provides direct evidence that this arthropod is a competent vector (4). We report the first identification and characterization of an HGE-like agent in a blacklegged tick collected in a tick-endemic area of Canada (6).

Sixty male and 60 female *I. scapularis* were collected from five white-tailed deer shot on Long Point Peninsula, Ontario, during November 1999. Live ticks were cut longitudinally into halves, and half of each specimen was placed in lysis buffer from a QIAamp DNA Mini Kit (Qiagen Inc., Canada); DNA was extracted per manufacturer's instructions. Five microliters of extracted tick DNA was then added to a polymerase chain reaction (PCR) mixture containing primers Ehr 521 and Ehr 790 (7), and the resulting amplification products were run on agarose gels. Extracted DNA from one male tick generated the expected 293-bp HGE agent amplicon. Preliminary DNA sequencing analysis of the putative granulocytic *Ehrlichia* PCR product indicated a high sequence identity with HGE agent 16S rDNA. To further genotype this HGE-like agent, an 894-bp portion of 16S rDNA was amplified by using primers ge3a, ge9f, ge10r (8), and primer mdge9r (5' ATGTCAAGGAGTG-GTAAGGT) in a nested PCR reaction.

Genetic characterization of the Long Point HGE-like agent (designated here as L3H) was carried out by sequencing an 849-bp portion of the rDNA gene and comparing it with other HGE-like agents in GenBank. Within the rDNA portion sequenced, L3H shares 99.6% identity with the HGE agent and *E. equi/E. phagocytophila*. In the 849-bp portion of the rDNA gene amplified and sequenced, the L3H strain differed from the HGE agent by three nucleotides. Comparison of L3H with HGE-like agents from the United States, Europe, and China suggests a high degree of sequence identity at the rDNA level; however, a number of nucleotide positions did show variation. (GenBank accession number for L3H is AF311343.)

This study documents for the first time (by rDNA sequence comparisons) that *I. scapularis* from a tick-endemic site in Canada can harbor an ehrlichia of the *E. equi* genogroup and is closely related to the HGE agent. The taxonomic significance of HGE-like agents that vary somewhat in their rDNA sequence is still unclear. HGE-like agents from diverse geographic locations and various hosts can exhibit nucleotide differences at a number of positions and still be >99% similar at the level of rDNA sequence identity. Recently it has been shown that sequencing of a more variable genomic region, such as the *ank* gene of granulocytic ehrlichia, can group these agents into different North American and European genetic clades or geno-groups (9). Whether all HGE-like "variants" that differ somewhat in their rDNA or *ank* gene sequences can cause human or animal disease remains to be determined.

The identification of an HGE-like agent further highlights the concern that *I. scapularis* may transmit a number of pathogens to humans or other animals in Canada. Public health officials and veterinarians should be aware of this finding and consider HGE in the differential diagnosis of patients or clients with relevant clinical presentations. Further studies documenting the prevalence of the HGE-like agent(s) in ticks from Canada and characterization of any agents identified are warranted to better define potential human and animal health risks.

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High Prevalence of Sin Nombre Virus in Rodent Populations, Central Utah: A Consequence of Human Disturbance?

To the Editor: Sin Nombre virus (SNV) (Bunyaviridae) is a newly discovered hantavirus responsible for hantavirus pulmonary syndrome (HPS) in humans. The deer mouse, *Peromyscus maniculatus,* is its primary reservoir (1,2). To address a gap in our understanding of the temporal dynamics of SNV, we conducted a longitudinal study in the Great Basin.

Our study site was the West Tintic Mountains, Jericho, Utah, 39°57' N, 112°22' W. We trapped on May 29 to 31, July 10 to 12, and October 7 to 9, 1999. Previous research (3) indicated woodrats (*Neotoma lepida*) were reservoirs for SNV; therefore, we concentrated our trapping efforts at woodrat middens. Middens (2 m diam) are structures of thousands of sticks built by woodrats and serve as nesting sites for a variety of small rodents, including deer mice (4,5). Each night of the 3-night trapping session, we set ~3 live traps baited with oats, peanut butter, and cotton at each of ~40 middens.

Captured rodents were collected each morning and anesthetized with Metafane (methoxyflurane). Animals were identified to species and ear-clipped for future identification. Scarring, body mass, sex, tail length, and reproductive status were recorded (data available by request).

Animals were bled via the retroorbital sinus. We performed an enzyme-linked immunosorbent assay for detection of hantavirus antibody (3).

We trapped six species of rodents; *P. maniculatus* was the most common, followed by *P. truei* and *N. lepida*. Other species captured infrequently were *Dipodomys ordii*, *Largurus curtatus*, and *Chaetodipus* sp.

Over three trapping periods, we captured 212 *P. maniculatus;* 63 were antibody-positive (29.7%). Prevalence of SNV was greater in males than in females (chi-square = 3.8, p = 0.05), and it varied little among sampling periods. Most of the variation was due to changes in prevalence in males, which was 28% to 42%; prevalence among female deer mice was 17% to 20%. *P. truei* also tested positive for SNV. Of 37 *P. truei* tested, 4 were antibody-positive (10.8%).

We found a high and relatively stable level of SNV prevalence in a population of deer mice in Central Utah. Mean antibody prevalence (29.7%) across 3 periods was up to 3 times higher than that of other locations. Prevalence of SNV in this population was comparable with that during the 1993 outbreak of HPS in the Four Corners region.

We propose that the high level of SNV prevalence could be due to disturbance by humans, primarily intensive use of all-terrain vehicles at the study site. Little Sahara Recreation Area, ~4 km from the study site, recorded 180,000 visitors during 1999, mostly all-terrain vehicle users (Bureau of Land Management statistics). Many visitors to Little Sahara camp and recreate on land in our study area. This heavy recreational use has produced numerous dirt roads and campsites. Vehicle movement has denuded the area of vegetation other than large (>1 m tall) shrubs and trees and has removed cryptogamic crust, resulting in compaction of sandy soil into roads, trails, and large open spaces. Open spaces caused by disturbance reduce habitat suitable for species such as *Peromyscus* (6,7) and may cause animal density to increase within a microhabitat. Increased intra- and interspecific interactions would favor the transmission of SNV. Thus, fragmentation of the landscape may alter behavior of deer mice in a manner that enhances transmission of SNV.

Four pieces of evidence corroborate our speculation that habitat disturbance increases prevalence of SNV. First, in experimentally fragmented habitats, the density of deer mice increased dramatically. In one study, density of deer mice in small patches (4 m x 8 m) was consistently 3 times higher during 7 years of the study than that of deer mice in larger patches (10 x 50 m) (6). These small patches are similar in size to patches created by vehicle movement at our study site. Second, deer mice in fragmented habitats travel much longer distances, on average 2 times as far, as deer mice in less fragmented habitats (6). Third, immuno-competence of small rodents may decline as population density increases, making rodents more susceptible to infection than at lower densities (8). These three factors taken together should enhance transmission of SNV by increasing interactions among deer mice with lowered immunocompetence. Finally, prevalence of SNV in deer mice is lower in populations from habitats less impacted but similar to our study site. Across four other sites in the Great Basin, prevalence of SNV was 11% (9). Although we have not quantified the disturbance in these other areas,

their general locations suggest they are not as disturbed by humans as the site near Little Sahara Recreational Area.

Further investigation of the effect of human disturbance on SNV prevalence is needed. We have presented several possible mechanisms that may be involved in a causal relationship between these two factors. Given that most HPS cases are contracted in areas where there has been human alteration to the landscape, future investigation of this hypothesis is warranted.

The prevalence and total numbers of infected rodents were much lower in *P. truei* than *P. maniculatus*. Adult *P. truei* are larger than *P. maniculatus* and tend to compete with *P. maniculatus* for food and nesting sites. Interspecific competition could lead to aggressive contact between these two species that could result in interspecific transmission of SNV. *P. truei* were regularly captured at the same middens on the same nights with *P. maniculatus*.

We suggest that the high level of disturbance at this study site could increase the probability of SNV transmission between species through the same mechanisms suggested for the high levels of prevalence within deer mice. Rodents at our study site may be living at higher densities than in other areas. The increased contact between species, especially when SNV prevalence is high in deer mice, could promote transmission to species other than *P. maniculatus*.

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Hantavirus Seroconversion of Wild-Caught *Peromyscus* During Quarantine

To the Editor: In 1993 an outbreak of unexplained respiratory deaths in the Four Corners region of the United States led to the discovery of Sin Nombre (SN) hantavirus and the associated human disease, hantavirus cardiopulmonary syndrome (HCPS). Numerous studies have shown that a series of hantaviruses similar to SN virus are maintained in natural reservoirs composed of Sigmodontine rodents, including deer mice (*Peromyscus maniculatus*), white-footed mice (*P. leucopus*), cotton rats (*Sigmodon hispidus*) and western harvest mice (*Reithrodontomys megalotis*) (1). Deer mice, however, are the principal reservoir of SN virus, the primary etiologic agent of HCPS in North America.

Some hantaviruses, thus far not including SN virus, have been transmitted in indoor animal-care facilities through the airborne route (2). The high case-fatality ratio of HCPS (40%), coupled with its airborne transmission by captive rodents, has led to classification of the agents of HCPS as biosafety level 3 (BSL-3) in tissue culture and BSL-4 in reservoir host rodents. Although deer mice mount an antibody response and develop chronic infection, the virus does not harm them. Deer mice are believed to shed SN virus in urine, feces, and saliva. Infection in humans occurs primarily by inhalation of aerosols from dried excreta containing infectious virus, particularly in closed spaces with poor ventilation (3).

Handling mice infected with SN virus in a laboratory requires BSL-4 conditions (4). However, outdoor standards greatly reduce costs and difficulties associated with handling infected rodents safely, since workers wearing respirators and protective clothing may handle infected mice outdoors (5). Thus, we have constructed outdoor quarantine facilities for the temporary housing of potentially infected mice (6). These facilities consist of a series of individual nest boxes enclosed by a partially buried steel plate fence. Mice are placed into individual nest boxes spaced 3 m apart, which prevents transmission of hantavirus among mice during quarantine (J. Botten and B. Hjelle, unpub. data). Each nest box is composed of an artificial burrow enclosed within a small steel container, which serves as a barrier to contain each mouse. These facilities allow safe handling of wild rodents at much lower cost than that associated with BSL-4 laboratories. Very few, if any, patients with HCPS contracted the virus in an open, outdoor environment (3).

Viral infections are characterized by a window period during which the host is infected but diagnostic test (e.g., antibody) results are negative. To detect infections reliably, it is important to conduct antibody tests after the host animal has been given sufficient time to mount a detectable immune response. Mills et al. (5) recommend testing captured rodents for hantavirus antibodies at the beginning and end of a 5-week quarantine period whenever potential reservoir species are used to establish laboratory colonies. Only upon completion of the second test can an animal be considered truly uninfected by a hantavirus.

We describe two cases of seroconversion in *Peromyscus* spp. that were undergoing such quarantine. These results support the use of a quarantine period in combination with hantavirus antibody testing to clear mice for indoor use.

We collected 132 white-footed mice from one southern and two northern areas of Illinois that have not previously been examined for the presence of hantavirus. The average seroprevalence among these populations was 1.5%. Forty-six of these mice were quarantined for 5 weeks (6), and one mouse underwent seroconversion as detected by strip immunoblot assay. The presence of viral RNA in this mouse was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) from lung tissue. In addition, we collected 69 deer mice from an area of New Mexico that had an overall seroprevalence of approximately 20% and placed them in quarantine (6).

One deer mouse delivered four pups while in quarantine and seroconverted 19 days after delivery (6,7). While all four pups were seropositive, viral RNA was detected in the dam by using RT-PCR for lung tissue and immunohistochemistry for heart, lung, and liver tissue (data not shown). Infectiousness of the virus from this mouse was demonstrated by successful passage through uninfected deer mice (7). The fact that the New Mexico pups had not become infected when they were euthanized at 21 days supports other epidemiologic data that suggest that deer mice do not transmit the virus vertically (9-11). These results strongly support the recommendations promulgated by Mills et al. (5) and the Centers for Disease Control and Prevention that wild rodents be used as colony founders only if they remain seronegative for hantavirus after a 5-week quarantine period.

Working in outdoor quarantine facilities is labor-intensive and requires routine maintenance and occasional repair. Building costs depend on the number of nest boxes, but the material cost of a substantial quarantine facility is $10,000 to $20,000. However, safety concerns and the difficulties of maintaining mice alive outdoors without bringing them indoors necessitate their use. A possible exception could be made for very temperate climates, where outdoor cages might be used temporarily.

Our finding that even a recently infected dam, one known to be infectious by horizontal route, did not transmit virus to her pups supports lack of vertical transmission of SN virus as argued previously by workers using less direct methods (9-11).

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