BLACK CREEK CANAL VIRUS INFECTION IN SIGMODON HISPIDUS IN SOUTHERN FLORIDA

GREGORY E. GLASS, WALTER LIVINGSTONE, JAMES N. MILLS, W. GARY HLADY, JOSHUA B. FINE, WILLIAM BIGGLER, TREvor COKE, DWIGHT FRAIZER, STEPHANIE Atherley, PIERRE E. ROLLIN, THOMAS G. KsIAZEK, C. J. PETERS, AND JAMES E. CHILDS

Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland; Department of Health and Rehabilitative Services, State of Florida, Miami, Florida; Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. A total of 1,500 small mammals were collected and tested for antibodies cross-reactive to Sin Nombre virus (Hantavirus: Bunyaviridae) at 89 sites in a 1,600 km² study area of southern Florida. More than 95% of the 123 seropositive animals were cotton rats (Sigmodon hispidus), suggesting infection by Black Creek Canal Virus, although seroreactive Rattus rattus (5 of 294) and Peromyscus gossypinus (1 of 39) also were captured. Crude seroprevalence in S. hispidus was 11%. Seroprevalence increased with body size and was more common in male (18%; n = 451) than in female (6%; n = 593) cotton rats. Infection within S. hispidus populations was widespread throughout the study area. Prevalence ranged from 0% to 60% at sites where more than five cotton rats were sampled but was not only a function of sample size. Sites with seropositive cotton rats were geographically clustered compared with sites with no seropositive cotton rats. Clustering was not due to the spatial distribution of sites with few animals, season of collection, or sex bias of animals captured at these sites. However, sites with no seropositive animals had an excess of animals in the intermediate size class (60–99 g) and a deficit of the largest and smallest animals. These data suggest that population structure within the habitat mosaic may play a significant role in the spatial distribution of hantavirus infection in local populations of reservoir species.

In the summer of 1993, the emergence of a previously unrecognized syndrome of acute respiratory distress, associated with high mortality, led to the identification of a previously unrecognized hantavirus (Family Bunyaviridae), the implication of a Sigmodontine rodent, Peromyscus maniculatus, as the reservoir, and the clinical and pathologic characterization of hantavirus pulmonary syndrome (HPS). Subsequently, several cases of HPS were identified in regions outside the geographic range of P. maniculatus. Based on previous evidence that most hantviruses tended to have a single rodent reservoir it was anticipated that other pathogenic hantviruses were responsible for these cases, and that other rodents served as their reservoirs.

Early in 1994, a case of HPS was identified in southern Florida and serologic studies showed reactivity between the patient’s sera and recombinant antigens developed against Sin Nombre virus (SNV) from P. maniculatus. Preliminary surveys of the rodent fauna in the area showed a high prevalence of reactivity to SNV in cotton rats (Sigmodon hispidus, Say and Ord) with low prevalences in the remaining species. Subsequently, the isolation and characterization of a hantavirus from two of these cotton rats was described. Partial sequence analysis showed the virus, named Black Creek Canal virus (BCCV), to be a unique member of the genus Hantavirus.

In studies of other reservoirs, hantavirus infection appeared to be transmitted horizontally so that the prevalence of infection increased with age in the reservoir animals. In addition, infection was geographically widespread, subsuming most of the reservoir’s distribution. Some studies noted that infection occurred with approximately equal frequency in both sexes, while in other reservoir species infection predominated in males. The purpose of this project was to characterize BCCV infection within the rodent fauna in southern Florida, especially its putative reservoir S. hispidus, in a more extensive manner than was possible during the preliminary surveys. Particular attention was focused on the spatial distribution of infection in local reservoir populations.

METHODS

Trapping of small mammals was undertaken from August to December 1994 at 110 sites throughout Dade County in southern Florida. Sampling methods, described elsewhere in more detail, consisted of lines of 48 Sherman live traps set in grassy, semi-wooded and residential areas throughout a region of approximately 1,600 km². Traps were baited with a mixture of bird seed and peanut butter and set for three consecutive nights, yielding an equal sampling effort of 144 trap-nights at each site. The study area was bounded on the east by Biscayne Bay, on the west by SW 217th Avenue, on the north by Sunset Drive, and on the south by Palm Drive. Results from 89 sites where at least one S. hispidus was captured are described.

Live-trapped animals were brought to a central processing location where they were anesthetized, identified to species, standard external measurements were recorded, blood was collected, and the animals were killed. During trapping and processing, recommended procedures were followed to reduce the risk of exposure for personnel. Collected sera were either sent to the Centers for Disease Control and Prevention (Atlanta, GA) or the Florida State Health Laboratory (Jacksonville, FL) for testing for antibodies to hantavirus. For consistency with the preliminary studies, SNV antigen was used as the test antigen in an ELISA format. Early studies showed there was relatively little loss in sensitivity, in cotton rats, using SNV antigen (16 of 90 positive), rather than BCCV antigen (17 of 90 positive), and that all cotton rats with amplifiable viral nucleic acids were infected with BCCV. Details of serologic testing were described previously. Briefly, an Escherichia coli recombinant SNV nucleocapsid antigen was coated on an ELISA plate. Negative
control wells used the *E. coli* without SNV nucleocapsid. Whole blood was diluted 1:100 in 5% skim milk with phosphate-buffered saline (PBS)-0.05% Tween and allowed to react with the antigen-coated wells. Bound IgG was detected with a mixture of goat anti-rat and goat anti-*Peromyscus* IgG conjugated to horseradish peroxidase. Optical densities (ODs) at 410 nm were recorded and the ODs from the negative control wells were subtracted from the ODs of the SNV recombinant antigen wells. In this paper a serologic response to SNV antigen was assumed to be caused by BCCV since *S. hispidus* has been implicated as the primary or sole reservoir to BCCV and no other hantavirus has been found among individuals of this species in this area.

Seroprevalence was calculated as the number of seropositive animals divided by the numbers of animals tested for selected strata of the data. Prevalence was examined for male and female rats separately, and the population was divided into mass classes, as a surrogate of age. The five mass classes were < 60 g, 60–99 g, 100–149 g, 150–199 g and ≥ 200 g. Cotton rats < 60 g are generally sexually immature while animals ≥ 100 g are usually reproductively mature. Differences in the seroprevalence by mass class and sex were tested by log-linear models using counts of seropositive and seronegative individuals.

The geographic distribution of sites with infected cotton rats was examined by the method of Cuzick and Edwards for sites with ≥ five sampled cotton rats. The $T_k$ statistic, a measure of spatial aggregation, was used in this study to assess whether sites with infected cotton rats were spatially clustered when compared with sites that did not have infected cotton rats. The correction of Simes was used for multiple tests of nearest neighbors. Sites with five collected *S. hispidus* were included because the proportion of these sites with infected rats (50%) was not different from sites where more rats were sampled, and sites with small numbers of collected cotton rats were not themselves spatially clustered. Tests for differences in proportions were used to determine if epizootiologic characteristics and season of collection contributed to spatial clustering of sites with infected cotton rats.

### Results

A total of 1,500 small mammals was trapped and tested during the survey (Table 1). *Sigmodon hispidus* was the predominant species captured (69.4%). This was, in part, influenced by the selection of the habitats associated with trapping sites, but even outside urban settings preliminary surveys showed that cotton rats predominated in the local fauna. *Sigmodon hispidus* (Table 1) had the highest seroprevalence to SNV antigen (11.2%) of any of the species tested. A single *P. gossypinus* (1 of 39) and five *Rattus rattus* (5 of 294) also tested positive for SNV antigen.

*Sigmodon hispidus* were caught at 89 of 110 sites that were sampled. The mean number of rats captured per site, where they occurred, was 11.7 (range = 1–51) and at least five *S. hispidus* were caught 65.1% (58 of 89) of these sites. Seropositive *S. hispidus* were trapped at 46.1% of the 89 sites, which represented a variety of land use types including agricultural, residential, recreational, and industrial sites (Florida Department of Health and Rehabilitative Services, unpublished data). Infected *S. hispidus* were found at 35 (60.3%) of 58 sites with five or more sampled cotton rats. The average seroprevalence of *S. hispidus* at these sites was 20.7%. Sites with seropositive *S. hispidus* occurred throughout the study area, but tended to be more common in the southern and western regions (Figure 1). Overall, sites with infected *S. hispidus* were geographically clustered. Except for sites immediately adjacent to sites with infected *S. his-

<table>
<thead>
<tr>
<th>Species</th>
<th>Number tested</th>
<th>Number positive</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sigmodon hispidus</em></td>
<td>1,041</td>
<td>117</td>
<td>11.2</td>
</tr>
<tr>
<td><em>Oryzomys palustris</em></td>
<td>22</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Peromyscus gossypinus</em></td>
<td>39</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Rattus rattus</em></td>
<td>294</td>
<td>5</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>40</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>64</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>1,500</td>
<td>123</td>
<td>8.2</td>
</tr>
</tbody>
</table>

![Figure 1](image-url)
pidus, the next seven nearest collections also were more likely to have infected S. hispidus than expected by chance (T2, \( \gamma < 0.05 \); Simes correction of Bonferroni method \( P = 0.047 \); Table 2). At greater distances, the distribution of sites with infected and uninfected cotton rats were randomly distributed with respect to one another.

Finding seropositive S. hispidus was not simply a reflection of trap success. At eight (34.8\%) of 23 sites where \( \geq 19 \) animals were tested, no seropositive S. hispidus were captured. There also was no significant difference in the proportion of negative and positive sites sampled throughout the three seasons of trapping (\( \chi^2 = 0.01, P = 0.997 \), degrees of freedom \( [df] = 2 \)). Sites with infected S. hispidus had a slightly higher proportion of male rats than negative sites (44.7\% versus 40.7\%) but the difference was not statistically significant (\( \chi^2 = 1.48, P = 0.224 \)). However, there was a significant difference in the size structures of the population of males collected at uninfected or infected sites (\( \chi^2 = 18.73, P < 0.001, df = 4 \)). Sites with no positive S. hispidus had fewer than expected small (< 60 g) male rats (15 observed, 23.7 expected), fewer of the largest (\( \geq 200 \) g) male S. hispidus (0 observed, 5.1 expected), and more of the males reaching sexual maturity (60–99 g) than expected (35 observed, 25.4 expected). The size structures of the female populations at infected and uninfected sites were not significantly different (\( \chi^2 = 7.92, P = 0.094 \)), although there also tended to be an excess of females reaching sexual maturity (60–99 g) at the uninfected sites (63 observed, 52.5 expected).

Seroprevalence increased with increasing body mass (Figure 2) for both sexes. In juvenile (< 60 g) and young adult (60–99 g) S. hispidus, seroprevalence was approximately 2–4\% and did not differ between males and females. In larger S. hispidus, seroprevalence increased with body mass (Figure 2) and increased more rapidly for males than females. The log linear model providing the best fit to the data (\( \chi^2 = 6.43, P = 0.09, df = 3 \)) indicated a statistically significant association between sex and seropositivity, a significant increase in the proportion of seropositive animals with size, and a significant increase in the proportion of large rats that were males. In the largest mass class (\( \geq 200 \) g), when 77.3% of the animals collected were male, nearly all male rats were seropositive (88.2\%) while only 20.0\% of females of this size were seropositive. A more detailed (< 30 g versus 31–59 g) examination of seroprevalence in the smallest mass class (< 60 g) showed a marked decrease in seroprevalence from the smallest to the larger mass (9.1\%; \( n = 55 \) versus 2.0\%; \( n = 101 \)) before the onset of reproduction.

**DISCUSSION**

This study supports a growing number of reports characterizing the epizootiology of hantavirus infections in natural reservoir hosts. As with previous studies, seroprevalence to hantavirus tended to be common only in a restricted number of sampled species (Table 1).3,10,16,17 For example, a recent survey of more than 3,000 small mammals, representing 69 different species, found antibodies to SNV in only nine species and in four of these antibody was found in only a single individual.10 To date, hantaviruses appear to have rodents of the family Muridae as their primary hosts.

As with other Sigmodontine reservoirs, seroprevalence in S. hispidus increases with size of the animal and is more common in males than females. The consequences of this pattern is that the prevalence of infection is affected by the size structures and sex ratios of local populations, which is influenced by population dynamics of the rodents. In addition to supporting the general picture of hantavirus epizootiology, this study demonstrates a mesoscale geographic clustering of hantavirus infection and establishes an association between population structure and the geographic distribution of hantavirus infection in the reservoir species.

Although we used heterologous antigen to detect antibody to BCCV, the excellent cross-reactivity between SNV and BCCV suggests that few seropositive S. hispidus were not identified. Conversely, interpreting positive serological results as indicative of BCCV infection is supported by the polymerase chain reaction amplification of viral nucleic acids and the previous isolation of BCCV from seropositive S. hispidus from these sites,8 as well as the relative paucity of seroreactive specimens from other species (Table 1).

The relationship between body size and seroprevalence (Figure 2) implies that collected samples with predominantly larger (older) animals are more likely to have infected in-

**TABLE 2**

<table>
<thead>
<tr>
<th>K</th>
<th>T1</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>0.352</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.022</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>0.020</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>0.041</td>
</tr>
<tr>
<td>5</td>
<td>117</td>
<td>0.031</td>
</tr>
<tr>
<td>6</td>
<td>143</td>
<td>0.009</td>
</tr>
<tr>
<td>7</td>
<td>162</td>
<td>0.028</td>
</tr>
<tr>
<td>8</td>
<td>181</td>
<td>0.060</td>
</tr>
<tr>
<td>9</td>
<td>202</td>
<td>0.079</td>
</tr>
<tr>
<td>10</td>
<td>219</td>
<td>0.172</td>
</tr>
</tbody>
</table>

* The test of Cuzick and Edwards was performed using sites where at least five cotton rats were tested. Sites with at least one seropositive animal served as case sites and sites where all were negative served as control sites. K represents the k-th order nearest site. T1 is the test statistic that measures the number of times a site with infected cotton rats was the k-th order neighbor of another site with infected rats, and P is the level of significance. A P < 0.05 indicates that there was a statistically significant clustering of case sites that were k-th neighbors.

† Overall significance \( P = 0.047 \).
individuals than samples that represent predominantly smaller (younger) animals. The somewhat higher seroprevalence observed in the smallest body size class is thought to reflect the presence of maternal IgG antibody in offspring that is catabolized, resulting in a decreasing seroprevalence from the smallest to the larger juvenile mass classes, until infection at a later age produces increased seroprevalence. The decrease in seroprevalence among S. hispidus from 9% to 2% in the two subadult size classes mirrors the patterns noted for SNV in P. maniculatus in which prevalence decreased from 14% to 1% in the smallest mass classes before increasing among adult mice. The strong skew towards seropositive males in older S. hispidus also is consistent with previous reports from Sigmodontine rodents. The higher rates of seropositivity in older male rodents has been hypothesized to be caused by aggressive intrasexual interactions among adult male Sigmodontine rodents and aggression among adult rodents is reported common. However, in the present study, no information was recorded on the presence or frequency of biting that would allow us to evaluate this hypothesis for the maintenance of BCCV.

The systematic sampling of this study showed that a large proportion of sites within the study area yielded no infected S. hispidus, even though the species was common. Earlier studies of P. maniculatus sampled during the HPS outbreak investigation of 1993 also indicated some sites had no captures of seropositive mice, an observation supported by smaller surveys in Kansas. A large serosurvey throughout the southwestern United States also found no seropositive deer mice at 20 of 41 sampling sites where the species was collected, although positive mice occurred throughout the geographic region.

Various hypotheses can be evaluated to explain sites without seropositive S. hispidus. One possible explanation could be that these areas merely reflected insufficient sampling. Although this may explain some of the sites where relatively few animals were sampled, it does not explain the absence of seropositive animals at many of the localities. Even at the 23 sites where ≥ 19 S. hispidus were captured, nearly 35% of those locations yielded no seropositive animals, a value not significantly different from the 40% of seronegative sampling sites when only ≥ five animals were tested. Another explanation for finding uninfected sites was the timing of collections because not all sites could be sampled within a single sampling interval. However, we think this is unlikely because there were no seasonal pattern in the proportion of seronegative sites that were sampled.

In the absence of methodologic reasons for this pattern, alternative explanations for sites without infected animals involve the interplay of viral transmission and reservoir population dynamics as they affect the persistence of hantavirus in local reservoir populations. The significant effects of sex, body size, and their interaction on seroprevalence suggest that differences in sex ratios among sites could influence local seroprevalence rates. However, we found no significant difference in the sex ratios of populations from sites without infected animals when compared to sites with infected animals and therefore conclude that differences in sex ratio were not responsible for the occurrence of uninfected sites.

The major feature that distinguished sites without seropositive S. hispidus was the higher than expected number of subadults captured at these locations, and, especially among males, the paucity of the largest and smallest size classes of animals. Thus, these sites may reflect a commonly reported phenomenon related to dispersal of maturing rodents. As juvenile rodents mature, they typically disperse from their natal burrows and establish their own territories or home ranges. Aggressive interactions with resident animals are thought to drive dispersal activity to new sites. Dispersal sinks for S. hispidus then represent areas where there are a disproportionate number of dispersing subadults (60–99 g). This is the size class with the lowest seroprevalence and if BCCV, like other hantaviruses, is horizontally transmitted, then these subadults would tend to be uninfected when they reached these sites.

The geographic clustering of sites with infected S. hispidus (Table 2) may reflect larger, more contiguous, patches of suitable habitats to the west and south of the Miami region because most of the human development occurs adjacent to the Atlantic coast where infection can be more easily maintained and transmitted by local movements of individuals. These patterns of focality of infection suggest that the presence of a reservoir species is necessary but not sufficient for hantaviral disease outbreaks and it may be possible to characterize habitats that support sufficient populations of animals for the persistence of hantaviruses, based on landscape patterns, by linking remotely sensed data to infection patterns in local populations.

The results of this and other recent studies point to the need for careful data collection, identification, and documentation of the characteristics of locally sampled populations of hantavirus reservoirs. The apparent epizootiology of the viruses is influenced by the species collected, the size structure and sex ratio of the sample collected, as well as the habitat in which the sampling is conducted. The success in understanding patterns of hantavirus maintenance and transmission in various species across studies will be limited unless researchers consider these potentially confounding factors that affect the patterns of infection.

Acknowledgments: We thank the many staff of District XI who assisted in the collection of small mammals, Dr. Michael S. Gaines (University of Miami, Miami, FL) for assistance in specimen identification and the Health Rehabilitative Services Laboratory (Gainesville, FL) for serologic testing. Timothy Shields assisted with the spatial analyses. Special appreciation is extended to Annie R. Neusman and Col. Rodney L. Bates (U. S. Air Force) for logistical support during this study.

Financial support: Gregory E. Glass was supported by an Inter governmental Personnel Agreement from the Centers for Disease Control and Prevention. Financial support was provided to the State of Florida Health and Rehabilitative Services District XI by the Centers for Disease Control and Prevention under grant award H5Q/CCG408954-01.

Authors’ addresses: Gregory E. Glass and Joshua B. Fine, Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205. Walter Livingstone, W. Gary Hiday, William Biggler, Trevor Coke, Dwight Frazier, and Department of Health and Rehabilitative Services, State of Florida, Miami, FL 33125. James N. Mills, Pierre E. Rollin, Thomas G. Ksiazek, C. J. Peters, and James E. Childs, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333.
BLACK CREEK CANAL VIRUS IN FLORIDA

REFERENCES