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White-footed Mice: Tick Burdens and Role in the Epizootiology of Potomac Horse Fever in Maryland

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ABSTRACT: One hundred ten white-footed mice (*Peromyscus leucopus*) were captured on horse farms in south-central Maryland, examined for ticks, and tested for specific antibodies to *Ehrlichia risticii*, the causative agent of Potomac horse fever. *Peromyscus leucopus* were consistently infested with immature American dog ticks (*Dermacentor variabilis*), with monthly prevalences as high as 80%. Sera from all 97 *P. leucopus* tested for antibodies to *E. risticii* were negative. This indicates that *P. leucopus* is not a reservoir of *E. risticii*, and suggests that immature *D. variabilis* do not acquire *E. risticii* in feeding upon white-footed mice.

Key words: *Ehrlichia risticii*, Potomac horse fever, *Peromyscus leucopus*, white-footed mice, American dog ticks, *Dermacentor variabilis*, serology, survey.

The possible involvement of wildlife as reservoirs of *Ehrlichia risticii*, the causative agent of Potomac horse fever (PHF) (Holland et al., 1985), remains problematical. An arthropod vector of this rickettsia has not been discovered (Schmidtmann et al., 1988), and exposure to *E. risticii* among non-equine mammals is just becoming evident. Serologic evidence of exposure to *E. risticii*, has been reported for cats, dogs, foxes (*Vulpes vulpes*) and a rabbit (*Sylvilagus virginianus*) (Sessions, 1988), but none of the rodents tested by Fletcher (1987) and Schmidtmann et al. (1988), including Norway rats (*Rattus norvegicus*) ($n = 130$), house mice (*Mus musculus*) ($n = 28$), meadow voles (*Microtus pennsylvanicus* ($n = 68$) and white-footed mice (*Peromyscus leucopus*) ($n = 40$), was seropositive. Nevertheless, Gordon et al. (1988) reported positive titers to *E. risticii* in three *P. leucopus* captured at horse farms in Ohio in 1986 and in three more *P. leucopus* tested in a preliminary study in 1985.

The white-footed mouse is a common inhabitant of hedgerows, woodlands and pasture-woodland interfaces, and serves as a major host for the immature (larval and nymphal) stages of the American dog tick (*Dermacentor variabilis*) and deer tick (*Ixodes dammini*) (Sonenshine et al., 1965; Carey et al., 1980). *Peromyscus leucopus* also is a reservoir for the etiological agents of Rocky Mountain spotted fever (*Rickettsia rickettsii*) (Sonenshine et al. 1966; Magnarelli et al., 1979), Lyme disease (*Borrelia burgdorferi*) (Levine et al., 1985; Donahue et al., 1987), and babesiosis (*Babesia microti*) (Spielman et al., 1985), which are transmitted by the aforementioned ticks. Because adult *D. variabilis* are known to attach and feed on horses at farms enzootic for PHF in Maryland (Carroll and Schmidtmann, 1986), it is important to resolve whether or not one of the two principal hosts for immature stages of *D. variabilis*, *P. leucopus*, is involved in the epizootiology of PHF. This study reports further serologic testing of *P. leucopus* from horse farms with histories of PHF in Maryland for antibodies to *E. risticii*, along with an assessment of immature ticks parasitizing the mice.

White-footed mice were captured with Sherman® live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida 32316, USA) on seven horse farms in Montgomery County, Maryland (USA; 39°05′N, 77°21′W). All seven farms experienced cases of PHF during the 3 yr prior to the study, including one intensively studied farm with 15 of 35 (43%) of the horses seropositive by indirect immunofluorescence antibody (IFA) assay (Ristic et al., 1986; Sessions, 1988). Three of the farms experienced cases of PHF during the trapping period. The traps were baited with peanut butter and

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cracked corn and set along hedgerows or <2 m into woodlands bordering horse pastures. Each farm was trapped at 2- or 3-wk intervals from March through September 1987. Ten to 15 traps were set over two consecutive 24 hr periods at each premises and checked after each 24 hr period. No more than four mice were removed from a given farm in any trapping period to reduce the impact on the *P. leucopus* populations. In the laboratory, animals were humanely euthanatized with CO_2 gas and serum samples were taken by cardiac puncture. Mice were examined for ticks under a dissecting microscope either immediately after death or they were placed in a sealable plastic bag and refrigerated for examination at a later date. The last 10 mice caught in September were not examined for ticks.

The presence of specific antibodies (IgG) against *E. risticii* was determined by the indirect immunofluorescent antibody (IFA) assay, as described by Robinson et al. (1976). Initially, field trapped *P. leucopus* were infected in the laboratory with *E. risticii* intraperitoneally and they seroconverted by day 14. Those sera were used in subsequent assays as positive controls. All *P. leucopus* sera (n = 97) were screened at a 1:40 dilution. The indicator antibody was a 1:20 dilution of fluorescein-bound goat anti-mouse IgG (Cappel Scientific, Malvern, Pennsylvania 19355, USA). A 2% (w/v) casein in a tris-buffered saline solution (Oaks et al., 1987) was used for all dilutions to decrease non-specific fluorescence.

One hundred of the 110 *P. leucopus* captured were examined for immature ticks. The only ticks observed were larvae (n = 266) and nymphs (n = 33) of *D. variabilis*, and they were present in all months of the study except March, when only two mice were captured (Table 1). The prevalence in 45 mice caught in April and May was 75%; in June, 50% of the animals had immature ticks. Numbers of ticks on mice in July and August were lower than in the spring months, but 83% of *P. leucopus* were parasitized by immature *D. variabilis* in August. All 97 serum specimens tested for antibodies to *E. risticii* were negative. Antibody titers to *E. risticii* laboratory-challenged white mice (*Mus musculus*, Balb C) and *P. leucopus* were generally similar, ranging from maximum values of 1:160 to 1:320.

Peromyscus leucopus was the only species of rodent captured. *Peromyscus maniculatus bairdii*, which prefers prairie, open fields and crop lands (Paradiso, 1969) was not captured. However, *P. maniculatus bairdii* has been collected from suitable habitats in Fairfax County, Virginia (Peacock, 1967), near Harrisburg, Dauphin County, Pennsylvania (Hamilton, 1950), and in Prince George's County, Maryland (Stickle, 1951). We did not capture *P. maniculatus bairdii*, possibly because of its scarcity or our selective placement of traps in hedgerows and woods edges.

The absence of specific antibodies to *E. risticii* in the white-footed mice we tested is not in agreement with the findings of Gordon et al. (1988), who reported positive antibody titers to *E. risticii* in six *P. leucopus* captured on two horse farms in Ohio where 20 of 37 horses (54%) had seroconverted. However, when our data are combined with other Maryland data, notably 40 other *P. leucopus* sera that were likewise negative for antibodies to *E. risticii* (Fletcher, 1987), it is apparent that the white-footed mouse is not involved in the epizootiology of PHF in Maryland. Our samples were broadly distributed throughout an area that has a persistent history of *E. risticii* infection in horses. Further, these *P. leucopus* were captured during months before and after clinical PHF occurred in horses on the premises and included 18 mice in September, the month when Gordon et al. (1988) reported seropositive *P. leucopus*.

The seronegative status of white-footed mice in Maryland indicates that *P. leu-*

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TABLE 1. Infestation of *Peromyscus leucopus* with *Dermacentor variabilis* larvae and nymphs, and results of indirect immunofluorescent antibody (IFA) tests for antibodies to *Ehrlichia risticii*.

Month	Numbers of mice	Prevalence (%)	Larvae $\bar{x} \pm$ SE	Larvae n	Nymphs $\bar{x} \pm$ SE	Nymphs n	Number of mice with antibodies to *E. risticii*/ number tested
March	2	0	0		0	0	0/2
April	21	71	3.86 ± 1.12	81	0.14 ± 0.08	3	0/13
May	24	79	6.63 ± 2.54	159	0.54 ± 0.30	13	0/21
June	16	50	0.31 ± 0.15	5	0.75 ± 0.27	12	0/15
July	17	18	0.29 ± 0.21	5	0.12 ± 0.08	2	0/16
August	12	83	2.25 ± 0.82	10	0.25 ± 0.13	3	0/12
September	18[a]	38	0.75 ± 0.41	6	0	0	0/18
Totals	110			266		33	0/97[b]

[a] Eight specimens were examined for ticks; all 18 were tested for antibodies.

[b] In April and May some mice died in traps and blood samples could not be taken, but the low ambient temperatures prevented tick detachment. One blood sample was lost in both June and July.

copus does not serve as a natural reservoir of *E. risticii*, and suggests that immature *D. variabilis* do not become infected with *E. risticii* by feeding on this species. This finding does not rule out infection of adult *D. variabilis* with *E. risticii*. Nonetheless, the seronegative status of *P. leucopus*, as well as *M. pennsylvanicus* (Fletcher, 1987), the two principal hosts of immature *D. variabilis* (Sonenshine et al., 1965), questions further whether adult *D. variabilis* is a vector of *E. risticii*. In a previous study, the feeding of large numbers of adult *D. variabilis*, many captured from the same farms as the present study, failed to expose horses to *E. risticii* (Schmidtmann et al., 1986).

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