EXPERIMENTAL INFECTION OF DUSKY-FOOTED WOOD RATS (NEOTOMA FUSCIPES) WITH EHRLICHIA PHAGOCYTOPHILA SENSU LATO

Authors: Foley, Janet E., Kramer, Vicki, and Weber, David

EXPERIMENTAL INFECTION OF DUSKY-FOOTED WOOD RATS (*NEOTOMA FUSCIPES*) WITH *EHRLICHIA PHAGOCYTOPHILA* SENSU LATO

Janet E. Foley,[1,4] **Vicki Kramer,**[2] **and David Weber**[3] [1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616-8782, USA; [2] California Department of Health Services, Sacramento, California 95814, USA; [3] Feline Retrovirology Research Laboratory, School of Veterinary Medicine, University of California, Davis, California 95616-8782, USA; [4] Corresponding author (e-mail: jefoley@ucdavis.edu)

ABSTRACT: Dusky-footed wood rats, *Neotoma fuscipes,* have been implicated in the natural maintenance of *Ehrlichia phagocytophila* sensu lato, the agent of human granulocytic ehrlichiosis (HGE), in northern California based on high seroprevalence and amplification of *E. phagocytophila* s.l. DNA from wood rat blood. In order to further assess granulocytic ehrlichiosis in wood rats, we examined wild-caught wood rats for infection and then performed experimental intra-peritoneal infections with *E. phagocytophila* s.l. in horse or wood rat blood, and tested animals for 120 days by polymerase chain reaction (PCR) and serology. Of 15 wood rats collected from northern California, three were antibody and PCR-positive for *E. phagocytophila* s.l. at the time of capture. The naturally infected wood rats remained PCR-positive for a mean of 52 days (±7 SD). Experimental IP passage of *E. phagocytophila* s.l. in wood rat blood was successful in three of four wood rats and the mean duration of PCR-positivity was 75 days (±21.2 SD). Experimental infection with *E. phagocytophila* s.l. in horse blood succeeded in all four of the recipients and the mean duration of PCR-positivity of 81 days (±17.5 SD). No infected individual appeared to be ill based on feeding behavior, activity, and hydration status. These data confirm that wood rats are susceptible to *E. phagocytophila* s.l., may develop prolonged infection without clinical ehrlichiosis, and may play a role in maintaining *E. phagocytophila* s.l. in nature.

Key words: dusky-footed wood rats, *Ehrlichia equi, Ehrlichia phagocytophila* sensu lato, human granulocytic ehrlichiosis, *Neotoma fuscipes.*

Human granulocytic ehrlichiosis (HGE) is an emerging tick-borne disease that was first reported in 1994 when ehrlichial inclusions were detected in granulocytes of patients with myalgia, fever, and thrombocytopenia (Bakken et al., 1994). The clinical presentation of HGE varies from inapparent infection, to severe flu-like dis-ease, to death (Dumler and Bakken, 1998). Hematologic and biochemical abnormalities may include thrombocytopenia, leukopenia, and elevated hepatic transaminases (Bakken et al., 1996).

The agent of HGE is indistinguishable from other granulocytic ehrlichiae of domestic animals, including *Ehrlichia equi* and *E. phagocytophila.* Although nomenclature is currently in revision, in this paper the agent will be designated *E. phagocytophila* sensu lato. Experimental inoculation of *E. phagocytophila* s.l. into horses produces a clinical syndrome identical to natural equine granulocytic ehrlichiosis (GE) (Madigan et al., 1995), *E. phagocytophila* s.l. and *E. equi* are genetically indistinct (Chae et al., 2000), and *E. phagocytophila* s.l. cross-reacts serologically and shares a western blot profile with *E. equi* (Dumler et al., 1995; Asanovich et al., 1997). The geographic distribution of HGE parallels that of Lyme borreliosis, including the upper midwest, the greater New York area, and low mountain regions of California. The vector of *E. phagocytophila* s.l. and *E. equi* is *Ixodes scapularis* east of the Rocky Mountains (Telford et al., 1996) and *I. pacificus* on the west coast (Richter et al., 1996).

Because the tick vectors and reservoirs of *E. phagocytophila* s.l. in California differ compared to vectors and reservoirs on the Atlantic seaboard and in the upper Midwest US, it is important that the western ecology be investigated independently in order to understand the natural maintenance of *E. phagocytophila* s.l. in the west and to evaluate and control for hu-

man risk. Dusky-footed wood rats, *Neotoma fuscipes,* were implicated in the natural maintenance of *E. phagocytophila* s.l. based on the high seroprevalence in wood rats in one location in the northern California coast range mountains (69 of 143; 48%) and 69% of the seropositive wood rats had *E. phagocytophila* s.l. DNA in their blood (Nicholson et al., 1999). However, it is not known whether wood rats remain infected for prolonged periods of time, facilitating tick acquisition of the agent, or whether wood rats themselves develop clinical signs of ehrlichiosis. This study was undertaken to determine the duration of patent infection after experimental challenge with *E. phagocytophila* s.l. and to evaluate clinical responses of the wood rats to the infectious agent.

Fifteen wood rats were live-trapped in spring 1999 in northern California. Four were from a county park in the low coast range mountains in Alameda County (Sunol, 215 m elevation, center of site: 37°35′N, 121°53′W), seven were from the Sonoma County site that was previously described as Sonoma B (elevation about 275 m, center of sites: 38°19′N, 122°34′W) (Nicholson et al., 1999), and four originated from the Diamond A study site, also in Sonoma County at the same GPS location, previously described (Fritz et al., 1997). An additional animal was born in captivity and reared to 1 yr of age. The field-caught animals varied in age from approximately 4-mo to 1-yr-old ; there were eight females and seven males. The fur of each wood rat was saturated one time with AQ Synerkyl (DVM Pharmaceuticals, Miami, Florida, USA) flea and tick spray to kill ectoparasites. Wood rats were bled retroorbitally (every other day for the first week and then biweekly until two consecutive PCR tests were negative) into tubes containing ethylenediaminetetraacetic acid (EDTA) for detection of antibodies and polymerase chain reaction (PCR). Total DNA was extracted from 50 µl whole blood using a kit (QiaAmp, Qiagen, Chatsworth, Massachusetts, USA). Nested PCR was performed

in a thermal cycler (MJ Research, Watertown, Massachusetts, USA) as described (Breitschwerdt et al., 1998) using primers Ehr-Out1 and Ehr-Out2 for the outer product and HE3-R and *E. equi* 3-IP2 for the inner product (Gibco Life Technologies, Rockville, Maryland, USA). The 395 bp PCR products were visualized by transillumination in 1.5% agarose gels stained with ethidium bromide.

Plasma anti-*E. phagocytophila* s.l. immunoglobulins were assayed by indirect immunofluorescence assay (IFA) as described previously (Barlough et al., 1995) but using *E. phagocytophila* s.l.-USG3 strain in HL60 cells as substrate (provided by W. Nicholson, Centers for Disease Control, Atlanta, Georgia, USA). Tests for antibodies against Sin Nombre virus (SNV) were performed at the California Department of Health Service Viral and Rickettsial Laboratory (Berkeley, California, USA). Three of fifteen field-caught wood rats at initial testing were positive for antibodies against *E. phagocytophila* s.l. The three antibody positive wood rats were also PCR-positive and all antibody negative wood rats were PCR-negative. All of the wood rats with evidence of exposure to *E. phagocytophila* s.l. were from Sonoma B. The PCR-positive wood rats remained PCR-positive from 45–59 days, mean 52 days (±7 SD). One of the three animals from Sunol had antibodies against SNV at the time of admission. A second animal from that site appeared unthrifty. Both of these wood rats were euthanatized with a barbiturate overdose and sent to a reference laboratory for further investigation.

Eight uninfected wood rats were experimentally inoculated with *E. phagocytophila* s.l. All had been held in confinement for 1 mo and were negative for antibodies and by PCR on nine separate occasions, documenting they were not infected. Four wood rats received 0.5 ml of *E. phagocytophila* s.l.-infected purified neutrophils from a horse that was experimentally infected with *E. phagocytophila* s.l. strain-

MRK at the UC Davis Center for Equine Health, prepared as described previously (Madigan et al., 1995). The other four wood rats were infected by pooling fresh blood from two PCR-positive wood rats and then immediately inoculating 0.5 ml IP. Following inoculation, PCR and tests for antibodies were performed every other day for a week and then biweekly for 3 mo. Three of four wood rats that received wood rat inoculum became PCR-positive, on days 34 post-infection (PI), day 45, and day 47. The duration of PCR-positivity was 60–90 days, mean 75 days ($\pm$21.2 SD). Antibodies were detected in all three wood rats 21 days after inoculation. For the wood rat that showed no evidence of infection, PCR and tests for antibodies were performed for 120 days before it was concluded that the animal had not acquired ehrlichiosis. All four wood rats infected by horse blood acquired ehrlichial infection and antibodies were detected between days 14 and 21 for all four and PCR positivity first detected from 21–41 days PI. These wood rats remained PCR-positive for a mean of 84 days ($\pm$18.0 SD). All four had detectable antibodies by day 21. No animals developed abnormal clinical signs.

This study compliments earlier work (Nicholson et al., 1998, 1999) that indicated dusky-footed wood rats in certain areas of California are frequently infected with *E. phagocytophila* s.l. and may be reservoirs. We compared the DNA sequence of wood rat *E. phagocytophila* s.l. isolates from northern California (previously reported by Nicholson et al., 1999) with human isolates from southern Humboldt County and found 99% sequence identity in the groESL heat shock protein gene (Foley, unpubl. data). In the present study, we found a high prevalence of infected animals from Sonoma B and showed that wood rats are easily infected experimentally with *E. phagocytophila* s.l. Naturally and experimentally infected wood rats exhibited no clinical signs.

The chronicity of *E. phagocytophila* s.l. infection in wood rats is comparable with that reported previously for other rodents. Infection with *E. phagocytophila* s.l. in *Peromyscus leucopus* resolved within 12 wk of infection (Levin and Fish, 2000). In laboratory mice, (C3H strain), *E. phagocytophila* s.l. can be detected by xenodiagnosis for up to 60 days (Sun et al., 1997; Hodzic et al., 1998). Other rodents, including species found on the west coast, may be more resistant to infection. For example, *P. maniculatus* appears to be a poor host of *E. phagocytophila* s.l., based on our unsuccessful attempts to experimentally infect them by IP injection of infected horse blood (Foley, unpubl. data) and low seroprevalence in sites where HGE is present (Nicholson et al., 1998, 1999). However, no study has comprehensively compared infectivity, duration of infection, and pathogenicity of different *E. phagocytophila* s.l. isolates across rodent species to determine whether there are specific interactions between hosts and disease agent that may facilitate more severe and more prolonged infections in some host-agent combinations.

The high frequency of infection in some populations, the ease of in vivo passage, and the relatively long period of ehrlichemia suggest that wood rats play a role in maintaining *E. phagocytophila* s.l. in nature. However, wood rats are only one species among a potentially large number of mammalian hosts of the HGE agent in California, given the broad host range of nymphal and adult *I. pacificus*, which includes lizards, birds, and a broad range of mammals (Furman and Loomis, 1984). Evidence for *E. phagocytophila* s.l. infection in western mammals has been observed in deer (*Odocoileus hemionus hemionus, O. hemionus columbianus*), elk (*Cervus elaphus nannodes*) (Foley et al., 1998), wood rats (*N. fuscipes*) (Nicholson et al., 1999), black bears (*Ursus americanus*) (Walls et al., 1997), mountain lions (*Puma concolor*) (Foley et al., 1999), striped skunks (*Mephitis mephitis*) (Foley, unpubl. data), coyotes (*Canis latrans*) (Pusterla et al., 2000), and horses (Madi-

gan, 1993). Other carnivores may become infected as well. Wood rats appear to be part of a complex cycle of infection among different vectors and hosts. In addition to *I. pacificus,* other non-human biting ticks such as *I. spinipalpis* have been suggested to transmit *E. phagocytophila* s.l. among wood rats (Nicholson et al., 1998).

The spatial distribution of *E. phagocytophila* s.l. in the west is patchy, based on the nonhomogeneous distribution of positive ticks tested by PCR (Barlough et al., 1997; Kramer et al., 1999), the concentration of cases in horses and dogs in the northern coast ranges and Sierra Nevada foothills (Vredevoe et al., 1999; Foley et al., in press), and the concentration of infected wood rats in Sonoma County but not in Placer and Santa Cruz Counties (Nicholson et al., 1998, 1999). However, the intensity of surveillance was not standardized in any of these studies. The contribution of wood rats to the ecology of *E. phagocytophila* s.l. may vary among patches. Thus this study and earlier research document the need for field-based research to assess the wood rat's contribution to *E. phagocytophila* s.l. maintenance in nature and the risk of exposure to people.

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