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Retrospective Serosurvey for Human Granulocytic Ehrlichiosis Agent in Urban White-footed Mice from Maryland

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ABSTRACT: Archived serum samples from 111 *Peromyscus leucopus*, collected 1984–88 in Baltimore City (Maryland, USA), were analyzed by indirect immunofluorescence assay. Sera from two (2%) individuals contained antibodies reactive to *Ehrlichia equi* and the human granulocytic ehrlichiosis (HGE) agent. This suggests that the HGE agent or an antigenically related organism has been present in rodent populations in this locality for more than a decade, and was present in the region at least 12 yr before a case of human disease was recognized.

Key words: *Ehrlichia equi*, human granulocytic ehrlichiosis, indirect immunofluorescence assay, *Peromyscus leucopus*, reservoir, white-footed mouse.

Human granulocytic ehrlichiosis (HGE), a potentially fatal undifferentiated febrile tick-borne illness (Bakken et al., 1994), is caused by an as-yet unnamed Gram-negative rickettsial organism. Based upon sequence analysis of the 16S ribosomal RNA gene, the HGE agent appears to be very similar, if not identical, to the granulocytotropic *Ehrlichia equi* and *E. phagocytophila* (Chen et al., 1994), previously presumed to be pathogens of nonhuman mammals. Human, as well as horse, dog and bovine antibodies to the HGE agent cross-react with *E. equi* and *E. phagocytophila* antigen, but not with *E. chaffeensis* (etiologic agent of human monocytic ehrlichiosis), when analyzed by indirect immunofluorescence assay (IFA) (Dumler et al., 1995). Evidence for the endemic maintenance of the HGE agent in white-footed mice (*Peromyscus leucopus*) by the black-legged tick (*Ixodes scapularis*) was provided by Telford et al. (1996) Walls et al. (1997) demonstrated HGE agent DNA by polymerase chain reaction (PCR) and antibodies to the HGE agent in approximately 10% of *P. leucopus* collected from areas of Minnesota (USA) where *I. scapularis* was abundant. Serologic evidence of HGE infection in four of 26 *P. leucopus* tested from Rhode Island (USA) also has been recently demonstrated (Yeh et al., 1997). In addition, white-tailed deer (*Odocoileus virginianus*) have been implicated in the HGE agent transmission-cycle, at least indirectly by way of supporting the adult ticks (Bakken et al., 1996). Although white-footed mice, white-tailed deer, and black-legged ticks are indigenous to eastern Maryland (USA), only recently has evidence been gathered indicating the presence of the HGE agent in this region. In 1996, a 16S rRNA gene sequence identical to that of the HGE agent was amplified from a questing *I. scapularis* nymph collected from Harford County (Maryland), and the first two human cases of HGE in Maryland were confirmed by serologic analysis (J. S. Dumler, unpublished data).

To examine the possible presence of HGE in the mid-Atlantic region of the United States prior to the description of human disease, a retrospective serologic study was performed on serum samples collected from *P. leucopus* in the mid 1980's in Baltimore (Maryland). The samples were obtained during studies of rodent-borne zoonoses (Childs et al., 1987; Korch et al., 1989). Because the samples were initially acquired to study other infectious agents in urban environments, the collections were not optimized to obtain animals from areas where *I. scapularis* were common. Mice were collected by Sherman (H. B. Sherman Traps, Tallahassee, Florida, USA) live-traps from four locations within the city of Baltimore. The animals were either marked and released

for recapture, or killed immediately. Briefly, 49 small mammal live traps were arranged in a seven by seven trapping station array with 10 m spacing between traps. Traps were set for three consecutive nights. Details of sampling protocols are provided in Childs et al. (1987).

Whole blood was collected from the retro-orbital sinus or by cardiac puncture, and serum was stored at −80 C until tested. Serological testing was based upon the methodology of Bakken et al. (1994). Upon thawing, samples were diluted 1:80 in 0.1 M phosphate-buffered saline (PBS), pH 7.4, with 0.5% non-fat dry milk (PBS-M). For the screening run, 10 μl of diluted sample was applied per well on IFA slides coated with leukocytes prepared from an experimentally *E. equi*-infected horse (courtesy J. Madigan, University of California, Davis, California, USA). Slides were incubated at room temperature for 60 min in a humidity chamber, washed three times in 0.1 M PBS, and then allowed to dry. Fluorescein isothiocyanate (FITC)-labeled goat anti-*P. leucopus* IgG (H + L), (Kierkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) diluted 1:50, was applied to each well and slides were again incubated at room temperature for 60 min. After washing in 0.1 M PBS twice, slides were counterstained with 0.025% Evan's blue for 5 min and then washed a final time in water. Each run included a positive control serum obtained from a naturally infected mouse identified in Connecticut (Magnarelli et al., 1997), and a laboratory-reared *P. leucopus* negative control serum (*Peromyscus* Stock Center, University of South Carolina, Columbia, South Carolina, USA). Sera that reacted at the screening dilution of 1:80 were titrated to endpoint by successive twofold dilutions using IFA slides coated with *E. equi*-infected equid neutrophils. Sera reactive with *E. equi* antigen were also titrated using a human-derived HGE agent antigen (Webster strain) propagated in tissue culture (host cells-HL60 promyelocytes).

Seropositive animals were found at two of four sites. Two of the 111 (2%) mice tested had antibodies that reacted with *E. equi*; both also reacted with the HGE agent antigen at equivalent titers. The first seropositive animal, an adult (20.0 g) nonreproductive male was one of 33 collected in early November 1984 and again in mid December 1984 from a park in northeastern Baltimore City (76°36′W, 39°24′N). No sera remained from the first capture for testing; the HGE agent antibody titer at second capture was 160. The second seropositive *P. leucopus,* one of 67 tested from this site, was a 16.5 g male first captured in mid December 1984 and recaptured in late February 1985 from a landfill in southern Baltimore City (76°36′W, 39°12′N). The first HGE agent antibody titer was 640 and at second capture the titer was 1,280. Eleven mice collected from two other locations were seronegative for *E. equi* antibodies.

Antibodies reactive with the agent of human granulocytic ehrlichiosis (HGE) were present in wild-caught *P. leucopus* from Baltimore City in 1984, 10 yr prior to the original description of HGE in the upper midwestern USA (Chen et al., 1994). HGE is an emerging infectious disease. This emergence is thought to be due in part to human activities including residing and recreating in HGE-endemic areas where the presumed reservoirs and vectors are present and abundant. Risk of becoming infected with the HGE agent is expected to increase in areas of high density deer populations that are parasitized by the tick vector, as is the case with Lyme borreliosis (Fish, 1995). The findings of this study are notable for several reasons. First, the two mice that tested positive by serology were trapped in two locations within Baltimore City limits, urban environments generally considered marginal habitats for these rodents. Consequently, an urban human population generally not considered to be at risk may be exposed to pathogens such as the HGE agent and *Borrelia burgdorferi*. Second, the evidence that the HGE agent may have been enzootic for

at least a decade prior to its characterization provides an example of how infectious agents that are commonly referred to as "emerging" may in fact be re-emerging, or simply more readily diagnosed.

The results demonstrate that the HGE agent, or an antigenically related organism, existed in Baltimore City, undetected either clinically or epidemiologically, before the disease came to public health importance. The relatively low prevalence of the HGE agent in *P. leucopus* observed in this study is to be expected in Baltimore City because poor habitat for mice and ticks likely limits the populations of both. However, one case of clinical human Lyme disease has been identified and linked to exposure in a city park (Schwartz et al., 1991). Nicholson and colleagues (W. Nicholson pers: commun.) recently found evidence confirming that *P. leucopus* in eastern Maryland have antibodies reactive with the HGE agent. These results also suggest that a large human population in Maryland may be at risk for infection with the agent of HGE. Although the individual risk may be lower than in more suitable mouse and tick habitats, the presence of this urban reservoir exposes a large human population to the potential burden of disease.

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