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Michael O. Favorov

Michael Y. Kosoy

Sergei A. Tsarev

James E. Childs
Centers for Disease Control and Prevention, Atlanta, james.childs@yale.edu

Harold S. Margolis

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Michael O. Favorov,[1,3] Michael Y. Kosoy,[2] Sergei A. Tsarev,[4] James E. Childs,[2] and Harold S. Margolis[1]

[1]Hepatitis Branch and [2]Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, and [3]Division of International Health, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Georgia; [4]Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC

The recent identification of antibody to hepatitis E virus (HEV) in pigs, sheep, and cattle and characterization of an HEV isolated from domestic pigs suggest animal reservoirs for this virus. To investigate whether rodents might be a natural reservoir of HEV, the prevalence of anti-HEV was determined among a variety of species throughout the United States. Serum samples were obtained from 806 rodents of 26 species in 15 genera. Anti-HEV prevalence was assessed by 2 EIAs (mosaic protein– and 55-kDa protein–based), which gave concordant results. The highest prevalence of antibody was found in the genus *Rattus* (59.7%; 166/278). Overall, rodents from urban habitats had a significantly higher prevalence of anti-HEV than did animals captured from rural areas. A high prevalence of anti-HEV was found in animals captured on mainland versus barrier islands. The results from this study provide convincing evidence of widespread HEV or HEV-like infection in rodents of the United States.

Hepatitis E virus (HEV) infection has been shown to be the cause of many large outbreaks of enterically transmitted hepatitis over the last 4 decades. Those parts of the world in which outbreaks have occurred include the Indian subcontinent, China, Africa, the Middle East, and Mexico. Sporadic cases of hepatitis E occur at a relatively high rate in many countries in these regions during intraepidemic periods. In countries where outbreaks have not been documented, cases of hepatitis E occur primarily among travelers returning from regions in which hepatitis E is endemic. However, sporadic cases have occurred in persons without a history of travel, and the mode of HEV transmission is not certain [1–7].

Epidemiologic studies indicate that HEV is transmitted by the fecal-oral route and that contaminated water has been the primary source of infection. However, several aspects of the epidemiology of HEV infection are not consistent with that of other enterically transmitted viral infections in developing countries. For instance, the age-specific incidence of infection peaks among young adults rather than young children [4, 8–11]. Also, low secondary attack rates have been observed in households with cases of hepatitis E, in contrast to the high rates of household transmission observed for hepatitis A [6, 12] and other enterically transmitted infections. These findings raise the question as to whether humans are the primary source or reservoir of HEV.

In contrast to other hepatitis viruses, experimental models of infection in animals other than nonhuman primates have been established. Experimental infections have been established in pigs, sheep, and laboratory rats [13, 14]. The recent identification of antibody to HEV in pigs, sheep, and cattle [15] and the isolation and characterization of an HEV from domestic pigs that is similar to human HEV [16, 17] suggest that animal reservoirs may exist for this virus.

To investigate whether rodents might be a natural reservoir of HEV or HEV-like viruses, we determined the prevalence of HEV infection, as measured by anti-HEV, among a variety of rodent species captured throughout the United States.

Materials and Methods

Rodent populations. Rodents belonging to 26 species in 15 genera were collected during 1994–1998 from 21 sites in 13 states: Alabama ($n = 8$ rodents), Arizona ($n = 30$), Colorado ($n = 88$), Florida ($n = 113$), Georgia ($n = 151$), Louisiana ($n = 33$), Maryland ($n = 127$), Nevada ($n = 8$), North Carolina ($n = 72$), New Mexico ($n = 60$), New York ($n = 24$), Pennsylvania ($n = 67$), and Texas ($n = 25$). Sites sampled included 6 urban locations ($n = 256$ rodents) and 15 rural sites that consisted of various types of habitats ($n = 550$).

Protocols for blood collection followed those of Mills et al. [18], and samples were stored at −70°C until tested.

Detection of IgG anti-HEV. Serum samples were tested for anti-HEV by use of 2 EIAs, each of which used a different recombinant-expressed HEV antigen. Each serum specimen ($n = 806$) was tested by EIA that used a mosaic protein (MPr) composed of recombinant

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Reprints or correspondence: Dr. Michael O. Favorov, 2877 Brandywine Road, M/S K72, Atlanta, GA 30341 (mof0@cdc.gov).

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Table 1. Demographic characteristics of rodents tested for antibody to hepatitis E virus infection, United States, 1994–1998.

Genus and species (common name)	No.	% of total	State (no.)
Clethrionomys gapperi (boreal red-backed vole)	6	0.7	North Carolina (6)
Citellus			
mexicanus (Mexican ground squirrel)	2	0.2	Texas (2)
variegatus (rock squirrel)	1	0.1	New Mexico (1)
Mus musculus (house mouse)	14	1.7	Alabama (3), Georgia (7), Texas (4)
Microtus pennsylvanicus (meadow vole)	9	1.1	Pennsylvania (9)
Neotoma			
albigula (white-throated wood rat)	22	2.7	Arizona (1), New Mexico (21)
mexicana (Mexican wood rat)	84	10.4	Colorado (84)
micropus (southern plains wood rat)	8	1.0	Texas (8)
Onychomys leucogaster (grasshopper mouse)	1	0.1	New Mexico (1)
Ochrotomys nuttalli (golden mouse)	11	1.4	Georgia (3), North Carolina (8)
Oryzomys palustris (rice rat)	41	5.1	Florida (12), Georgia (29)
Perognathus penicillatus (desert pocket mouse)	10	1.3	Arizona (10)
Peromyscus			
boylei (brush mouse)	24	3	Arizona (9), New Mexico (15)
difficilis (rock mouse)	3	0.4	Colorado (3)
eremicus (cactus mouse)	7	0.9	Arizona (6), New Mexico (1)
gossiypinus (cotton mouse)	4	0.5	Florida (1), Georgia (3)
leucopus (white-footed mouse)	53	6.6	Alabama (2), Arizona (2), Georgia (12), North Carolina (18), Pennsylvania (19)
maniculatus (deer mouse)	91	11.3	Arizona (1), Colorado (1), Georgia (5), North Carolina (40), Pennsylvania (37), New Mexico (7)
polionotus (oldfield mouse)	3	0.4	Georgia (3)
truei (pinon mouse)	15	1.9	Arizona (1), New Mexico (14)
Pitymus pinetorum (pine vole)	2	0.2	Georgia (2)
Rattus			
norvegicus (Norway rat)	197	24.5	Alabama (1), Florida (1), Georgia (10), Louisiana (28), Maryland (127), Nevada (8), New York (22)
rattus (black rat)	81	10.1	Alabama (1), Florida (36), Georgia (28), Texas (11), Louisiana (5)
Sigmodon hispidus (cotton rat)	113	14	Alabama (1), Florida (63), Georgia (49)
Sciurus carolinensis (eastern gray squirrel)	2	0.2	New York (2)
Zapus hudsonius (meadow jumping mouse)	2	0.2	Pennsylvania (2)
Total	806	100	

proteins from immunoreactive epitopes of HEV open-reading frame (ORF) 2 and ORF 3 [19, 20]. Those specimens with adequate volume ($n = 612$) were also tested for anti-HEV by an EIA for which the antigen was a recombinant 55-kDa ORF 2 protein expressed by baculovirus in insect cells (55KAg) [21].

Both EIAs were modified from those described elsewhere [20, 21] in that horseradish peroxidase–conjugated rabbit anti-rat IgG (Boehringer Mannheim, Indianapolis), diluted 1 : 1000, was used to detect anti-HEV, and serum specimens were diluted to 1 : 50 in PBS, pH 7.4, supplemented with 10% normal goat serum, 1% bovine serum albumin, and 0.01% Tween 20.

Cutoff values to define an initially reactive specimen were derived from the frequency distribution of optical density (OD) values obtained from 61 randomly selected Norway rats. The OD cutoff for the MPr assay was 0.110, which approximated the mean value (0.01) of the negative specimens +3.2 SD units (SD = 0.028). The cutoff for the 55KAg assay was 0.166, which approximated the mean value (0.025) of the negative specimens +3.2 SD units (SD = 0.044).

Serum specimens ($n = 5$) from rats negative for anti-HEV in both EIAs were pooled and used as a standard negative control. A standard positive control was produced from pooled serum specimens of guinea pigs ($n = 2$) immunized with affinity-purified MPr antigen [19]. When each assay was performed, the OD value of the standard positive control at a 1 : 50 dilution had to be ≥ 0.5, and the OD value of the standard negative control had to be 0.01–0.07 for the test results to be considered valid.

Immunoblot analysis. Selected specimens were analyzed by immunoblot to confirm the specificity of antibody reactivity to the mosaic protein used as the antigen in the MPr assay. A recombinant fusion mosaic protein [19] was subjected to SDS-PAGE, and the separated proteins were transblotted to BAS 83 nitrocellulose (Schleicher & Schuell, Keene, NH) by means of a TE70 SemiPhor (Hoefer Scientific Instruments, San Francisco). The immunoblot assay conditions were used as described elsewhere [2].

Statistical analyses. For univariate and stratified analyses, statistical testing was done by use of the Pearson test, the Mantel-Haenszel test, or the test for trend, as appropriate. Statistical significance and relative risk estimates were determined by calculating P values with Yates's correction factor, exact 95% confidence interval (CIs), or Taylor series 95% CIs, as appropriate. Logistic regression analysis was conducted by use of LOGIST, a procedure accessible through the SAS system (SAS Institute, Cary, NC).

Results

Rodent captures. Serum samples were obtained from 806 rodents of 26 species in 15 genera (table 1). Urban sites provided 256 animals (31.8%), of which the majority ($n = 196$, 76.6%) were Norway rats *(Rattus norvegicus)* or black rats *Rattus rattus* ($n = 53$, 20.7%). The Norway rat was the most common species captured in Baltimore, New Orleans, Reno, and New York City, whereas *R. rattus* was the most common species captured in Atlanta and Miami (table 2). The remainder of rats

Table 2. Prevalence of antibody to hepatitis E virus (HEV) among rodents from urban and rural areas as detected by recombinant mosaic protein, United States.

State	City or county	Year(s)	Total no. tested	Genus and species (no. tested)	Total no. (%) anti-HEV–positive	Genus and species (no. anti-HEV–postive)
Urban areas						
Georgia	Atlanta	1994	43	*Peromyscus leucopus* (2), *Rattus norvegicus* (10), *Rattus rattus* (28), *Sigmodon hispidus* (3)	19 (44.2)	*R. norvegicus* (3), *R. rattus* (15), *S. hispidus* (1)
Maryland	Baltimore	1995	81	*Rattus norvegicus* (81)	63 (77.8)	*R. norvegicus* (63)
Maryland	Baltimore	1997	46	*Rattus norvegicus* (46)	42 (91.3)	*R. norvegicus* (42)
Florida	Miami	1997	21	*Rattus rattus* (20), *Rattus norvegicus* (1)	4 (19)	*R. rattus* (3), *R. norvegicus* (1)
Louisiana	New Orleans	1995	33	*Rattus norvegicus* (28), *Rattus rattus* (5)	10 (30.3)	*R. norvegicus* (8), *R. rattus* (2)
Nevada	Reno	1994	8	*Rattus norvegicus* (8)	2 (25)	*R. norvegicus* (2)
New York	New York	1997	24	*Rattus norvegicus* (22), *Sciurus carolinensis* (2)	15 (62.5)	*R. norvegicus* (15)
Total			256	*Rattus norvegicus* (196)	134 (68.4)	
				Rattus rattus (53)	20 (37.7)	
				Sigmodon hispidus (3)	1 (33.3)	
				Other species (4)	0	
Rural areas						
Alabama	Chilton	1996	8	*Mus musculus* (3), *Peromyscus leucopus* (2), *Rattus norvegicus* (1), *Rattus rattus* (1), *Sigmodon hispidus* (1)	1 (12.5)	*R. norvegicus* (1)
Arizona	Yavapai	1995	30	*Neotoma albigula* (1); *Perognathus penicillatus* (10); *Peromyscus* species: *P. boylei* (9), *P. eremicus* (6), *P. leucopus* (2), *P. maniculatus* (1), *P. truei* (1)	6 (20)	*N. albigula* (1), *P. boylei* (2), *P. eremicus* (3)
Colorado	Larimer	1997–1998	88	*Neotoma mexicana* (84), *Peromyscus difficilis* (3), *Peromyscus maniculatus* (1)	49 (55.7)	*N. mexicana* (48), *P. maniculatus* (1)
Florida	Dade, Nassau	1994, 1996	92	*Peromyscus gossiypinus* (1), *Oryzomys palustris* (12), *Rattus rattus* (16), *Sigmodon hispidus* (63)	42 (45.2)	*O. palustris* (10), *R. rattus* (8), *S. hispidus* (24)
Georgia	Barrow, Chatham, Walker, Walton	1994–1995	108	*Mus musculus* (7); *Ochrotomys nuttalli* (3); *Oryzomys palustris* (29); *Peromyscus* species: *P. gossiypinus* (3), *P. leucopus* (10), *P. maniculatus* (5), *P. polionotus* (3); *Pitymus pinetorum* (2); *Sigmodon hispidus* (46)	18 (16.7)	*M. musculus* (2), *P. leucopus* (2), *P. maniculatus* (2), *S. hispidus* (12)
North Carolina	Macon	1996	72	*Clethrionomys gapperi* (6), *Ochrotomys nuttalli* (8), *Peromyscus leucopus* (18), *Peromyscus maniculatus* (40)	11 (16.2)	*C. gapperi* (4), *P. leucopus* (2), *P. maniculatus* (5)
New Mexico	McKinley, Socorro, Taos	1995	60	*Citellus variegatus* (1); *Neotoma albigula* (21); *Onychomys leucogaster* (1); *Peromyscus* species: *P. boylei* (15), *P. eremicus* (1), *P. maniculatus* (7), *P. truei* (14)	12 (20)	*N. albigula* (12)
Pennsylvania	Monroe	1996	67	*Microtus pennsylvanicus* (9), *Peromyscus leucopus* (19), *Peromyscus maniculatus* (37), *Zapus hudsonius* (2)	3 (4.5)	*P. leucopus* (1), *P. maniculatus* (2)
Texas	Kleberg	1994	25	*Citellus mexicanus* (2), *Mus musculus* (4), *Neotoma micropus* (8), *Rattus rattus* (11)	4 (16)	*N. micropus* (1), *R. rattus* (3)
Total			550	*Clethrionomys gapperi* (6)	4 (66.7)	
				Mus musculus (14)	2 (14.3)	
				Neotoma albigula (22)	13 (59.1)	
				Neotoma mexicana (84)	48 (57.1)	
				Neotoma micropus (8)	1 (12.5)	
				Oryzomys palustris (41)	10 (24.4)	
				Peromyscus boylei (24)	2 (8.3)	
				Peromyscus eremicus (7)	3 (42.9)	
				Peromyscus leucopus (50)	5 (10)	
				Peromyscus maniculatus (91)	10 (11)	
				Rattus norvegicus (1)	1 (100)	
				Rattus rattus (28)	11 (39.3)	
				Sigmodon hispidus (110)	36 (32.7)	
				Other species (64)	0	

captured in urban areas belonged to 3 other species (*Peromyscus leucopus, Sciurus carolinensis,* and *Sigmodon hispidus*).

Rural sites consisting of natural habitat were sampled in 9 states and provided 550 rodents of 25 species (table 2). The most common species varied with the type of habitat and included white-throated wood rats (*Neotoma albigula; n* = 22) and mice of the genus *Peromyscus* (*n* = 148) from arid habitats in Arizona and New Mexico; Mexican wood rats (*Neotoma mexicana*) from Colorado; white-footed mice (*P. leucopus*) and deer mice (*Peromyscus maniculatus*) from forest habitats of Georgia, North Carolina, and Pennsylvania; meadow voles (*Microtus pennsylvanicus*) from grassy areas in Pennsylvania; and cotton rats (*S. hispidus*) and rice rats (*Oryzomys palustris*) from grassy and marshy areas of Georgia and Florida.

EIA performance. The MPr assay was positive for anti-HEV in 303 (38%) of 806 rodents. Antibody was detected by the 55KAg assay in 244 (40%) of the 612 rodents tested. Overall, the prevalence of anti-HEV detected by the 2 assays was not different (odds ratio [OR], 0.95; 95% CI, 0.83–1.08; $P > .4$); however, there were differences in the genus *Rattus* (table 3). Among these rodents, prevalence of anti-HEV among animals tested with the 55KAg assay was 76.4% (146/191), compared with 59.7% (166/278) for the same animals tested with the MPr assay (OR, 2.19; 95% CI, 1.4–3.4; $P < .001$).

For animals in all genera tested by both assays, the concordance for both positive and negative test results was 88.9% (544/612), and for specimens positive in either assay the concordance was 74.7% (201/269) (table 3). When anti-HEV prevalence was defined as reactivity in both assays (33%; 201/612), this did not differ from the prevalence obtained by positivity in the MPr assay alone (38%; 303/806) (OR, 1.24; 95% CI, 0.99–1.6; $P = .07$) and was less than the seroreactivity by 55KAg alone (40.0%; 244/612) (OR, 1.36; 95% CI, 1.1–1.7; $P = .01$). When anti-HEV prevalence was defined on the basis of seropositivity in either assay (44.0%; 269/612), this exceeded the anti-HEV prevalence by MPr alone (OR, 1.3; 95% CI, 1.04–1.62; $P = .02$) but was not different from prevalence as defined by seropositivity in the 55KAg assay (OR, 1.18; 95% CI, 0.99–1.49; $P = .16$).

Immunoblot analysis. Of 81 Norway rats tested in the MPr assay, serum specimens from 26 were randomly selected for further analysis of antibody specificity by immunoblot analysis. Among 22 serum specimens positive by the MPr assay, 21 (95.5%) were positive by immunoblot analysis of the MPr. All 4 EIA-negative serum samples were negative by immunoblot testing.

Antibody prevalence. Serum samples from 192 rodents were not of sufficient quantity to be tested by both the 55KAg and MPr assays. Of those animals not tested by the former assay, there was no significant difference in trap location, species, or year of collection from animals tested by both assays (data not shown). Because the anti-HEV prevalence results obtained by the 2 EIAs were statistically similar and generally gave concordant results, subsequent analysis was limited to antibody results obtained for all animals tested with the MPr assay. The highest prevalence of antibody was found among the genus *Rattus* (59.7%; 166/278), with a significantly higher prevalence among *R. norvegicus* (68.5%; 135/197) than *R. rattus* (38.3%; 31/81) (OR, 3.5; 95% CI, 1.98–6.25; $P < .0001$). The majority of *Rattus* specimens tested came from urban sites (table 2), although the prevalence of anti-HEV antibody (41.3%; 12/29) in *Rattus* species from rural locations (table 2) was similar to that of urban sites (OR, 3.5; 95% CI, 0.9–4.9; $P = .08$). Among rodents captured from rural environments, 146 (26.5%) of 550 were antibody-positive and belonged to 13 species (table 2). Of the seropositive animals from rural sites, the majority (67.1%; 98/146) belonged to 2 genera: *Neotoma* (*N. albigula, N. mexicana,* and *Neotoma micropus*), with 54.4% (62/114) positive, and *Sigmodon* (*S. hispidus*), with 32.7% (36/110) positive. Although only 3 animals were obtained from an urban environment, antibody prevalence in *S. hispidus* from urban sites (33%) was the same as that from rural locations (33%) (table 2). Of animals belonging to 6 species of *Peromyscus,* however, only 19 (9.8%) of 194 were antibody-positive, although these were spread among 4 species (*P. leucopus, P. maniculatus, P. boylei,* and *P. eremicus*). Of the remaining rodents, seropositive animals were identified in 3 additional species: the rice rat, *O. palustris* (10/41; 24%); the red-backed vole, *Clethrionomys gapperi* (4/6; 67%); and the house mouse, *Mus musculus* (2/14; 14.3%).

Table 3. Reactivity to antibody to hepatitis E virus (HEV) by EIA based on recombinant HEV antigens (mosaic protein [MPr]; 55-kDa protein [55KAg]) among rodents, by genus.

Rodent genus	Reactivity to HEV antigens					
	Positive by MPr	Positive by 55KAg	*P*, MPr vs. 55KAg	Positive by both MPr and 55KAg	Positive by either MPr or 55KAg	*P*, both vs. either
Neotoma	62/113 (55)	12/22 (55)	1	9/22 (41)	16/22 (73)	.08
Oryzomys	10/41 (24)	15/41 (37)	.3	10/41 (24)	15/41 (37)	.3
Peromyscus	21/200 (10)	22/196 (11)	.6	14/192 (7)	27/192 (14)	.05
Rattus	166/278 (60)	146/191 (76)	<.001	134/191 (70)	155/191 (81)	.02
Sigmodon	37/112 (33)	40/109 (37)	.7	28/109 (26)	48/109 (44)	.007
Other	7/62 (12)	9/53 (17)	.5	6/53 (11)	10/53 (19)	.4
Total	303/806 (38)	244/612 (40)	.43	201/612 (33)	269/612 (44)	<.001

NOTE. Data are no./total tested (%).

Demographic characteristics. The prevalence of anti-HEV–positive animals was >2-fold higher among rodents captured from urban areas (60.5%; 155/256) than among those originating from rural areas (26.5%; 146/550) (OR, 4.2; 95% CI, 3–5.9; $P < .001$), primarily because of the contribution of Norway rats to the urban sample.

In rural habitats of the southeastern region of the United States, the most commonly seropositive rodent was the cotton rat *(S. hispidus)*. This rodent had an overall anti-HEV prevalence of (32.7%; 37/113) and constituted 60.0% (36/60) of the animals reactive to MPr in Florida and Georgia. Wood rats (*Neotoma* species) contributed the highest proportion (95/106; 89.6%) of seropositive animals in the Southwest (Arizona, Colorado, New Mexico), with the highest anti-HEV prevalence in *N. albigula* (13/22; 59.1%) and *N. mexicana* (48/84; 57.1%).

Differences in prevalence of anti-HEV were also observed between rodents captured on the mainland versus animals captured on barrier islands. Of 29 rice rats *(O. palustris)* captured from 2 neighboring islands (Cockspur and McQueen Islands, Chatham County, GA), none was anti-HEV–positive, whereas all 10 rodents of the same species captured from the mainland of southern Florida was positive ($P < .001$). Similarly, whereas none of 18 cotton rats from Amelia Island in northern Florida were positive, 8 (32.0%) of 25 cotton rats captured from a mainland site (Social Circle, Walton County, GA) were positive ($P < .01$).

The prevalence of antibody differed from year to year during the 5-year collection period (analysis of trend: $P < .001$; $\chi^2 = 134$; $df = 4$). Antibody prevalence significantly varied by year when analyzed by species: Among *Neotoma* species, prevalence was 68% (40/59) in 1997 and 14% (1/7) in 1994; among *Oryzomys* species, 100.0% (10/10) in 1994 and 0 (0/20) in 1995; among *Rattus* species, 64.0% (73/114) in 1995 and 48% (46/95) in 1994; and among *Sigmodon* species, 51.0% (25/49) in 1995 and 0 (0/17) in 1996. Much of this variation may be due to the selection of different sampling sites in different years.

Logistic regression analysis. Factors that univariate analysis showed were statistically associated with anti-HEV seroreactivity among rodents were included in a logistic regression model. In this analysis, differences in anti-HEV activity by species, location (e.g., islands vs. mainland), and by assay showed that urban-rural variations were independently associated with anti-HEV seroprevalence. Year-to-year prevalences of anti-HEV among animals and by species were not significantly associated with anti-HEV seroreactivity.

Discussion

This study demonstrated the widespread occurrence and high prevalence of antibody reactive with HEV recombinant antigens among rodents captured in a variety of locations in the United States. Although antibody prevalence varied across species and habitats, infected animals were found in virtually all of the locations sampled (figure 1). These data provide strong evidence that an agent identical or closely related to HEV is circulating among rodent populations throughout the United States.

The 2 antigens used in the different HEV EIAs provided consistent results among those animals tested by both antigens. The concordance of the serologic findings between the 2 assays was high (88.9%). This high level of agreement was exceptional, as simultaneous testing of human serum specimens by these same 2 assays has shown a high degree of discordance [22, 23]. In addition, the antibodies in a subset of animals positive by the EIAs showed specific activity to purified HEV proteins in immunoblot assays. The single specimen positive in the EIA but negative by immunoblot probably reflected a low titer of antibody not detected by immunoblot assay, which has a lower sensitivity [2].

The high prevalence of antibody found among rodents (44% by either antigen, 33% by both antigens) indicated a high rate of infection with HEV or an HEV-like agent. Although it is possible that the anti-HEV detected in these animals represented a false-positive result, this is highly unlikely because of the specificity of the immunoblot data and the results of experimental HEV infection in rodents [24]. In addition, an HEV strain has been isolated from rodents captured in Nepal that was similar to HEV sequences obtained from patients with HEV infection in the same country [25]. The high prevalence of seropositive rodents, their widespread distribution, and the consistency of serologic findings support the hypothesis that rodents could serve as a reservoir of HEV or HEV-like viruses and that transmission to humans could occur in the right setting or set of circumstances. Future efforts must characterize the rodent HEV, define the epidemiology of HEV infection among rodents, and determine the relationship of this infection to humans.

Seropositive animals were found in all rodent communities in which sufficient numbers of animals were tested; however, significant variation in the prevalence of IgG antibody reactive with HEV was apparent. The highest prevalence occurred in urban areas among rats of the genus *Rattus,* whereas *Peromyscus* species had lower prevalence of antibody reactive to HEV antigens, regardless of locale. The reason for the high prevalence of anti-HEV among rats sampled from urban environments is unclear. Norway rats reached very high population densities in some urban locations, which may facilitate HEV transmission. It is interesting to note that rodents of the genera *Rattus* and *Mus* were introduced into the New World. The origin of the genus *Rattus* is a Central Asian area in which hepatitis E in humans is highly endemic [26]. As HEV isolates or sequences become available from rodent sources in the New World, it will be interesting to determine if the HEV-like viruses introduced to rodents are similar to Old World viruses. Recently identified HEV in pigs from the United States shared ~79%–80% nucleotide and 90%–92% amino acid sequence iden-

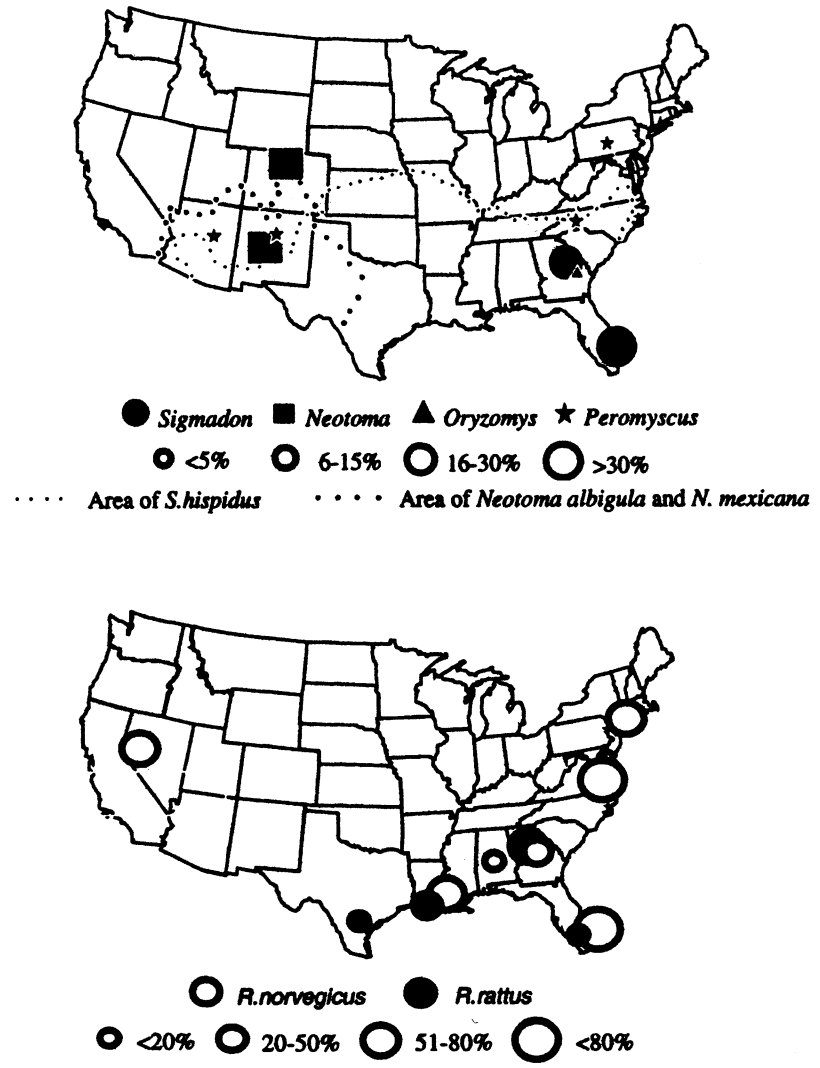

Figure 1. Distribution and prevalence of antibodies to hepatitis E virus among rats of genus *Rattus (bottom)* and rodents of 4 genera *(top)*

tity with human HEV strains; swine HEV cross-reacted with antibody to the human HEV antigens [27]. The Norway and black rats are already recognized threats to human health because of their association with a number of zoonotic infections, including plague, Seoul virus (genus *Hantavirus*), leptospirosis, and murine typhus [28].

The presence of anti-HEV among rodents indigenous to the United States and living in natural habitats suggests that infection occurs in a wide range of New World rodent species, such as *Neotoma* in the Southwest and cotton rats *(S. hispidus, O. palustris)* in the southeastern United States. It is also known that infection in rodents can be focal [28]. Particularly striking was the complete lack of anti-HEV among *S. hispidus* and *O. palustris* on islands, in contrast with seropositivity among animals of both species on the mainland. This result suggests either that the population of rodents on these islands is too small to sustain HEV transmission or that the virus was never introduced into those settings. Seropositive rodents were found in the Sevilleta National Wildlife Refuge, New Mexico. This finding provides a good example that HEV-related viruses may exist in remote areas that are well removed from humans and domestic animals. The exact nature of the HEV or HEV-like virus associated with rodents awaits their isolation and characterization. Nevertheless, the results from this study provide convincing evidence of widespread HEV or HEV-like infection in rodents of the United States.

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Addendum. Since this paper was submitted and accepted for publication, Kabrane-Lazizi et al. have reported similar findings of a high prevalence of antibody to hepatitis E virus among rats of the genus *Rattus* (Kabrane-Lazizi Y, Fine JB, Elm J, et al. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. Am J Trop Med Hyg 1999;61:331–5). Antibody prevalence, determined by ELISA, was reported as 78% ($n = 108$) for *Rattus norvegicus,* 90% ($n = 113$) for *Rattus rattus,* and 83% ($n = 18$) for *Rattus exulans.*