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HANTAVIRUS ANTIBODY OCCURRENCE IN BANK VOLES (*CLETHRIONOMYS GLAREOLUS*) DURING A VOLE POPULATION CYCLE

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ABSTRACT: Puumala virus, genus *Hantavirus,* is the etiologic agent of nephropathia epidemica, a mild form of hemorrhagic fever with renal syndrome. The bank vole (*Clethrionomys glareolus*) is the natural reservoir species of this hantavirus. We initiated sampling of bank voles at sites of recently identified human nephropathia epidemica cases and paired control sites in the fall of 1995 in coastal areas of northern Sweden. Sites were trapped annually in spring and fall until 1999. Prevalence of antibody to Puumala virus was similar among local bank vole populations in the two types of sites over time. During peak years, however, the absolute number of bank voles was higher in case sites than control sites. Consequently, the likelihood of Puumala virus exposure was increased at case sites during population highs. This would imply that the risk of Puumala virus exposure to conspecifics and humans is habitat and site dependent with a temporal component.

Key words: Bank vole, *Clethrionomys glareolus,* habitat, hantavirus, nephropathia epidemica, population dynamics, Puumala virus, rodents.

INTRODUCTION

Hantaviruses are the etiologic agents of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in people. Hantaviruses are chiefly transmitted by inhalation of aerosols containing the virus excreted from infected rodents (Schmaljohn et al., 1985; Lee, 1996; Young et al., 1998).

The annual number of clinically manifested hantavirus infections in humans may be as many as 150,000 worldwide (Lee, 1996). The number of humans infected with hantaviruses, and other rodent borne diseases, is expected to rise due to increases in human population density, altered populations of rodents in disturbed environments, and climate change (Epstein, 1995; Global Change and Terrestrial Ecosystems, 1999).

In Europe, the most widespread hantavirus is Puumala virus (PUU), which is associated with bank voles (*Clethrionomys glareolus*) and causes nephropathia epidemica (NE) in humans (Brummer-Korvenkontio et al., 1980; Yanigihara et al., 1984). Nephropathia epidemica is a milder form of HFRS and has a fatality of less than 0.5% (Linderholm et al., 1991; Brummer-Korvenkontio et al., 1999). Bank voles are common in forest regions and in northern Fennoscandia density fluctuations may show up to 500-fold changes from peak to decline phases during 3 to 4 yr cycles (Hansson and Henttonen, 1985; Hörnfeldt, 1994; Korpimäki and Krebs, 1996).

Whatever the ultimate cause of population fluctuations, variation in bank vole numbers is correlated with the yearly incidence of human PUU-infections in northern Fennoscandia (Lähdevirta, 1971; Nyström, 1977; Niklasson et al., 1995; Brummer-Korvenkontio, 1999). The average incidence of serologically confirmed cases in northern Sweden normally ranges between 20 to 40 cases per 100,000 inhab-

itants depending on the phase of bank vole population dynamics (Settergren et al., 1988). An earlier study showed that approximately 80% of human hantavirus infections in the region remain unrecognized (Ahlm et al., 1994).

We previously found indications that bank vole populations showed higher PUU antibody prevalence near sites of recent NE, as compared to control sites during a bank vole population peak (Ahlm et al., 1997). The study suggested that differences seemed to be related to significantly higher vole population numbers at the case sites. In the present study, we continued to investigate bank vole population dynamics and hantavirus antibody occurrence at these sites for an additional 4 yr, thus covering a full vole population cycle over two peaks. Specifically, we investigated whether this site dependent pattern from the initial peak year was: 1) accidental, 2) consistent throughout the study period, or 3) re-occurring at particular bank vole densities, indicating a stochastic hantavirus circulation or the presence of permanent or temporal hantavirus refuges (determined from PUU antibody occurrence) as discussed by Hjelle and Yates (2001).

MATERIAL AND METHODS

Sampling of bank voles was initiated in the vicinity of three human dwellings where NE was serologically confirmed 3–10 wk prior to the first sampling in fall of 1995. These sites were situated approximately 40 km apart (south-to-north 80 km), and denoted "South," "Center" and "North" according to location within the sampling region. Randomly selected sites with no previous known human hantavirus infection served as paired controls to South, Center, and North. To select these control sites, isoclines of 10 km radius were drawn on a map around each case site. The first sites that fell under the isoclines and were of equivalent composition as the case sites and accessible from adjacent roads, as later identified in the field, were then chosen.

Habitats at sampling sites consisted of managed and mixed conifer forests dominated by Scotch pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) of similar stand age (40–60 yr), with considerable undergrowth of bilberry (*Vaccinium myrtíllus*) and lingonberry (*V. vitis-idaéa*), and were ≥5 ha. Although exposure to PUU virus occurred in or near a dwelling at each case site, rodent sampling was performed in surrounding forests because these constituted the bank vole's natural habitats, rather than around constructions or arrangements of anthropogenic origin. Sampling sites are situated along the coastal area of Västerbotten county in northern Sweden (63°45′–63°20′N, 20°00′–21°00′E) (Ahlm et al., 1997).

Bank voles were sampled by snap trapping in May–June during 1995–99, as soon as possible after snowmelt, and in September–October when reproduction normally was completed. At every site, 30 snap-traps baited with dry apple were placed at 10 m intervals in each of six transects at each site. These 180 snap-traps were set during four nights constituting 720 trap nights at each of the six sites, a number known to adequately determine the relative abundance of local microtine populations (Myllymäki et al., 1971; Hanski et al., 1994). Success in sampling effort was expressed as trap indices that represented the number of voles captured per 100 trap-nights, i.e., a reflection of the relative population density on each sampling occasion (Hanski et al., 1994). Because of being short-handed in the initial phase of the project, not all areas were subjected to the same effort of sampling in the autumn of 1995. The number of traps used on this single occasion was 90, 165, and 135 at the South, Center and North control sites, giving raise to 360, 660, and 540 trap-nights respectively. This has been taken into account in all analyses and graphic presentations, hence use of trap indices and not absolute numbers. All sites were sampled within a 3 wk period per season and each pair of case and control sites was sampled simultaneously.

Collected voles were kept on ice, transferred to −70 C freezers, and processed in a laboratory (biosafety level three). Total body weight was obtained to the nearest 0.1 g with weights of fetuses subtracted. Blood was collected using Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Strips were dried and then eluted with 500 μl of phosphate-buffered saline at room temperature for 1 hr, where estimated dilution was 1:12.5. Detection of PUU virus specific immunoglobulin G (IgG), hereafter denoted as PUU antibody, was performed by enzyme-linked immunosorbent assay (ELISA) according to previous protocols (Elgh et al., 1996; Ahlm et al., 1997).

We used analysis of variance (ANOVA; SAS Institute, 2000) to test for differences between years, types and pairs of sampling sites, and the

interactions of types and pairs vs. type and year of sampling sites based on spring and fall samplings separately. The response variables were: 1) trap indices for total catch of bank voles per site (total trap index), 2) trap indices for catch of PUU antibody positive bank voles per site (PUU trap index), and 3) antibody prevalence measured as proportion of catch of PUU antibody positive bank voles within total catch per site (PUU prevalence). Where significant F-values were observed, we tested for differences within model effects by Tukey test. Data was normalized by arcsine transformation (Zar, 1984).

To evaluate the demographic composition of sampled populations, the age of each individual bank vole captured during 1997–1999 was determined according to criteria of molar root growth (Tupikova et al., 1968; Gustafsson et al., 1982) and assigned to discrete age classes, see Olsson et al. (2002) for details. Age classes used on fall captures were sexually immature juvenile/sub adult specimens <3 mo of age, sexually mature adults born in year of sampling 3–6 mo of age, and over-wintered adults >11 mo of age.

RESULTS

In total we captured 1,568 bank voles during 36,840 trap-nights. The overall mean PUU antibody prevalence to PUU was 15.4%, with the male-to-female ratio equal to 1.0:1.0, but sex related antibody prevalence ratio differed 1.36:1.0 (chi square=6.57, df=1, P=0.01). Peak years in bank vole abundance as determined from total trap indices were observed in 1995 and 1998. In 1996, 1997, and 1999, bank vole trap indices were considerably lower (Fig. 1). The trap indices of voles were generally lower in the spring.

There were no differences in spring and fall samples, trap indices, or antibody prevalence on ANOVA terms of sampling pairs, interactions of pair and type or year and type. In spring samples, there were no significant differences in indices between case and control sites. On the effect of sampling year, total trap index and PUU trap index were significantly higher in 1998 compared to all other spring samples (ANOVA; F-value=12.24, df=3, P<0.001; versus F-value=14.15, df=3, P<0.001: Tukey P<0.05, Fig. 1). The PUU antibody prevalence in spring revealed no differences for any term analyzed.

In fall samples, a significantly higher total trap index was observed within case sites compared to control sites (ANOVA; F-value=16.80, df=1, P<0.001). This was also evident for the PUU trap index (ANOVA; F-value=9.67, df=1, P<0.001). The total trap index and the PUU trap index were significantly higher in fall 1998 than in all other fall samples (Tukey P<0.05). The peak fall 1995 differed from fall 1996 in total trap index, and from fall 1999 in PUU trap index (Tukey P<0.05). Antibody prevalence in fall showed significant influence from sampling year only between two years (1998>1999) (ANOVA; PUU prevalence: F-value=3.77, df=4, P=0.02, Tukey P<0.05) but with no other term.

The demographic composition was not different between types of sites during the population increase in 1997 and the decline in 1999. In the peak fall of 1998, the difference in demographic composition was consistent between case and control sites where more adult females were captured at case sites (mean=10.33 adult females/site) than at control sites (mean=4.33 adult females/site). Also juveniles <3 mo of age were more abundant at case sites (mean=108.00 juveniles/site) than at control sites (mean=56.33 juveniles/site). However, the number of adult males captured that fall was strikingly low and was similar among sampling sites (case sites' mean=2.00 adult males/site; control sites' mean=1.33 adult males/site).

DISCUSSION

The relationship between hantavirus antibody prevalence and sex was similar to that found in other studies (Bernshtein et al., 1999; Mills et al., 1999) and these findings are possibly the result of behavioral differences where frequent male-male contacts lead to higher antibody prevalence among male bank voles. The pattern of inter-annual changes in bank vole numbers agreed with 3 to 4 yr periodicity in population dynamics of small mammals in

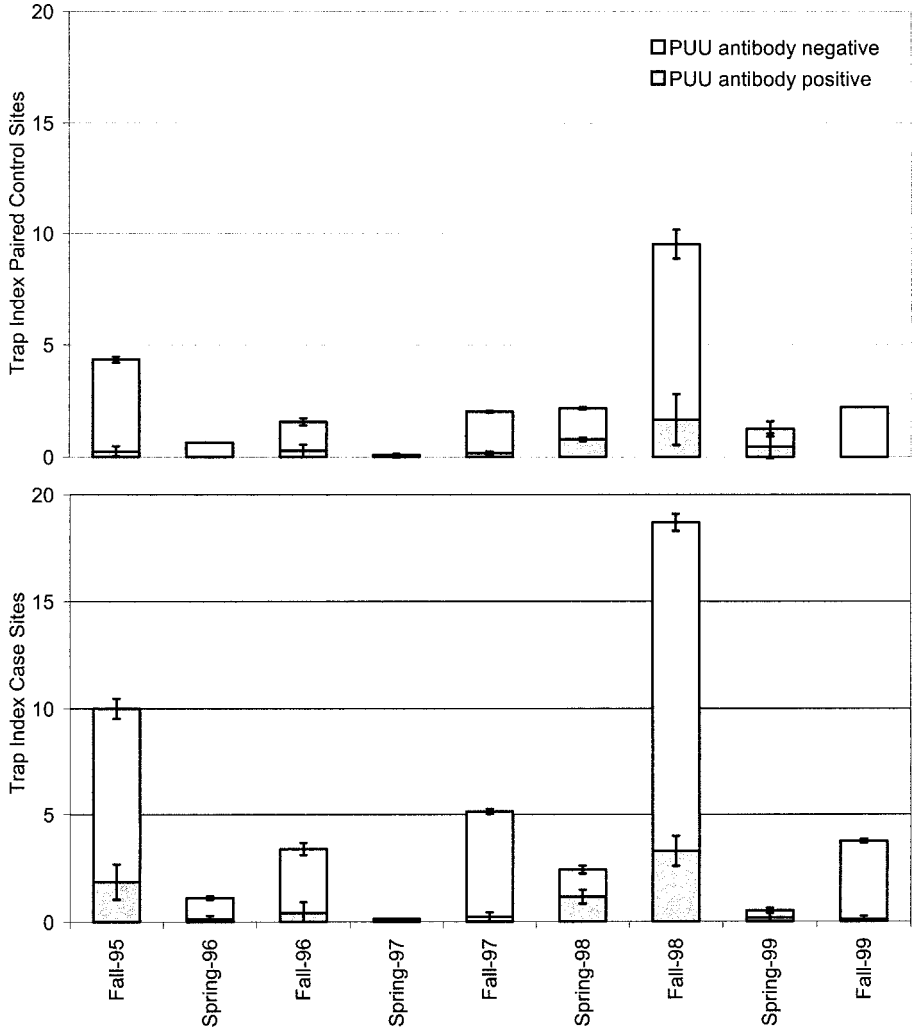

FIGURE 1. Mean trap index (±SE) of sampled bank vole populations for case sites (lower graph) vs. control sites (upper graph) starting fall 1995 and ending fall 1999. The lower, shaded part of the bars represents trap indices of Puuluma virus (PUU) antibody positive voles, while upper parts represent PUU antibody negative bank voles on each sampling occasion.

this region (Hörnfeldt, 1994; Niklasson et al., 1995). The annual number of confirmed cases of NE per 100,000 inhabitants per year within the region (i.e., the incidence), was correlated with the population dynamics of the bank voles studied. According to the Swedish Institute for Infectious Disease Control (1999, 2000) yearly incidences in the county of Västerbotten were 34.2 in 1995, 21.9 in 1996, 18.1 in 1997, 73.1 in 1998, 58.8 in 1999, and 17.6 in 2000. The high incidence in 1999, during which bank vole trap indices were generally low in both spring and fall, was explained by the fact that most cases of identified NE appeared during winter, the season when bank voles approach and invade human dwellings. In the study region, winters last on average from mid-October to mid-April (Raab and Vendin, 1995), and thus the number of NE in early 1999 showed an effect of the bank vole peak from fall 1998.

Assuming that hantavirus infections are transmitted horizontally, are non-virulent, and do not affect the natural rodent host's

condition or survival, a more effective spread of the PUU virus in the bank vole populations would be expected at increased densities due to more frequent contacts with conspecifics (Anderson and May, 1991; Dobson and Hudson, 1995; Grenfell and Harwood, 1997). Thus the PUU trap index of bank voles would be a density-dependent function of the total trap index, which should also be observed as proportionally higher PUU prevalence, either direct or with a time lag (Mills et al., 1999). Fall samples coincided with intra-annual peaks when juveniles and subadults were abundant. As such, these animals probably diluted the effect of density dependence observed on population scale, because they were either not exposed, or were infected within 3–4 wk prior to sampling and thus did not yet have IgG antibodies to PUU. However, analysis of individual bank voles captured during 1997–99, revealed there was increased likelihood of being PUU antibody positive in the peak year (1998) and the year of population decrease (1999), as compared to the increase year (1997), strongly implying direct and also delayed density dependence (Olsson et al., 2002).

Though spring indices appeared similar between case and control sites at the regional scale, processes operating on the local scale gave raise to higher fall densities at case sites. We suggest that the similar spring population densities among the sites, as reflected in the total trap index, was a result of disproportional mortality due to higher attractiveness of case sites to specialist avian and mustelid predators (Hanski et al., 1991; Klemola et al., 1999). This follows predator-prey theory suggesting that predators capture prey with decreasing efficiency, thus leveling out spatial dissimilarities in prey numbers (Ims and Steen, 1990; Hansen et al., 1999). The greater rate of population increase at case sites compared to control sites during the reproductive period could be a function of either higher reproductive output per area unit; increased survivorship; greater immigration; or a combination of the above (Hansson, 1994; Löfgren, 1995).

Although PUU antibody prevalence was similar between types of sites, actual trap indices of PUU antibody positive bank voles were higher in case sites compared to control sites, and therefore the likelihood of PUU-exposure by encountering an infected bank vole increased. This pattern was significant in the peak year of 1995 when the three NE case sites were identified (Ahlm et al., 1997) and also during 1998, and was consistent but not significant for the remaining fall samples. These site-specific differences in bank vole dynamics probably reflect crucial attributes of the case sites (Hansson, 1990, 1999; Löfgren, 1995;). The population dynamics of bank voles and occurrence of PUU virus have previously been shown to be spatially heterogeneous at regional and local scales (Verhagen et al., 1986; Escutenaire et al., 2000). Because the pattern was consistent over time in the present study, these results emphasize the importance and relevance of identifying habitat features that are likely to be associated with increased risks of exposure to hantavirus.

It was shown previously that cyclic population variation of bank voles in the region had a profound effect on the risk for humans to acquire PUU virus infection (Nyström, 1977; Niklasson et al., 1995). Here we show that the proportion of voles with IgG to PUU virus was not different between local fall populations during the whole population cycle, with one exception. The discrepancy appeared only at the population peaks, when the case sampling areas also supported much higher bank vole numbers. This same pattern at the second peak in the study strongly implies the influence of one or several environmental factors not yet identified. In conclusion there was a general temporal influence on bank vole numbers following regional vole dynamics, but on a spatial scale within sampling site. The patterns observed in peak years revealed recurring

risk of hantavirus exposure for bank voles and humans.

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