (5).

We have no evidence that the harvest mouse hantavirus infects humans. *R. megalotis* occurs in a large portion of the western United States. RT-PCR studies of the tissues of HPS patients within that range has failed to reveal any viruses other than those of the peromyscine FCV clade (3, 10). *Reithrodontomys* animals do not normally enter buildings (25, 26). However, the similarity between the *R. megalotis* hantavirus and FCV suggests that workers in the fields of agriculture, forestry, or mammalogy should regard this rodent as a potential vector for human disease.

We thank R. Parmenter and R. Yanagihara for helpful discussions and D. Walters, D. Tibbs, D. Taclindo, C. Smith, D. Sesline, P. Reynolds, M. Jay, L. Jaeyan, J. Jackson, L. Hart, W. Gannon, C. Freeman, R. Enscore, B. Enge, R. E. Emmons, D. Cottam, G. L. Challet, T. Brown, L. Bronson, S. G. Bennett, R. Bailas, and B. Anderson for assistance with obtaining, processing, and testing rodent samples. M. Platt, M. Lipscomb, and R. Lyons provided assistance with computers.

This work was supported by Blood Systems Foundation grant 38-3 and by the New Mexico Department of Health.

REFERENCES

1. **Arikawa, J., H. F. Lapenotiere, L. Iacono-Conners, M. L. Wang, and C. S. Schmaljohn.** 1990. Coding properties of the S and the M genome segments of Sapporo rat virus: comparison to other causative agents of hemorrhagic fever with renal syndrome. Virology **176**:114–125.
2. **Centers for Disease Control and Prevention.** 1993. Update: hantavirus disease—United States, 1993. Morbid. Mortal. Weekly Rep. **42**:612–614.
3. **Centers for Disease Control and Prevention.** 1994. Update: hantavirus pulmonary syndrome—United States, 1993. Morbid. Mortal. Weekly Rep. **43**:45–48.
4. **Centers for Disease Control and Prevention.** 1994. Newly identified hantavirus—Florida, 1994. Morbid. Mortal. Weekly Rep. **43**:99, 105.
5. **Childs, J. E., T. G. Ksiazek, C. F. Spiropoulou, J. W. Krebs, S. Morzunov, G. O. Maupin, K. L. Gage, P. E. Rollin, J. Sarisky, R. E. Enscore, J. K. Frey, C. J. Peters, and S. T. Nichol.** 1994. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J. Infect. Dis. **169**:1271–1280.
6. **Chu, Y. K., C. Rossi, J. W. LeDuc, H. W. Lee, C. S. Schmaljohn, and J. M. Dalrymple.** 1994. Serological relationships among viruses in the *Hantavirus* genus, family Bunyaviridae. Virology **198**:196–204.
7. **Elliott, R. M., C. S. Schmaljohn, and M. S. Collett.** 1991. Bunyavirus genome structure and gene expression. Curr. Top. Microbiol. Immunol. **169**:91–141.
8. **Hillis, D. M., and D. D. Bull.** 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42**:182–189.
9. **Hillis, D. M., J. P. Huelsenbeck, and C. W. Cunningham.** 1994. Application and accuracy of molecular phylogenies. Science **264**: 671–677.
10. **Hjelle, B., S. Jenison, G. Mertz, F. Koster, and K. Foucar.** 1994. Emergence of hantavirus disease in the southwestern United States. West. J. Med., in press.
11. **Hjelle, B., S. Jenison, N. Torrez-Martinez, T. Yamada, K. Nolte, R. Zumwalt, K. McInnes, and G. Myers.** 1994. A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States: evolutionary relationship to known hantaviruses. J. Virol. **68**:592–596.
12. **Jenison, S., T. Yamada, C. Morris, B. Anderson, N. Torrez-Martinez, N. Keller, and B. Hjelle.** 1994. Characterization of human antibody responses to Four Corners hantavirus infections among patients with hantavirus pulmonary syndrome. J. Virol. **68**:3000–3006.
13. **Koerner, T. J., J. E. Hill, A. M. Myers, and A. Tzagoloff.** 1991. High

expression vectors with multiple cloning sites for construction of trpE fusion proteins: pATH vectors. Methods Enzymol. **194:**477–490.

14. **Lee, P.-W., H. L. Amyx, D. C. Gajdusek, R. Yanagihara, D. Goldgaber, and C. J. Gibbs, Jr.** 1982. New haemorrhagic fever with renal syndrome-related virus in indigenous wild rodents in United States. Lancet **ii:**1405.
15. **Maddison, W. P., and D. R. Maddison.** 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Sunderland, Mass.
16. **Nerurkar, V. R., K.-J. Song, D. C. Gajdusek, and R. Yanagihara.** 1993. Genetically distinct hantavirus in deer mice. Lancet **342:** 1059.
17. **Nichol, S. T., C. F. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, and C. J. Peters.** 1993. Genetic identification of a novel hantavirus associated with an outbreak of acute respiratory illness in the southwestern United States. Science **262:**914–917.
18. **Parrington, M. A., and C. Y. Kang.** 1990. Nucleotide sequence analysis of the S genomic segment of Prospect Hill virus: comparison with the prototype hantavirus. Virology **175:**167–175.
19. **Puthavathana, P., M. Dobbs, L.-J. Baek, Y.-K. Chu, H. W. Lee, and C. Y. Kang.** 1993. Comparison of nucleotide sequences among hantaviruses belonging to the same serotype: an analysis of amplified DNA by thermal cycle sequencing. Virus Res. **30:**161–169.
20. **Robbins, L. W., and R. J. Baker.** 1980. G- and C-banded studies on the primitive karyotype for *Reithrodontomys*. J. Mammal. **61:**708–714.
21. **Schmaljohn, C. S., G. B. Jennings, J. Hay, and J. M. Dalrymple.** 1986. Coding strategy of the S genome of Hantaan virus. Virology **155:**633–643.
22. **Spiropoulou, C. F., S. Morzunov, H. Feldmann, A. Sanchez, C. J. Peters, and S. T. Nichol.** 1994. Genome structure and variability of a virus causing hantavirus pulmonary syndrome. Virology **200:** 715–723.
23. **Swofford, D. L.** 1991. PAUP: phylogenetic analysis using parsimony. Version 3.1.1. Illinois Natural History Survey, Champaign, Ill.
24. **Vapalahti, O., H. Kallio-Kokko, E. M. Salonen, M. Brummer-Korvenkontio, and A. Vaheri.** 1992. Cloning and sequencing of Puumala virus Sotkamo strain S and M segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. J. Gen. Virol. **73:**829–38.
25. **Webster, W. D., and J. K. Jones, Jr.** 1982. Reithrodontomys megalotis. Mamm. Species **167:**1–5.
26. **Wilson, D. E., and D. A. M. Reeder (ed.).** 1993. Mammal species of the world, 2nd ed. Smithsonian Institution Press, Washington, D.C.
27. **Xiao, S.-Y., J. W. LeDuc, Y.-K. Chu, and C. S. Schmaljohn.** 1994. Phylogenetic analysis of virus isolates of the genus hantavirus, family bunyaviridae. Virology **198:**205–217.
28. **Xiao, S.-Y., K. W. Spik, D. Li, and C. S. Schmaljohn.** 1993. Nucleotide and deduced amino acid sequences of the M and S genome segments of two Puumala virus isolates from Russia. Virus Res. **30:**97–103.
29. **Yanagihara, R., and D. C. Gajdusek.** 1987. Hemorrhagic fever with renal syndrome: global epidemiology and ecology of hantavirus infections, p. 171–214. *In* L. M. de la Maza and E. M. Peterson (ed.), Medical virology VI. Elsevier Science Publishers, Washington, D.C.
30. **Yates, T. L., R. J. Baker, and R. K. Barnett.** 1979. Phylogenetic analysis of karyological variation in three genera of peromyscine rodents. Syst. Zool. **28:**40–48.