Prevalence of Infection in Ticks Submitted to the Human Tick Test Kit Program of the U.S. Army Center for Health Promotion and Preventive Medicine

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Abstract

Ticks removed from humans are sent by Department of Defense medical personnel/clinics to the Tick-borne Disease Laboratory of the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) for identification and testing for pathogens by polymerase chain reaction (PCR). *Amblyomma americanum* are tested for *Ehrlichia chaffeensis* and *Borrelia burgdorferi*, *Ixodes scapularis* are tested for *B. burgdorferi* and the agent of human granulocytic ehrlichiosis (HGE), and *Dermacentor variabilis* are tested for *Rickettsia rickettsii*. In 1998, only ticks received alive were PCR'd; in 1999, testing was expanded to include dead *I. scapularis*. In 1998, 346 *A. americanum* were tested. Of these, 1% contained *B. burgdorferi* and 3% contained *E. chaffeensis*. Of the 96 *I. scapularis* tested in 1998, 22% were positive for *B. burgdorferi* and 4% were positive for the agent of HGE. Of the 367 *D. variabilis* tested, 16 (4%) were positive for SFG rickettsiae. RFLP analysis identified 15 as *R. montana* and one as WB-8-2. In 1999 (as of 15 Nov) 101/346 (29%) *I. scapularis* have been found positive for *B. burgdorferi* and 29/346 (8%) for the agent of HGE. *A. americanum* infection rates in 1999 are 1.5% (7/444) for *E. chaffeensis* and 0.2% for *B. burgdorferi*. Of 304 *D. variabilis* tested, 14 contain *R. montana*. Different tick-borne diseases have similar early symptoms, therefore species i.d. and infection status assist health care providers in making treatment decisions.

INTRODUCTION

Ticks removed from humans are sent by Department of Defense medical personnel/clinics to the Tick-borne Disease Laboratory of the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) for identification and analysis. Results of tick identification are reported by telephone by telephone within 1 day to the tickbite patient's healthcare provider and results of analysis are subsequently reported by telephone within 3-8 working days. Since different species of ticks transmit different diseases, and since most tick-borne diseases have very similar early symptoms, knowing the species and infection status of the tick enhances the physician's ability to accurately diagnose and treat the patient.

MATERIALS & METHODS

DNA Extraction & PCR Analysis

- DNA from individual ticks was extracted using the IsoQuick nucleic acid extraction kit (ORCA Research, Bothell, WA).
- The quality and quantity of the extracted DNA from each tick was assessed with tick mitochondrial DNA primers 16S+2 and 16S-1 (Black & Piesman 1994).
- A second PCR was performed to reconfirm each positive test. If possible, a primer set targeting a different gene was used.
- RFLP analysis was performed on samples PCR- positive for SFG rickettsiae.

Tick Species & Target Pathogen

Ixodes scapularis
- *Borrelia burgdorferi*
- Agent of Human Granulocytic Ehrlichiosis (HGE)

Amblyomma americanum
- *Borrelia burgdorferi*
- *Ehrlichia chaffeensis*

Dermacentor variabilis
- Spotted fever group rickettsia (SFGR), *Rickettsia rickettsii*

PCR Primers

Borrelia burgdorferi
- Chromosomal Osp gene (Rosa et al. 1991)
- Flagellin gene (Johnson et al. 1992)

Ehrlichia chaffeensis
- 16S rRNA gene (Anderson et al. 1992, R.F. Massung, unp.)
- VLPT (Variable-lenght PCR target) gene (Paddock et al. 1997, Sumner et al. 1999)
- 120 kDa protein gene (Yu et al. 1997)

Rickettsia rickettsii
- 120kDa antigen gene (Eremeeva et al. 1994)
- 190kDa antigen gene (Regnery et al. 1990)

Agent of HGE
- 16S rDNA gene (Chen et al. 1994, R.F. Massung et al. 1998)

In 1997 and 1998, only ticks received alive were PCR'd; in 1999, testing was expanded to include dead *I. scapularis*. There was no significant difference in *B. burgdorferi* and agent of HGE infection rates in 65 live and 208 dead *I. scapularis* (Table 1)

Table 1. Comparison of infection rates of live *I. scapularis* vs dead *I. scapularis* tested by PCR, 1999

	no. positive/no.tested (%)		Probability (P)[a]
	Live	Dead	
Nymphs PCR-positive for aoHGE	1/34 (3%)	4/94 (4%)	0.735
Adults PCR-positive for aoHGE	4/31 (13%)	15/114 (13%)	0.970
Nymphs PCR-positive for *B. burgdorferi.*	11/34 (32%)	16/94 (17%)	0.060
Adults PCR-positive for *B. burgdorferi*	11/31 (35%)	45/114 (39%)	0.686

a Percentages within a row are not significantly different when P > 0.05; Fisher's exact test for comparison of two proportions

RESULTS

A small percentage (Table 2) of our PCR's are performed on pooled ticks because we occasionally receive ticks from patients who have experienced multiple concurrent tickbites. In these cases, ticks of the same species from the same patient are pooled for testing. Each pool of ticks is counted as a single sample and infection rates of the pools are calculated using a minimun infection rate of one tick per pool (Tables 3-23).

Table 2. Tick pool size and proportion of tests that were performed on pooled ticks

	I. scapularis		*A. americanum*		*D. variabilis*	
	no. pools/no. tests (%)	average pool size	no. pools/no. tests (%)	average pool size	no. pools/no. tests (%)	average pool size
1998	0/96 (0%)	no pools	44/346 (13%)	2	13/367 (4%)	2
1999[a]	8/346 (2%)	2	54/444 (12%)	2	16/304 (5%)	2.5

[a] Tick numbers as of 15 Nov 1999

1998

Ixodes scapularis

Table 3. *I. scapularis* ticks that were PCR-positive using primers specific for a chromosomal target sequence of *B. burgdorferi* (Rosa et al. 1991) and primers specific for the 16S rDNA gene of the agent of HGE (Massung et al. 1998)

Sex/stage	No. positive/no. tested (%)		
	B. burgdorferi[a]	Agent of HGE[b]	Coinfected
Female	18/62 (29%)	2/62 (3%)	0/62 (0%)
Male	0/1 (0%)	1/1 (100%)	0/1 (0%)
Nymph	3/30 (10%)	2/30 (7%)	2/30 (7%)
Larva	0/3 (0%)	0/3 (0%)	0/3 (0%)
Total	21/96 (22%)	4/96 (4%)	2/96 (2%)

[a] Positive PCR reconfirmed using primers specific for the flagellin gene of *B. burgdorferi* (Johnson et al. 1992)
[b] Positive PCR reconfirmed by a repeat 16S rDNA PCR

Table 4. Geographic origin of *I. scapularis* PCR-positive for *B. burgdorferi*

County acquired	No. positive/no. tested			
	Female	Male	Nymph	Total
New London Co., CT	5/7	0	0/1	5/8
Harford Co., MD	4/22	0	0/13	4/35
Morrison Co., MN	1/4	0/1	2/2	3/7
Burlington Co., NJ	2/6	0	0/3	2/9
Fairfax Co., VA	2/2	0	0/2	2/4
Monmouth Co., NJ	1/2	0	0/1	1/3
Morris Co., NJ	1/3	0	0	1/3
Baltimore Co., MD	0	0	1/2	1/2
Sussex Co., NJ	1/1	0	0	1/1
Unknown	1/1	0	0	1/1

Table 5. Geographic origin of *I. scapularis* PCR-positive for the agent of HGE

County acquired	No. positive/no. tested			
	Female	Male	Nymph	Total
Morrison Co., MN	0/4	1/1	2/2	3/7
Burlington Co., NJ	1/6	0	0/3	1/9

Table 6. Geographic origin of *I. scapularis* PCR-positive for *B. burgdorferi* and the agent of HGE

County acquired	No. positive/no. tested			
	Female	Male	Nymph	Total
Morrison Co., MN	0/4	0/1	2/2	2/7

Table 7. *I. scapularis* ticks that were PCR-positive using primers specific for a chromosomal target sequence of *B. burgdorferi* (Rosa et al. 1991) and primers specific for the 16S rDNA gene of the agent of HGE (Massung et al. 1998)

Sex/stage	No. positive/no. tested (%)		
	B. burgdorferi[a]	Agent of HGE[b]	Coinfected
Female	69/195 (35%)	20/195 (10%)	10/195 (5%)
Male	3/11 (27%)	1/11 (9%)	0/11 (0%)
Nymph	27/134 (20%)	6/134 (5%)	3/134 (2%)
Larva	0/4 (0%)	0/4 (0%)	0/4 (0%)
Pools of mixed sex and/or stage	2/2 (100%)*	2/2 (100%)	*
Total	101/346 (29%)	29/346 (8%)	13/346 (4%)

[a] Positive PCR reconfirmed using primers specific for the flagellin gene of *B. burgdorferi* (Johnson et al. 1992)
[b] Positive PCR reconfirmed by a repeat 16S rDNA PCR
*Pools excluded (coinfection of individual ticks is impossible to determine if ticks are pooled)

Table 8. Geographic origin of *I. scapularis*.PCR-positive for *B. burgdorferi*

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Mixed pools	Total
Morrison Co., MN	33/69	2/5	11/31	2/2	48/107
Burlington Co., NJ	14/36	0	5/28	0	19/64
Harford Co., MD	6/24	1/1	1/17	0	8/42
New London Co., CT	4/16	0/2	5/15	0	9/33
Monmouth Co., NJ	4/10	0	0/8	0	4/18
Ann Arundel Co., MD	2/7	0	1/6	0	3/13
Fairfax Co., VA	2/4	0	0	0	2/4
Morris Co., NJ	0/1	0	1/3	0	1/4
Monroe Co., WI	1/2	0	0/2	0	1/4
Baltimore Co., MD	0	0	1/1	0	1/1
Howard Co., MD	0	0	1/1	0	1/1
(Unknown), MD	0	0	1/1	0	1/1
Cecil Co., MD	1/1	0	0	0	1/1
(Unknown), MA	1/1	0	0	0	1/1
York Co., PA	1/1	0	0	0	1/1

Table 9. Geographic origin of *I. scapularis* PCR-positive for the agent of HGE

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Mixed pools	Total
Morrison Co., MN	13/69	1/5	3/31	2/2	19/107
Burlington Co., NJ	2/36	0	1/28	0	3/64
Harford Co., MD	2/24	0/1	0/17	0	2/42
New London Co., CT	1/16	0/2	0/15	0	1/33
Monmouth Co., NJ	1/10	0	0/8	0	1/18
Monroe Co., WI	1/2	0	0/2	0	1/4
Morris Co., NJ	0/1	0	1/3	0	1/4
Bucks Co., PA	0	0	1/1	0	1/1
Lebanon Co. PA	1/1	0	0	0	1/1

Ixodes scapularis (cont'd)

Table 10. Geographic origin of *I. scapularis* PCR-positive for *B. burgdorferi* and for the agent of HGE

County acquired	No. positive/no. tested			
	Female	Male	Nymph	Total
Morrison Co., MN	6/69	0/5	2/31	8/107
Burlington Co., NJ	1/36	0	0/28	1/64
Harford Co., MD	1/24	0/1	0/17	1/42
New London Co., CT	1/16	0/2	0/15	1/33
Monmouth Co., NJ	1/10	0	0/8	1/18
Monroe Co., WI	1/2	0	0/2	1/4

Amblyomma americanum

Table 11. *A. americanum* ticks that were PCR-positive using primers specific for the VLPT gene of *E. chaffeensis* (Paddock et al. 1997, Sumner et al. 1999)

Sex/stage	No. positive/no. tested (%)	
	E. chaffeensis	*B. burgdorferi*
Female	2/70 (3%)	0/70 (0%)
Male	5/62 (8%)	0/62 (0%)
Nymph	3/196 (2%)	3/196 (2%)
Larva	0/2 (0%)	0/2 (0%)
Pools of mixed sex and/or stage	0/16 (0%)	0/16 (0%)
Total	10/346 (3%)	3/346 (0.8%)

Table 12. Geographic origin of *A. americanum* ticks PCR-positive for *E. chaffeensis*

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Mixed pools	Total
Caroline Co., VA	1/24	1/18	1/70	0/6	3/118
Burlington Co., NJ	0/9	2/11	1/40	0/2	3/62
Sebastion Co., AR	1/2	1/2	0/2	0/1	2/7
Carteret Co., NC	0	1/1	0	0	1/1
St. Clair Co., IL	0	0	1/1	0	1/1

Table 13. Geographic origin of *A. americanum* ticks PCR-positive for *B. burgdorferi*

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Mixed pools	Total
Caroline Co., VA	10/24	0/18	2/70	0/6	2/118
Newport News, VA	0/5	0/7	1/17	0/1	1/30

Table 14. Comparison of PCR-detected *E. chaffeensis* DNA sequences in *A. americanum*

Accession #	County acquired	Sex/Stage	VLPT PCR[a]	120 kDa PCR[b]
010798	Carteret Co., NC	M	4 repeats	3 repeats
019198	Burlington Co., NJ	N	4 repeats	3 repeats
026198	Burlington Co., NJ	M	4 repeats	4repeats
039798	Caroline Co., VA	M(2)	5 repeats	no test
049898	St. Clair Co, IL	N	5 repeats	4 repeats
053298	Caroline Co., VA	F	4 repeats	no test
060598	Burlington Co., NJ	M	4 repeats	4 repeats
082898	Caroline Co., VA	N(2)	4 repeats	3 repeats
093598	Sebastian Co., AR	F	5 repeats	4 repeats
100398	Sebastian Co., AR	M	5 repeats	4 repeats

[a] PCR using primers specific for the VLPT gene of *E. chaffeensis* which amplify a tandem repeat region that differs in number of repeats (Paddock et al. 1997, Sumner et al. 1999).

[b] PCR using primers specific for 120-kDa protein gene of *E. chaffeensis* which amplify a tandem repeat region that differs in number of repeats (Yu et al 1997).

1999

Amblyomma americanum

Table 15. *A. americanum* ticks that were PCR-positive using primers specific for the VLPT gene of *Ehrlichia chaffeensis* (Paddock et al. 1997, Sumner et al. 1999)

Sex/stage	No. positive /no. tested (%)	
	E. chaffeensis	*B. burgdorferi*
Female	3/76 (4%)	0/76 (0%)
Male	1/93 (1%)	0/93 (0%)
Nymph	1/242 (0.4%)	1/242 (0.4%)
Larva	0/5 (0%)	0/5 (0%)
Pools of mixed sex and/or stage	2/28 (7%)	0/28 (0%)
Total	7/444 (2%)	1/444 (0.2%)

Table 16. Geographic location *A. americanum* ticks PCR-positive for *E. chaffeensis*

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Mixed pools	Total
Caroline Co., VA	3/26	1/37	0/53	2/15	6/131
Burlington Co., NJ	0/9	0/11	1/40	0/2	1/62

Table 17. Geographic location *A. americanum* ticks PCR-positive for *B. burgdorferi*

County acquired	No. positive/no. tested				
	Female	Male	Nymph	Larva	Total
Harford Co., MD	0/6	0/11	1/30	0/2	1/49

Table 18. Comparison of PCR-detected *E. chaffeensis* DNA sequences in *A. americanum*

Accession #	County acquired	Sex/Stage	VLPT PCR[a]	120 kDa PCR[b]
009599	Caroline Co., VA	M(4),F(1)	4 repeats	4 repeats
012799	Caroline Co., VA	F	4 repeats	4 repeats
022499	Caroline Co., VA	F	5 repeats	3 repeats
035599	Caroline Co., VA	N(3),M(1)	4 repeats	3 repeats
095399	Caroline Co., VA	M	4 repeats	4 repeats
151699	Caroline Co., VA	F	4 repeats	4 repeats
155799	Burlington Co., NJ	N	5 repeats	4 repeats

[a] PCR using primers specific for the VLPT gene of *E. chaffeensis* which amplify a tandem repeat region that differs in number of repeats (Paddock et al. 1997, Sumner et al. 1999).

[b] PCR using primers specific for 120-kDa protein gene of *E. chaffeensis* which amplify a tandem repeat region that differs in number of repeats (Yu et al 1997).

Dermacentor variabilis

Table 19. *D. variabilis* ticks that were PCR-positive using primers specific for the 120 kDa antigen gene of *R. rickettsii* (Eremeeva et al. 1994) and identified by RFLP with endonuclease *Rsa* I as non-pathogenic SFG rickettsiae

Sex/stage	No. positive/no. tested (%)[a]
Female	11/207 (5%)
Male	5/160 (3%)
Pools of mixed sex and/or stage	0/5 (0%)
Total	16/367 (4%)

[a] PCR results were reconfirmed by testing with primers specific for the 190 kDa antigen gene (Regnery et al. 1990) and RFLP with endonuclease *Pst* I

Table 20. Geographic origin of *D. variabilis* (n=367) that were PCR-positive using primers specific for the 120kDa antigen gene of *R. rickettsii* (Eremeeva et al. 1994)

Accession #	County acquired	Sex	Engorgement	RFLP[a]
011798	Monmouth Co.,NJ	F	Flat	WB-8-2
015098	Harford Co., MD	F	Part	*R. montana*
023598	Morris Co., NJ	F	Part	*R. montana*
037698	Fairfax Co., VA	F	Part	*R. montana*
046898	Monmouth Co.,NJ	F	Flat	*R. montana*
050098	Harford Co., MD	F	Flat	*R. montana*
072498	Morrison Co., MN	F	Part	*R. montana*
072598	Morrison Co., MN	M	Flat	*R. montana*
072698	Morrison Co., MN	M	Flat	*R. montana*
088198	Burlington Co., NJ	M	Flat	*R. montana*
100398	Sebastian Co., AR	F(2)	Flat	*R. montana*
110098	Morrison Co., MN	F	Flat	*R. montana*
110998	Morrison Co., MN	F	Flat	*R. montana*
113198	Morrison Co., MN	F	Flat	*R. montana*
131398	Morrison Co., MN	M	Flat	*R. montana*
131498	Morrison Co., MN	M	Flat	*R. montana*

[a] RFLP performed with restriction enzyme *Rsa* I.
WB-8-2, an undescribed species of non-pathogenic SFG rickettsiae.

Dermacentor variabilis

Table 21. *D. variabilis* ticks that were PCR-positive using primers specific for the 190kDa antigen gene of *R. rickettsii* (Regnery et al. 1990) and identified by RFLP with endonuclease *Pst* I as *R. montana*.

Sex/stage	No. positive/no. tested (%)[a]
Female	9/156 (6%)
Male	5/140 (3%)
Mixed pools	0/8 (0%)
Total	14/304 (5%)

[a] PCR results were reconfirmed by testing with primers specific for the 120kDa antigen gene (Eremeeva et al. 1994)and RFLP with endonuclease *Rsa* I

Table 22. Geographic origin of *D. variabilis* ticks (n=304) that were PCR-positive for SFG rickettsia using primers specific for the 190kDa antigen gene of *R. rickettsii* (Regnery et al. 1990)

Accession #	County acquired	Sex	Engorgement	RFLP[a]
019599	Monmouth Co., NJ	F	Flat	*R. montana*
061699	Morrison Co., MN	F	Flat	*R. montana*
061899	Morrison Co., MN	F	Flat	*R. montana*
065199	Morrison Co., MN	M	Flat	*R. montana*
065499	Morrison Co., MN	F	Flat	*R. montana*
072599	Morrison Co., MN	F	Flat	*R. montana*
073299	Morrison Co., MN	M	Flat	*R. montana*
074599	Monroe Co., WI	M	Flat	*R. montana*
115899	Annville Co., PA	F	Part	*R. montana*
144999	Meade Co., TN	F	Flat	*R. montana*
147999	Meade Co., TN	M	Flat	*R. montana*
156799	Harford Co., MD	F	Flat	*R. montana*
175899	Norfolk Co., VA	M	Flat	*R. montana*
179999	Burlington Co., NJ	F	Flat	*R. montana*

[a] RFLP performed with restriction enzyme *Pst* I.

Summary of Results, 1998 &1999

Table 23. Comparison of infection rates in ticks, 1998 and 1999 (through 15 Nov 1999)

Tick & target	No. positive/no. tested (%)	
	1998	1999
A.a & *B.b.*	3/346 (1%)	1/444 (0.2%)
A.a. & *E.c.*	10/346 (3%)	7/444 (1.5%)
I.s. & *B.b.*	21/96 (22%)	*101/346 (29%)
I.s. & aoHGE	4/96 (4.5%)	29/346 (8%)
D.v. & SFGR	16/367 (4%)	13/304 (4%)

DISCUSSION

B. Burgdorferi Infection in *A. Americanum*

The disease transmission potential of a tick species is a primary consideration in determining the potential impact of tickbite on a patient. *I. scapularis* is considered to be the major vector of *B. burgdorferi* as *A. americanum* is of *E. chaffeensis* and *D. variabilis* is of *R. rickettsii* (a SFG rickettsia). The potential of *A. americanum* to transmit *B. burgdorferi*, or a related borrelia, is the subject of current study and controversy. The Tick-borne Disease Laboratory has found *B. burgdorferi* in *A. americanum* since the inception of the Tick Test Program in 1989. Hopefully, the status of *A. americanum* as vector of *B. burgdorferi* will soon be defined.

Lack of *R. rickettsii* infection in *D. variabilis*

D. variabilis is the major vector of *R. rickettsii*, but our analysis identified only non-pathogenic species of rickettsia, *R. montana* and WB-8-2. A low prevalence of *R. rickettsii* in *D. variabilis*, even in areas endemic for Rocky Mountain spotted fever, has been previously reported, but is not yet understood. In the case of another anthropophilic North American tick, *Dermacentor andersoni*, infection with a non-pathogenic SFG rickettsia, *Rickettsia peacockii*, blocked the ability of ticks to transmit *R. rickettsii* transovarially. This mechanism of transovarial interference may be occuring in other Dermacentor species (e.g., *D. variabilis*), mediated by other non-pathogenic SFG rickettsia (e.g., *R. montana*; WB-8-2).

Outcome of Tick Test Kit Program

Benefits to doctor & patient

- Different tick-borne diseases have similar early symptoms, therefore species i.d. and infection status assist health care providers in making treatment decisions.

Benefits to the Preventive Medicine (PM) team

- Installation-specific data can be combined with environmental survey data to assess the threat of tick-borne disease.

Indirect benefits to doctor, patient and PM team

- Telephone communication provides season-long dialog on tick biology, tick-borne diseases, repellents and tick-bite prevention.

Benefits to Tick-Borne Disease Laboratory

- The large data sets amassed each year are used for investigating trends in known and emerging tick-borne diseases. Tick DNA is archived and used for practicing new techniques of analysis. These efforts improve the Lab's services and in turn benefit the doctor, patient and PM team.

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