

# Comparative Vector Competence of *Dermacentor variabilis* and *Ixodes scapularis* (Acari: Ixodidae) for the Agent of Human Granulocytic Ehrlichiosis

FRANKA DES VIGNES, MICHAEL L. LEVIN, AND DURLAND FISH

Department of Epidemiology and Public Health, Yale School of Medicine, 60 College Street, P.O. Box 208034, New Haven, CT 06520-8034

J. Med. Entomol. 36(2): 182-185 (1999)

**ABSTRACT** Vector competence of *Ixodes scapularis* Say and *Dermacentor variabilis* Say for the agent of human granulocytic ehrlichiosis (HGE) was compared. Five white-footed mice, *Peromyscus leucopus* Rafinesque, were inoculated intra-peritoneally with blood from a mouse infected with the agent of HGE. Approximately 100 *I. scapularis* and *D. variabilis* larvae were placed on each mouse and allowed to feed to repletion. Fed larvae were collected, separated according to species and allowed to molt to nymphs. Twenty-six per cent of *I. scapularis* (34/131) and 11% of *D. variabilis* (11/96) tested positive for the agent of HGE by polymerase chain reaction (PCR) after molting to nymphs. Positive *I. scapularis* nymphs transmitted the agent of HGE to *P. leucopus* mice in 5 of 5 trials. In contrast, the positive *D. variabilis* nymphs did not transmit the agent of HGE in any of 6 trials. In a 2nd experiment, 3 *P. leucopus* mice were infested with *I. scapularis* nymphs that were infected with the agent of HGE. All 3 mice became infected with the agent of HGE and  $\approx 300$  *D. variabilis* larvae were placed on each mouse and allowed to feed to repletion. Larvae were collected and allowed to molt to nymphs as before. Approximately 8% (5/60) of the nymphs became positive for the agent of HGE as determined by PCR. Twenty-five of these nymphs were then placed on each of 9 *P. leucopus* mice and allowed to feed to repletion. Evidence of transmission was not observed in any of 9 mice exposed to *D. variabilis* nymphs. These results demonstrate that although *I. scapularis* is a competent vector of the agent of the HGE, *D. variabilis* is not.

**KEY WORDS** *Dermacentor variabilis*, *Ixodes scapularis*, *Ehrlichia*, human granulocytic ehrlichiosis

HUMAN GRANULOCYTIC EHRLICHIOSIS (HGE) is a newly recognized tick-borne disease in the United States (Chen et al. 1994) and the blacklegged tick *Ixodes scapularis* Say has been reported to be a vector for the agent of HGE (des Vignes and Fish 1997). The contribution of other tick species to the maintenance of the agent of HGE in nature and in transmission of this agent to humans is unknown.

The white-footed mouse, *Peromyscus leucopus* Rafinesque, a natural reservoir host of the agent of HGE (Telford et al. 1996) is fed upon also by the American dog tick, *Dermacentor variabilis* Say. These human biting ticks may acquire infection from infected mice in their immature stages and transmit the agent of HGE through the bite of adults. Both *D. variabilis* and *I. scapularis* feed upon humans in areas where HGE is endemic (Falco and Fish 1988), and field evidence suggests that *D. variabilis* may be involved in the transmission of the agent of HGE in Connecticut (Magnarelli et al. 1995).

The ability of *D. variabilis* ticks to acquire, maintain and transmit efficiently the agent of HGE to reservoir mice and humans may enable this tick species to play an important role in the epidemiology of HGE in the United States. In this study, we compared the competence of *D. variabilis* with *I. scapularis* for the ability

to acquire and transmit the agent of HGE to a natural reservoir host, *P. leucopus*.

## Materials and Methods

*Ehrlichia*-free *P. leucopus* mice, 6-8 wk old, from our laboratory colony were each infected with an isolate of the agent of HGE by intraperitoneal (IP) inoculation of 0.1 ml of EDTA-anticoagulated blood from an infected severe combined immunodeficient (SCID) mouse. The NCH-1 strain used to inoculate mice originated from an infected human inhabiting Nantucket Island, MA (Telford et al. 1996).

To produce infected *I. scapularis* and *D. variabilis* nymphs, each of these mice was infested simultaneously with  $\approx 100$  *I. scapularis* and  $\approx 100$  *D. variabilis* larvae derived from uninfected laboratory colonies 7 and 14 d after inoculation with infected blood. Larvae were allowed to feed to repletion, collected, and allowed to molt to nymphs at 24°C and 95% RH in an environmental chamber. Nymphs were sorted according to species and kept separate according to the infected mouse they fed upon.

Twenty *I. scapularis* nymphs derived from larvae that fed on inoculated mice were placed on each of 5 naive *P. leucopus* mice 3-4 wk after molting. Thirteen

to 25 *D. variabilis* nymphs, derived from larvae fed on inoculated mice, were placed on a 2nd group of 7 naive mice 3–4 wk after molting. Fed nymphs that survived the molt to adults, 41 *I. scapularis* and 43 *D. variabilis* ticks, were tested by PCR to determine whether transstadial transmission occurred.

To determine if mice exposed to potentially infected nymphs acquired infection, blood was collected from the retro-orbital sinus of each naive mouse before infestation, and at 7 and 14 d after infestation. Blood and sera were stored for testing for the agent of HGE by PCR and indirect fluorescence antibody tests. Additionally, 100 *I. scapularis* larvae were placed on each mouse 7 d after infestation for xenodiagnosis, and a pool of 10 *I. scapularis* larvae that fed on each mouse was tested for the agent of HGE by PCR.

A 2nd experiment was performed to evaluate the ability of *D. variabilis* to acquire and transmit a Westchester County, NY, strain of the agent of HGE. This colony of infected nymphs was derived from uninfected larvae that fed upon laboratory-reared *P. leucopus* mice infected previously by field-caught nymphs from an endemic site in Westchester County, NY (Schwartz et al. 1997a). The prevalence of infection with the agent of HGE in these laboratory-maintained nymphs was estimated at 50% by PCR assay of a random sample of 50 ticks.

Three *P. leucopus* mice were infested each with 20 *I. scapularis* nymphs from the infected laboratory population. The nymphs were allowed to feed to repletion, and the 3 mice were bled 7 and 21 d after nymphal infestation to confirm infection. Approximately 300 uninfected *D. variabilis* larvae from our laboratory colony were placed on each mouse 7 d after nymphal infestation to obtain infected nymphs.

The *I. scapularis* nymphs used to infect these mice and the *D. variabilis* larvae fed on the mice were allowed to molt to adults and nymphs, respectively. Twenty-five of these *D. variabilis* nymphs were placed on each of 9 naive mice which were previously bled. *D. variabilis* nymphs were allowed to feed to repletion, and mice were bled again at 7, 14, and 21 d after infestation to confirm infection. Subsequently, 100 *I. scapularis* larvae were placed on each mouse for xenodiagnosis 7 d after *D. variabilis* nymphs fed to repletion. A pool of 20 replete *I. scapularis* larvae from each mouse was tested for infection with the agent of HGE.

Ticks collected from mice were assayed for the presence of the agent of HGE by PCR. The positive control used for PCR was the original isolate of the agent of HGE from the Westchester County canine (USG3) maintained in the promyelocytic cell line HL-60 (Aquila, Worcester, MA). The negative control used for PCR was distilled water. DNA was extracted from ticks using Isoquick, a commercial DNA/RNA extraction kit (Orca Research, Bothell, WA) to maximize sensitivity (Schwartz et al. 1997). Primers EHR 521 (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') and EHR 747 (5'-GCA CTC ATC GTT TAC AGC GTG-3') were used to amplify a 247-bp fragment of 16S ribosomal DNA as described previously by Pan-

**Table 1.** Transtadial passage of the NCH-1 strain of the HGE agent in *I. scapularis* and *D. variabilis* nymphs fed as larvae

Mouse no.	No. nymphs positive/No. nymphs tested					
	189	190	191	192	193	Total
Species						
<i>D. variabilis</i>	3/25	5/19	0/14	1/23	2/15	11/96
<i>I. scapularis</i>	11/28	4/24	3/15	6/29	10/35	34/131

choli et al. (1995). The amplification products were electrophoresed in 2% agarose gels stained with ethidium bromide and visualized with a UV transilluminator.

An indirect fluorescence antibody test developed by Aquila using antigens derived from the agent of HGE in culture obtained from Westchester County was performed on mouse sera. Sera were screened at a dilution of 1:40 in phosphate-buffered saline (pH 7.4) on spot slides of the *Ehrlichia* antigen.

**Results**

All 5 mice inoculated with blood infected with the NCH-1 strain became infected with the agent of HGE, as determined by indirect fluorescence antibody tests on mouse sera collected 21 d after inoculation. Also, both *I. scapularis* and *D. variabilis* nymphs fed as larvae on these mice were positive by PCR, 3–4 wk, after molting to nymphs. The percentage of positive ticks was 26% (34/131) for *I. scapularis* nymphs compared with 11% (11/96) for *D. variabilis* (Table 1).

All 5 mice fed upon by *I. scapularis* nymphs acquired infection with the NCH-1 strain, as determined by indirect fluorescence antibody tests of mouse sera collected 21 d after nymphal infestation. Furthermore, xenodiagnostic *I. scapularis* larvae from all 5 mice tested positive by the PCR.

*Ixodes scapularis* nymphs transmitted the agent of HGE in 5/5 trials to *P. leucopus* mice. At least 2–5 nymphs that fed on each mouse were positive as determined by the PCR assay after molting to adults (Table 2).

In contrast, none of 7 mice fed upon by *D. variabilis* nymphs positive for the NCH-1 strain became infected, as determined by indirect fluorescence antibody testing on mouse serum collected 21 d after nymphal infestation. Furthermore, xenodiagnostic ticks from each mouse all tested negative for the agent of HGE by PCR. Between 4 and 10 *D. variabilis* nymphs fed on each mouse; none of these nymphs

**Table 2.** Ability of *I. scapularis* nymphs to transmit the NCH-1 strain of the HGE agent to *P. leucopus*

Mouse no.	Replete nymphs/nymphs placed	Positive adults/adults tested	IFA	Xenodiagnosis
1	11/20	5/7	+	+
2	15/20	3/10	+	+
3	7/20	3/6	+	+
4	13/20	2/9	+	+
5	12/20	4/9	+	+

**Table 3.** Ability of *D. variabilis* nymphs to transmit the NCH-1 strain of the agent of HGE to *P. leucopus*

Mouse no.	Replete nymphs/ nymphs placed	Positive adults/ adults tested	IFA	Xenodiagnosis
6	5/25	0/5	-	-
7	10/25	0/9	-	-
8	8/16	0/8	-	-
9	7/16	0/6	-	-
10	4/10	0/3	-	-
11	7/17	0/7	-	-
12	5/13	0/5	-	-

tested positive for the agent of HGE after molting to adults (Table 3).

In the 2nd experiment, all 3 mice became infected with the Westchester County, NY, strain after being fed upon by infected *I. scapularis* nymphs using the same criterion as above. Approximately 8% (5/60) of *D. variabilis* nymphs fed as larvae on these 3 mice were positive with the agent of HGE (Table 4). All 9 mice fed upon by *D. variabilis* nymphs remained uninfected as determined by indirect fluorescence antibody tests on sera collected 21 d after nymphal infestation and by xenodiagnosis (Table 5).

**Discussion**

After feeding as larvae on mice inoculated with the NCH-1 strain, *I. scapularis* nymphs were positive for the agent of HGE 2-3 times more frequently than *D. variabilis* (Table 1). Therefore, *I. scapularis* is more efficient at acquiring and maintaining this infection than is *D. variabilis*. Subsequently, the NCH-1 strain was transmitted to *P. leucopus* by *I. scapularis* in 5 of 5 trials (Table 2). However, *D. variabilis* nymphs were unable to transmit this strain to *P. leucopus* mice in 7 trials (Table 3). Furthermore, *D. variabilis* nymphs positive for a wild Westchester County, NY, strain by PCR were unable to transmit it to mice in 9 additional trials (Table 5).

It is possible in some trials with *D. variabilis* that no infected *D. variabilis* nymphs fed on some mice, given the prevalence of infection in *D. variabilis* nymphs, was estimated at only 11% (11/96) for those fed on mice inoculated with the NCH-1 strain and 8% (5/60) for those fed on mice infected with the Westchester County, NY, strain. However, 46 nymphs were collected from 7 mice in the 1st experiment (Table 3) and 89 replete nymphs were collected from 9 mice in the

**Table 4.** Acquisition of the Westchester County, NY, strain of the agent of HGE by *D. variabilis* nymphs fed as larvae on *P. leucopus*

Mouse no.	<i>I. scapularis</i> nymphs collected/ nymphs placed	<i>I. scapularis</i> positive adults/ adults tested	IFA	Proportion of positive <i>D. variabilis</i> nymphs fed as larvae
333	7/20	3/6	+	1/20
335	14/20	5/11	+	2/20
336	13/20	5/11	+	2/20

**Table 5.** Ability of *D. variabilis* nymphs to transmit the Westchester County, NY, strain of the agent of HGE to *P. leucopus* mice

Mouse no.	Nymphs collected/ nymphs placed	IFA	Xenodiagnosis
13	12/25	-	-
14	14/25	-	-
15	16/25	-	-
16	10/25	-	-
17	6/25	-	-
18	4/25	-	-
19	11/25	-	-
20	10/25	-	-
21	6/25	-	-

2nd experiment (Table 5). Therefore, a total of 135 *D. variabilis* nymphs fed to repletion and were unable to transmit either strain of the agent of HGE to *P. leucopus* mice.

*Dermacentor variabilis* ticks have been found to be naturally infected with *Borrelia burgdorferi*, the agent of Lyme disease (Anderson et al. 1985). However *D. variabilis* is incompetent to transmit *B. burgdorferi* (Piesman and Sinsky 1988). *D. variabilis* may be found with evidence of infection with the agent of HGE (Magnarelli et al. 1995). However, field-caught ticks naturally infected with the agent of HGE may not be competent to transmit the pathogen. Nymphs that are PCR positive for the agent of HGE may be because of DNA of the HGE agent being retained in the blood residue after the larval to nymphal molt and is not evidence of an established infection in the tick. In addition, laboratory studies demonstrated that *D. variabilis* adults were unable to transmit a related granulocytic form of *Ehrlichia* to dogs (Anziani et al. 1990).

The results of this study suggest that *D. variabilis* lost infection during the nymph to adult molt, and that *D. variabilis* nymphs are not competent to maintain and transmit the agent of HGE to vertebrate hosts in nature. Consequently, *D. variabilis* appears not to contribute to the maintenance of the agent and does not serve as a secondary vector of the agent of HGE to humans.

**Acknowledgments**

We thank Michele Papero and David Zakur for their assistance. We also thank Richard Coughlin (Aquila Pharmaceuticals) for providing IFA slides and a culture of HGE for our assays. This research was sponsored by National Institutes of Health Grant No. AI8956 and a grant from the G. Harold and Leila Y. Mathers Charitable Foundation.

**References Cited**

Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde. 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). J. Clin. Microbiol. 22: 36-38.

Anziani, O. S., S. A. Ewing, and R. W. Barker. 1990. Experimental transmission of a granulocytic form of the tribe

- Ehrlichiae by *Dermacentor variabilis* and *Amblyomma americanum* to dogs. *Am. J. Vet. Res.* 51: 929–931.
- Chen, S.-M., J. S. Dumler, J. S. Bakken, and D. H. Walker. 1994. Identification of a granulocytic *Ehrlichia* species as the etiologic agent of human disease. *J. Clin. Microbiol.* 32: 589–595.
- des Vignes, F., and D. Fish. 1997. Transmission of the agent of human granulocytic ehrlichiosis by host-seeking *Ixodes scapularis* (Acari: Ixodidae) in southern New York State. *J. Med. Entomol.* 34: 379–382.
- Falco, R., and D. Fish. 1998. Ticks parasitizing humans in a Lyme disease endemic area of southern New York State. *Am. J. Epidemiol.* 128: 1146–1152.
- Magnarelli, L. A., K. C. Stafford III, T. N. Mathers, M. T. Yeh, K. D. Horn, and J. S. Dumler. 1995. Hemocytic rickettsia-like organisms in ticks serologic reactivity with antisera to Ehrlichiae and detection of DNA of the agent of human granulocytic ehrlichiosis by PCR. *J. Clin. Microbiol.* 33: 2710–2714.
- Pancholi, P., C. P. Kolbert, P. D. Mitchell, K. D. Reed, Jr., J. S. Dumler, J. S. Bakken, S. R. Telford III, and D. H. Persing. 1995. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. *J. Infect. Dis.* 172: 1007–1012.
- Piesman, J., and R. J. Sinsky. 1988. Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). *J. Med. Entomol.* 25: 336–339.
- Schwartz, I., S. Varde, R. B. Nadelman, G. P. Wormser, and D. Fish. 1997. Inhibition of efficient PCR amplification of *Borrelia burgdorferi* DNA in blood-fed ticks. *Am. J. Trop. Hyg.* 56: 339–342.
- Telford, S. R. III, J. E. Dawson, P. Katavolos, and C. K. Warner. 1996. Perpetuation of the agent of the human granulocytic ehrlichiosis in a deer tick–rodent cycle. *Proc. Natl. Acad. Sci. U.S.A.* 93: 6209–6214.

Received for publication 25 June 1998; accepted 21 October 1998.

---