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

In 1997, >1000 ticks were received in the Human Tick Test Kit Program. Those received alive were tested for pathogens by polymerase chain reaction (PCR). A total of 222 *Amblyomma americanum* were tested for *Ehrlichia chaffeensis* and *Borrelia burgdorferi*. Results of the initial nested PCRs indicate that 33 (15%) of these contained *E. chaffeensis* and 26 (12%) contained *B. burgdorferi*. Six were co-infected with both organisms. A total of 13/308 (4%) *Dermacentor variabilis* were PCR-positive for spotted fever group (SFG) rickettsiae. Restriction fragment length polymorphism (RFLP) analysis identified all as *Rickettsia montana*. One hundred twenty seven *D. variabilis* from Monroe County, WI, were tested for *B. burgdorferi* and 14 (11%) were positive. Twenty four *Ixodes scapularis* ticks were received alive and tested by PCR. Of these, five (21%) contained *B. burgdorferi* and one contained the agent of human granulocytic ehrlichiosis.

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 and analysis are then reported to the tickbite patient's health care provider. 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 greatly enhances the physician's ability to accurately diagnose and treat the patient.

DNA extraction & PCR analysis:

1. DNA from individual ticks was extracted using the IsoQuick nucleic acid extraction kit (ORCA Research, Bothell, WA).
2. 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).
3. A second PCR was performed to reconfirm each positive test. If possible, a different primer set targeting a different gene was used.
4. RFLP analysis was performed on samples PCR-positive for SFG rickettsiae.

PCR primers:

The following primers were used in PCR analysis:

1. *B. burgdorferi:* Chromosomal Osp gene (Rosa et al. 1991): flagellin gene (Johnson et al. 1992)
2. *E. chaffeensis:* 16S rRNA gene (Anderson et al. 1992, R.F. Massung, unp.); 120 kDa gene (Yu et al. 1997)
3. *R. rickettsii:* 120kDa antigen gene (Eremeeva et al. 1994) & 190kDa antigen gene (Regnery et al. 1990)
4. Agent of human granulocytic ehrlichiosis: 16S rDNA gene (Chen et al. 1994, R.F. Massung, unp.)

Results: See Tables 1 - 4.

Discussion: The disease transmission potential of a tick species is a primary consideration in determining the potential impact of tickbite on a patient. *D. variabilis* is the major vector of *R. rickettsii*, but our analysis identified only a non-pathogenic species of rickettsia, *R. montana*. 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. *A. americanum* is considered to be the major vector for *E. chaffeensis*, but the potential of *A. americanum* to transmit *B. burgdorferi* is the subject of current study and controversy. We have found *B. burgdorferi* in *A. americanum* since the inception of the Tick Test Program in 1989. Hopefully, the status of *A. americanum* as a vector of *B. burgdorferi* will soon be defined. The discovery of *B. burgdorferi* in unengorged adult *D. variabilis* was unexpected and warrants further investigation.

Further molecular characterization:

1. Samples of DNA from *A. americanum* positive for *E. chaffeensis* will be selected for

sequence analysis and comparison with published ehrlichia sequences.
2. Samples of DNA from *A. americanum* positive for *B. burgdorferi* will be selected for sequence analysis and comparison with published borrelia sequences.
3. Samples of DNA from *D. variabilis* positive for *R. montana* will be selected for sequence analysis and comparison with published rickettsia sequences.
4. Samples of DNA from *D. variabilis* positive for *B. burgdorferi* will be selected for sequence analysis and comparison with published borrelia sequences.
5. DNA from *D. variabilis* from locations other than Monroe County, WI will be analyzed by PCR for *B. burgdorferi.*

References:

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Table 1. ***Amblyomma americanum*** **ticks (n = 222) that were PCR-positive using primers specific for the 16S rRNA gene of** ***Ehrlichia chaffeensis*** **(Anderson et al. 1992, R.F. Massung, unp.)**

	CHPPM #	County acquired	Sex/Stage	Engorgement	2nd PCR[b]	3rd PCR[c]	Coinfection
1	none	Hoke Co., NC	no data	no data	+	-	
2	003097	Okalossa Co., FL	F	Flat	-	-	
3	003197	Monmouth Co., NJ	F	Full	+	-	
4	003897	Calhoun Co., AL	F	Part	+	-	*B. b.*
5	005897	Fairfax Co., VA	M	Flat	+	-	*B. b.*
6	006597	Jefferson Co., AK	F	Part	+	-	
7	007297	Ocean Co., NJ	F	Flat	+	-	*B. b.*
8	007497	Ocean Co., NJ	F(3), N[a]	Flat	+	-	*B. b.*
9	010497	Harford Co., MD	F	Part	+	-	
10	010597	Harford Co., MD	N	Part	-	-	
11	014097	Burlington Co., NJ	F	Flat	+	-	
12	014197	Burlington Co., NJ	F	Part	-	-	
13	014397	Burlington Co., NJ	N	Full	-	-	
14	024797	Burlington Co., NJ	F, N[a]	Flat	+	-	
15	028097	Caroline Co., VA	M	Flat	+	-	
16	032297	Burlington Co., NJ	F	Flat	+	+A	*B. b.*
17	032497	Burlington Co., NJ	M	Flat	-	-	
18	033097	Harford Co., MD	N	Full	+	+S	
19	035297	Caroline Co., VA	M	Flat	+	+S	
20	036497	Caroline Co., VA	F	Flat	+	+A	
21	056897	Caroline Co., VA	F	Flat	+	-	
22	071197	Burlington Co., NJ	M	Flat	+	+A	
23	073997	Riley Co., KS	M	Flat	-	-	
24	081297	Nemaha Co., KS	N	Flat	+	-	
25	103297	Burlington Co., NJ	N (2)[a]	Flat	-	-	
26	105397	Burlington Co., NJ	N	Part	+	-	
27	106797	Burlington Co., NJ	N	Part	+	-	
28	107897	Burlington Co., NJ	N	Full	-	-	
29	109097	Burlington Co., NJ	N	Flat	-	-	
30	109297	Burlington Co., NJ	N	Full	+	-	
31	110497	Fairfax Co., VA	L (2)[a]	Flat	+	-	
32	111297	Harford Co., MD	L	Part	+	-	*B. b.*
33	111997	Lee Co., NC	N	Flat	+	-	

[a] Ticks were pooled for PCR
[b] 2nd PCR using primers specific for 16S rRNA gene of *E. chaffeensis* (Anderson et al. 1992, R.F. Massung, unp.)
[c] 3rd PCR using primers specific for 120-kD gene of *E. chaffeensis* (Yu et al 1997), which amplify a tandem repeat region that differs in number of repeats between the Arkansas and Sapulpa strain of *E. Chaffeensis.*
A, Arkansas strain; S, Sapulpa strain

Table 2. *Amblyomma americanum* ticks (n = 222) that were PCR-positive using primers specific for a chromosomal target sequence of *Borrelia burgdorferi* (Rosa et al. 1991)

	CHPPM #	County acquired	Sex/Stage	Engorgement	2nd PCR[b]	3rd PCR[c]	Coinfection
1	003897	Calhoun Co., AL	F	Part	+	-	*E. c.*
2	005897	Fairfax Co., VA	M	Flat	+	-	*E. c.*
3	006097	Calhoun Co., AL	N	Part	+	-	
4	006997	Fairfax Co., VA	F	Flat	+	-	
5	007297	Ocean Co., NJ	F	Flat	+	-	*E. c.*
6	007497	Calhoun Co., AL	F(3), N[a]	Flat	+	-	*E. c.*
7	009297	Fairfax Co., VA	F	Part	no data	-	
8	010197	Halifax Co., NC	N	Full	no data	-	
9	010797	VA	N	Full	+	-	
10	011197	Harford Co., MD	M	Flat	+	-	
11	015497	Burlington Co., NJ	N	Part	-	-	
12	018597	Caroline Co., VA	N	Full	-	-	
13	019497	Harford Co., MD	F	Part	+	-	
14	032297	Burlington Co., NJ	F	Flat	+	-	*E. c.*
15	039997	Dinwiddie Co., VA	F	Flat	+	+	
16	041597	Burlington Co., NJ	F, M, N[a]	Flat	+	-	
17	070597	Burlington Co., NJ	F	Part	+	+	
18	073197	Burlington Co., NJ	F	Flat	+	+	
19	073297	Durham Co., NC	N	Flat	+	+	
20	081597	Montgomery Co., TN	N	Full	+	+	
21	088797	Pender Co., NC	M	Flat	+	+	
22	089497	Monmouth Co., NJ	N	Flat	+	+	
23	096697	Burlington Co., NJ	N	Full	+	-	
24	102197	Somerset Co., MD	N (5)[a]	Full	+	-	
25	111197	Monmouth Co., NJ	N	Full	+	-	
26	111297	Harford Co., MD	L	Part	+	-	*E. c.*

[a] Ticks were pooled for PCR.
[b] 2nd PCR using primers specific for a chromosomal target sequence of *B. burgdorferi* (Rosa et al. 1991).
[c] 3rd PCR using primers specific for the flagellin gene of *B. burgdorferi* (Johnson et al 1992).

Table 3. *Dermacentor variabilis* (n = 308) that were PCR-positive using primers specific for the 120kDa antigen gene of *Rickettsia rickettsii* (Eremeeva et al. 1994)

	CHPPM #	County acquired	Sex	Engorgement	2nd PCR[b]	RFLP[c]
1	003697	NJ	M	Flat	+	*R. montana*
2	006297	Comanche Co., OK	M	Flat	+	*R. montana*
3	007797	Ocean Co., NJ	F	Flat	+	*R. montana*
4	007897	Monmouth Co., NJ	M	Flat	+	*R. montana*
5	017097	Fairfax Co., VA	F	Flat	+	*R. montana*
6	021997	Morrison Co., MN	F(3)[a]	Flat	+	*R. montana*
7	051897	Morrison Co., MN	M	Flat	+	*R. montana*
8	072597	Morrison Co., MN	F	Flat	+	*R. montana*
9	074697	Morrison Co., MN	F	Flat	+	*R. montana*
10	090397	Monroe Co., WI	F	Flat	+	*R. montana*
11	093697	Burlington Co., NJ	F	Flat	+	*R. montana*
12	100697	Monroe Co., WI	F	Flat	+	*R. montana*
13	104397	Morrison Co., MN	F	Flat	+	*R. montana*

[a] Ticks were pooled for PCR.
[b] 2nd PCR using primers specific for the 190kDa antigen gene of *R. rickettsii* (Regnery et al. 1990).
[c] RFLP performed with restriction enzymes *Pst* I and *Rsa* I.

Table 4. ***Dermacentor variabilis*** **(n = 127) that were PCR-positive using primers specific for a chromosomal target sequence of** ***Borrelia burgdorferi*** **(Rosa et al. 1991)**

	CHPPM #	County acquired	Sex	Engorgement	2nd PCR[b]	3rd PCR[c]	4th PCR[d]
1	038697	Monroe Co., WI	M	Flat	+	+	
2	038997	Monroe Co., WI	M	Flat	-	-	
3	044197	Monroe Co., WI	M	Flat	-	-	
4	044597	Monroe Co., WI	M	Flat	+	no test	
5	066097	Monroe Co., WI	F	Flat	+	+	
6	075997	Monroe Co., WI	F	Flat	+	+	
7	092897	Monroe Co., WI	M,F[a]	Flat (M), Full (F)	+	+	
8	094697	Monroe Co., WI	F	Part	+	+	+
9	096297	Monroe Co., WI	F	Flat	+	+	
10	096597	Monroe Co., WI	F	Flat	-	-	
11	097397	Monroe Co., WI	F	Flat	+	+	+
12	097697	Monroe Co., WI	M	Flat	+	-	
13	101597	Monroe Co., WI	F	Flat	+	-	
14	101897	Monroe Co., WI	F	Flat	+	-	

[a] Ticks were pooled for PCR.
[b] 2nd PCR using primers specific for a chromosomal target sequence of *B. burgdorferi* (Rosa et al. 1991).
[c] 3rd PCR using primers specific the flagellin gene of *B. Burgdorferi* (Johnson et al. 1992).
[d] 4th PCR using primers 149/319 specific for the Osp A gene of *B. Burgdorferi* (gift of David Persing, Mayo Clinic). The two positives (946, 973) were visualized by agarose gel electrophoresis; however, these primers typically require hybridization/Southern blot for detection of product. Further analysis of these samples by hybridization/Southern blot will be performed.