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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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Mycobacterium tuberculosis Isolates of Beijing Genotype in Thailand

To the Editor: Anh and colleagues recently reported that in new tuberculosis (TB) cases from Vietnam, *Mycobacterium tuberculosis* isolates of the Beijing genotype are associated with younger age and, in isolates from Ho Chi Minh City, with resistance against isoniazid and streptomycin (1). However, occurrence of Beijing genotype *M. tuberculosis* strains may be different in other Southeast Asian countries such as Thailand.

From May 1999 to June 2000, we obtained 244 *M. tuberculosis* isolates from TB patients at Ramathibodi Hospital, Bangkok, Thailand (the hospital treats approximately 625 TB patients annually). The isolates have been prospectively analyzed by DNA-fingerprinting with the spoligotyping method (2). Drug-resistance testing and recording of patients' data (sex, age, geographic origin) were completed for 204 of 244 patients. The 204 patients originated from all six regions of Thailand, although the central region (comprising the Bangkok area) and the northeast region predominated (59% and 23.5%, respectively). Altogether 111 male and 93 female patients with a median age of 34 years (1 to 89 years) were included. Status of BCG vaccination or HIV infection was not assessed.

The Beijing genotype was found in 90 (44.1%) of the 204 isolates analyzed in detail, without significant differences regarding the respective patients' geographic origin or sex. Thus in Thailand, the frequency of the Beijing type is somewhere between the frequency in Vietnam (53%) (1) and in peninsular Malaysia (estimated at 24%) (3). Using the same age groups as Anh and colleagues, we did not find an association of Beijing genotype with young age (p = 0.41; chi-square test for trend). Although Beijing type isolates were more frequent among patients <25 years (18 [56%] of 32) than among those >25 years (69 [43%] of 161), this was not significant (p = 0.13). This association remained not significant, if only isolates from the central or the northeast region were analyzed.

Of 204 isolates, 62 (30%) showed resistance to ≥ 1 of 4 drugs tested (isoniazid, 8.8%; rifampicin, 6.4%; streptomycin, 19.6%; ethambutol, 4.9%). However, overall drug resistance, resistance to single drugs, and multidrug resistance were not associated with the Beijing genotype. The frequency of resistance was similar in distribution but overall lower than reported for the Ho Chi Minh City isolates (isoniazid, 24%; rifampicin, 2%; streptomycin, 31%; ethambutol, 2%) (1).

In both studies, the highest percentage of drug resistance was found for streptomycin. In our sample, this was not associated with particular spoligotypes or with geographic origin of the patient. Furthermore, streptomycin-resistant isolates were not more frequent in older age groups, although there was a nonsignificant trend (p = 0.12; chi-square$_{trend}$). Streptomycin is still used for standard quadritherapy in Thailand, and occurrence of resistant strains can reflect selection or transmission recently or in the past. This differs from occurrence of streptomycin resistance in countries where streptomycin is no longer used in standard therapy (4).

In the original description of the Beijing family of strains in 1995, Beijing genotype isolates were found in 7 (37%) of 19 Thai isolates (5). In a subsequent IS*6110* restriction fragment length polymorphism analysis, 80 (38%) of 211 isolates from central Thailand collected in 1994 to 1995 belonged to the Beijing family (6). Whether 90 (44%) of 204 among our recent isolates reflect a reliable increase in Beijing type transmission over the last 5 years, is difficult to state. However, the fact that no correlation of Beijing type with (young) age of the patient was observed in the previous analysis (6) supports the notion that increasing incidence of the Beijing strain in Thai cases is not due to recent transmission.

The *M. tuberculosis* population appears to be considerably more heterogeneous in Thailand than in the large urban areas of Vietnam. In our study, the three most common spoligopatterns besides Beijing, S156, S153, and S22 (according to the nomenclature of Soini et al. [7]), together comprised 20% of 244 isolates. However, the second most frequent spoligopattern, the "Vietnam genotype" (S10 according to Soini), reportedly shared by 27% of the Vietnamese isolates, was not found in our sample of