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Frequency of rickettsia sps. in dermacentor variabilis and amblyomma americanum in central Hanover County, Virginia

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FREQUENCY OF RICKETTSIA SPS. IN
DERMACENTOR VARIABILIS AND AMBLIOMMA AMERICANUM
IN CENTRAL HANOVER COUNTY, VIRGINIA

By

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B.S., UNIVERSITY OF UTAH, 1982

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INTRODUCTION

In the eastern United States, Dermacentor variabilis is the primary vector of Rickettsia rickettsii, the etiological agent for Rocky Mountain Spotted Fever. Cases of Rocky Mountain Spotted Fever in eastern United States increased steadily from 1959 to 1979 to a peak of one case per 200,000 people in 1979 (Morbidity and Mortality Weekly Report, 1985). In 1976, 98.9% of the reported cases of Rocky Mountain Spotted Fever were east of the 100th meridian with 1.1% occurring west of the 100th meridian (Burgdorfer, 1977). Hanover County, Virginia is an endemic area for this disease (Sonenshine, 1971); the last reported case was in 1984 (Personal communication, Dept. of Epidemiology, Va., 1989). The present study was to determine if rickettsial infection was present in the tick population at three sites in Hanover County, Virginia.

MATERIALS AND METHODS

Three study sites were chosen in Hanover County, Virginia, an area endemic for Rocky Mountain Spotted Fever. In 1988, from April to September, in a total of 38 collection days, sites were flagged with a 142 cm x 142 cm white flannel cloth attached to a yardstick (Benach et al., 1977). The cloth was dragged over the vegetation and ticks that adhered were removed with tweezers and placed in a small vial. Ticks were kept alive on moist cotton until a hemolymph test was performed. Sites usually

were flagged in the late morning or early afternoon to avoid morning dew. Ticks also were obtained by removing them from humans and dogs. Ticks were identified following Sonenshine (1979).

The establishment of rickettsial infection was determined by the hemolymph test (Burgdorfer, 1970). The distal portion of the third leg of the tick was amputated with a scalpel and a drop of hemolymph placed on a slide. The slide was air dried, fixed with heat, stained with the Gimenez method and examined under oil immersion (1,600 x). Rickettsiae are primarily located within the cytoplasm of hemocytes, which stains green whereas the rickettsiae appear as pink or red spheres (Burgdorfer, 1970). The species of rickettsiae cannot be determined with this method.

SITE DESCRIPTIONS

Site 1 was a 119 m stretch of access road to a sewer pump station off of Atlee Station Road, 5 km southeast of Ashland, Virginia and 3.06 km from the intersection of Route 301 and Atlee Station Road. (Figure 1). A 1 m wide area of brush and grass on both sides of the road was flagged (Benach et al., 1977). Site 1 A was an additional 99 m of road added to the area in site 1. Ground vegetation consisted of grasses (Family: Gramineae) growing to an approximate height of 1 m. Trees, mainly white oak (Quercus alba) and pine (Pinus sp.) with a scattering of

beech (Fagus grandifolia), hickory (Carya sp.) and holly (Ilex opaca), provided a canopy over the road. During April and May, large sections of the low lying ground adjacent to the road were covered with water.

Site 2 was a 320 m stretch of a private gravel road in a five year old housing development off of Route 301, 11 km southeast of Ashland, Virginia and 0.8 km west of the end of Route 752 (Figure 1). A 0.5 m wide area on the north side of the road was flagged. Ground vegetation consisted of grasses and huckleberry (Gaylussacia sp.) bushes, reaching a height of 0.3 to 0.5 m. Trees along the north side of the road were predominantly white oak and pine with some holly and dogwood (Cornus florida).

Site 3 was a 63 m stretch along an abandoned, overgrown road on a private farm off of Atlee Station Road, 4.5 km southeast of Ashland, Virginia and 4.7 km from the intersection of Route 301 and Atlee Station Road (Figure 1). A 0.5 m wide area on both sides of the road was flagged. Vegetation consisted of honeysuckle vines (Lonicera sp.) and periwinkle (Vinca sp.), approximately 0.3 m tall with white oak and pine trees overhanging the road.

RESULTS

A total of 388 ticks was collected by flagging at the three sites from April to September (Table 1). The predominant

species of tick at all three sites was Dermacentor variabilis (94%, 85%, and 75%). Site 3 had the highest incidence of Amblyomma americanum (25%) and site 2 was the only location where an Ixodes sp. tick occurred.

Hemolymph tests for the presence of rickettsiae (Burgdorfer, 1970) showed that site 2 was the only area where the organism was found. The incidence rate was 2.68%, where three female D. variabilis and one female A. americanum were infected (Table 2). The species of rickettsiae was not determined.

Of the 388 ticks collected, 16 had bacillary organisms present intracellularly and extracellularly in their tissues. The bacteria stained red with the hemolymph test. Two of the 16 ticks were collected at site 2; 14 were at site 1. Dermacentor variabilis composed 7.7% of the infected ticks and A. americanum 1.3% (Table 3).

DISCUSSION

The distribution of ticks in this study is consistent with studies by Sonenshine (1971) in Virginia. Of the 3323 ticks collected from animal hosts in his work 97.8% were D. variabilis and 2.2% were A. americanum which is similar to the results in the present study except at site 3 where 75% were D. variabilis and 25% A. americanum. The high percentage of A. americanum at site 3 is not significant ($P=0.5$) and may be

the result of a small data pool of only 15 ticks, five of which were A. americanum and ten were D. variabilis (See Appendix I). Amblyomma americanum is known to secrete aggregation pheromones (Harwood and James, 1979), and it is possible this influenced the clustering of these ticks in the same spot on the same day.

The 2.68% incidence of rickettsiae in this study is similar to results reported in other endemic areas. For example, Burgdorfer (1974) found a 3.5% incidence of rickettsiae in D. variabilis in a study in the Tennessee Valley Region; in South Carolina, Burgdorfer et al. (1975) reported a 4% incidence of rickettsiae in D. variabilis and Magnarelli (1979) found a 2.9% incidence of rickettsiae in D. variabilis in Connecticut.

Small mammals, e. g. meadow mice and rabbits, are primary hosts for the larval and nymphal stages of D. variabilis and A. americanum (McDade and Newhouse, 1986). After molting, the adult tick attaches to larger animals such as dogs, raccoons, foxes, and deer (McDade and Newhouse, 1986). Since rickettsiae, were found only at site 2, a possible explanation might relate to habitat disturbance. In 1980, a housing development was started at this site. In undisturbed areas, like site 1 and site 3, fewer small mammals should be present and a lesser chance for immature ticks to feed on rickettsemic hosts would be expected. In a disturbed area, i.e. site 2, more small mammals were expected because of an increase in suitable habitat (Pianka, 1979), thus

raising the chance of an immature tick feeding on a rickettsemic host and acquiring the rickettsiae.

All four ticks harboring the rickettsiae were females. The apparent sex bias in harboring rickettsiae reflects a small data pool as both male and female D. variabilis are equally susceptible to the rickettsial infection (Ricketts, 1907). Rickettsia rickettsii has never been recovered from a naturally infected A. americanum but rickettsiae related to the spotted fever group have been identified in this species (Burgdorfer et al., 1974). These rickettsiae react with anti-R. rickettsii conjugates in a fluorescent stain but do not produce a detectable infection (high fever or scrotal swellings) in laboratory animals (Burgdorfer et al., 1974). These rickettsiae may not be pathogenic to man, as a one year old boy in South Carolina, who was bitten by an A. americanum infected with this species, did not become ill (Burgdorfer et al., 1975).

At site 1, bacillary organisms were present at 7.7%, a frequency comparable to the 8.9% observed by Burgdorfer et al. (1974) in the Tennessee Valley Region. In South Carolina, bacillary organisms were discovered in 25 D. variabilis (Burgdorfer and Brinton, 1975). These organisms produced an infection throughout the tick and were passed transovarially by females to their progeny. They were not pathogenic to laboratory animals nor to humans (Burgdorfer et al., 1975).

These bacillary organisms might be an inhibiting factor for rickettsial distribution at site 1. It has been shown that once a tick is infected with a species of rickettsia, it will

not retain or accept a second species (Burgdorfer et al., 1981). Burgdorfer et al. (1981) discovered that the "East side agent", a rickettsia found only on the eastern side of the Bitter Root Valley, an area free of Rocky Mountain Spotted Fever, was retained by D. variabilis when challenged with R. rickettsii. The competition between R. rickettsii and the "East side agent" for tick hosts is thought to be responsible for relegating R. rickettsii to the western side of the Bitter Root Valley, Montana.

At site 1, where no rickettsiae were found, 7.7% of all ticks collected harbored the bacillary organism. At site 2, where there was a 2.68% incidence of rickettsiae, only 1.3% of the ticks collected had the bacillary organism. Possibly the bacillary organism was competing with the rickettsiae for tick hosts at site 1, limiting the distribution of rickettsiae. Only further study similar to that conducted with the "East side agent" can show if the bacillary organism is a factor in rickettsial distribution at these sites.

CONCLUSION

The incidence of Rocky Mountain Spotted Fever in Hanover County is similar to other endemic areas. Of the 388 ticks collected, four ticks has a rickettsia present in their tissues. There was a 2.68% infection rate at site 2 which could be the result of an increased small mammal population, a result of a disturbed habitat.

The bacillary organism found in 7.7% of D. variabilis collected at site 1 might be competing with rickettsiae for hosts and may be a limiting factor in the distribution of rickettsiae to certain areas of Hanover County, Virginia.

Figure 1. a) Map of Hanover County, Virginia; The three test sites are located within the red square. b) Enlargement for area in red square (a). Three test sites are designated by red numbers.

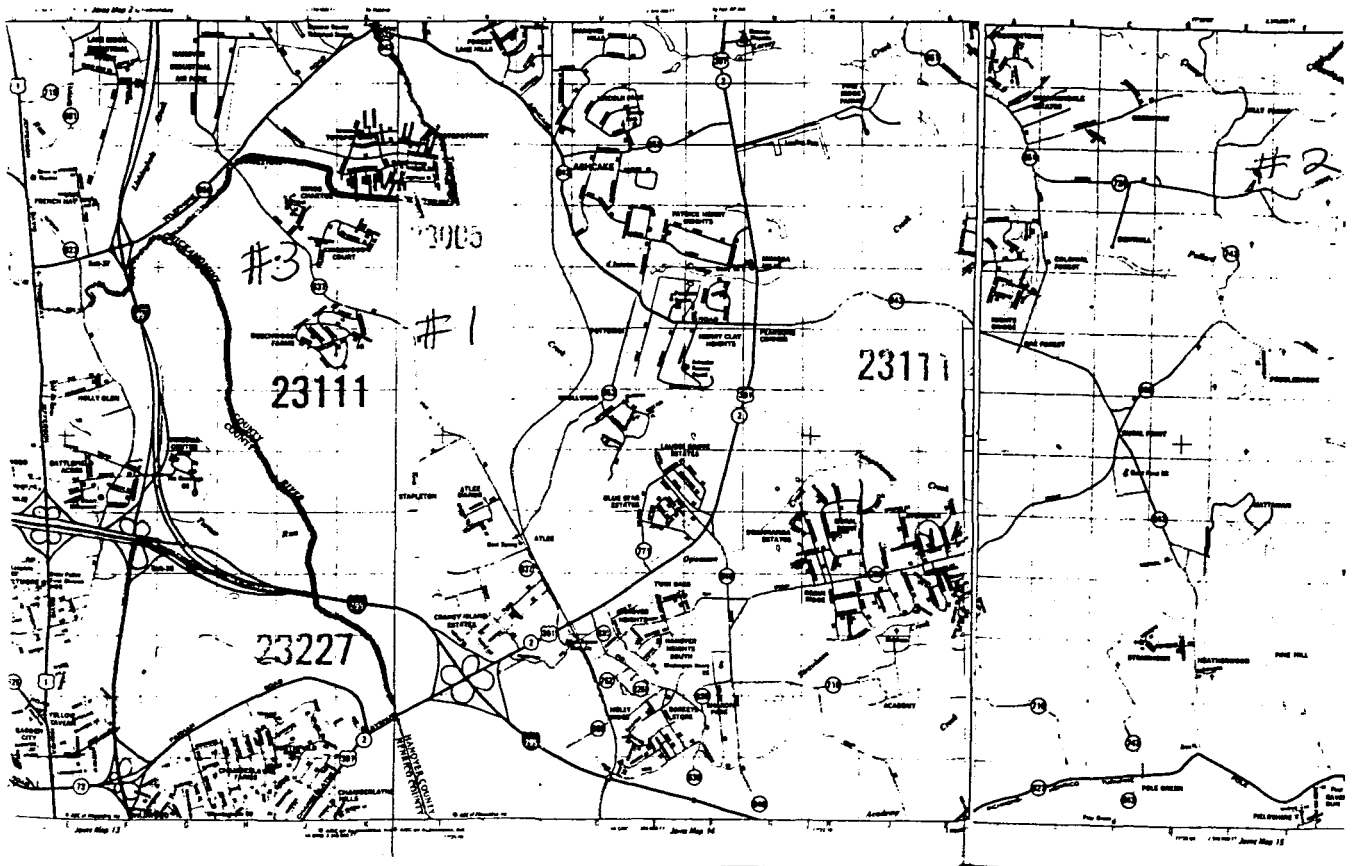


Table 1. Distribution of tick species collected by flagging at test sites in Hanover County, Virginia from April to September 1988.

Site	<u>Dermacentor</u> <u>variabilis</u>		<u>Amblyomma</u> <u>americanum</u>		<u>Ixodes</u>	
	No.	%	No.	%	No.	%
1	171	94	11	6	0	0
1 A	36	97	1	3	0	0
2	126	84.5	22	14.8	1	0.67
3	15	75	5	25	0	0

Table 2. Frequency of ticks infected with Rickettsia spp. collected by flagging in Hanover County, Virginia from April to September of 1988.

Site	<u>Rickettsia</u> spp.	
	No.	%
1	0	0
1 A	0	0
2	4	2.68
3	0	0

Table 3. Frequency of ticks infected with bacillary organisms, collected in Hanover County, Virginia from April to September 1988.

Site	Bacilli Present No.	%	<u>Dermacentor</u> <u>variabilis</u> NO.	<u>Amblyomma</u> <u>americanum</u> NO.
1	14	7.7	11	3
1 A	0	0	0	0
2	2	1.3	2	0
3	0	0	0	0

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Appendix I. Collection data from test sites in Hanover
County, Virginia from April to September, 1988.

+ - present

- - absent

* - Bacillary organism present

* - Hemolymph test positive

Location Site: #1

4/29/88

Conditions

12:30 p.m.,
76°F,
Clear, Shady

Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
15	+			A	M	+			-	-	-
16	+			A	M	+			-	-	-
17	+			A	M	+			-	-	-
18	+			A	F	+			-	-	-
19	+			A	M	+			-	+	-
20	+			A	F	+			-	+	-
21	+			A	F	+			-	+	-
22	+			A	M	+			-	+	-
23	+			A	M	+			-	+	-
24	+			A	M	+			-	+	-
25	+			A	F	+			-	+	-
26	+			A	F	+			-	+	-
27	+			A	F	+			-	+	-
28	+			A	F	+			-	+	-
29	+			A	M	+			-	+	-

Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
1:15 p.m., 72°F, Cloudy, COOL	5/27/88	45	+			A	F	+			-	+	-	
		46	+			A	F	+			-	-	-	
	5/28/88	47	+			A	M		+			-	-	-
		48	+			A	F	+				-	+	-
		49	+			A	F	+				-	+	-
		50	+			A	M	+				-	-	-
		51	+			A	M	+				-	+	-
		52	+			A	M	+				-	-	-
		53	+			A	F	+				-	+	-
		54	+			A	M	+				-	+	-
		55	+			N		+				-	+	-
		56	+			A	FF	+				-	-	-
		57	+			A	M	+				-	-	-
		58	+			A	M	+				-	+	-
59	+			A	F	+				-	+	-		

Location Site:

Conditions

5:00 pm, 80°F
Sunny

Date

5/28/88
5/29/88

Specimen #

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

Flagging

+

+

+

+

+

+

+

+

+

+

+

+

+

+

+

Human

Dog

Life Stage

A

A

A

A

A

A

A

A

A

A

A

A

A

A

A

Sex

M

F

F

F

F

F

F

M

M

M

F

F

F

F

F

D. variabilis

+

+

+

+

+

+

+

+

+

+

+

+

+

+

+

A. americanum

Ixodidae

Hemolymp Test

-

-

-

-

-

-

-

-

-

-

-

-

-

-

-

Unidentified
Extracellular
Bacteria

+

+

+

+

+

+

+

+

+

+

+

+

+

+

+

Bacillary Organism

-

-

-

-

-

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-

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Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<i>D. variabilis</i>	<i>A. americanum</i>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
6:45 pm, 80°F Shady, Hot	5/29/88	75	+			A	F	+			-	+	-	
		76	+			A	F		+		-	+	-	
		77	+			A	F	+			-	+	-	
	5/30/88	78	+			A	M	+				-	+	-
		79	+			A	F	+				-	+	-
		80	+			A	F	+				-	+	-
		81	+			A	F	+				-	+	-
		82	+			A	F	+				-	+	-
		83	+			A	M	+				-	+	-
		84	+			A	F	+				-	+	-
		85	+			A	F	+				-	+	-
		86	+			A	F	+				-	+	-
		87	+			A	M	+				-	+	-
		88	+			A	M	+				-	+	-
89	+			A	M	+				-	+	-		

Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
11:30 am, 89°F Clear	5/30/88	90	+			A	F		+		-	+	-
		91	+			A	M		+		-	+	-
		92	+			A	F	+			-	+	-
		93	+			A	M	+			-	-	-
		94	+			A	M	+			-	-	-
		95	+			A	F	+			-	-	-
		96	+			A	M	+			-	-	-
		97	+			A	F	+			-	-	-
		98	+			A	M	+			-	-	-
		99	+			A	F	+			-	-	-
		100	+			A	M	+			-	-	-
		101	+			A	M	+			-	-	-
		102	+			A	M	+			-	-	-
		103	+			A	F	+			-	-	-
104	+			A	F	+			-	-	-		

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
	6/12/88	120	+			A	F	+			-	-	-
		121	+			A	M	+			-	+	-
	6/13/88	122	+			A	F	+			-	+	-
		123	+			A	M	+			-	+	-
		124	+			A	F	+			-	-	-
		125	+			A	M	+			-	+	-
		126	+			A	M	+			-	+	-
	6/15/88	127	+			A	F	+			-	+	-
		128	+			A	M	+			-	+	-
		129	+			A	M	+			-	+	-
		130	+			A	M	+			-	+	-
	6/17/88	131			+	A	M	+	+		-	+	-
	6/23/88	132	+			A	F	+			-	+	-
		133	+			A	F	+			-	+	-
		134	+			A	M	+			-	+	+

8:20 am, 64°F
Shady, Cool

11:00 am, 84°F
Sunny

9:00 am, 94°F
Hot

Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<i>D. variabilis</i>	<i>A. americanum</i>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
	6/23/88	135	+			A	M	+			-	+	-
		136	+			A	M	+			-	+	-
		137	+			A	M	+			-	+	-
		138	+			A	F	+			-	+	*
		139	+			A	M	+			-	+	*
		140	+			A	M		+		-	+	*
	6/27/88	141	+			A	F	+			-	+	*
10:45 am, 72°F COOL		142	+			A	F	+			-	+	*
		143	+			A	M	+			-	+	*
		144	+			A	F	+			-	+	*
		145	+			A	F	+			-	+	-
	6/30/88	146	+			A	F		+		-	+	*
9:45 am, 68°F COOL		147	+			A	F	+			-	+	-
	7/2/88	148			+	A	M	+			-	-	-
	7/4/88	149			+	A	F	+			-	-	-

Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<i>D. variabilis</i>	<i>A. americanum</i>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
	7/18/88	165	+			A	F	+			-	+	-
		166	+			A	F	+			-	+	-
		167	+			A	M	+			-	-	-
9:00 am, 75°F Windy, Cool	7/21/88	168	+			A	F	+			-	+	-
	7/24/88	169		+		A	M	+			-	+	+
1:30 pm, 85°F Wet, Warm	7/25/88	170	+			A	F	+			-	+	-
12:30 pm, 82°F Wet	7/27/88	171	+			A	F	+			-	+	-
		172	+			A	F	+			-	+	+
		173			+	A	M	+			-	+	-
		174			+	A	F	+			-	+	-
		175			+	A	M	+			-	+	-
		176			+	A	M	+			-	+	-
11:30 am, 88°F Warm, Shady	7/29/88	177	+			A	F	+			-	+	-
11:30 am, 82°F Cloudy	8/3/88	178	+			A	F	+			-	+	-
11:00 am, 90°F Hot	8/8/88	179	+			A	M	+			-	+	-

Location Site: #1

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
6:45p.m., 84° F, Shady	8/13/88	180	+			A	F	+			-	+	-	
		181	+			A	F	+			-	+	-	
		182	+			A	F	+				-	-	
9:45 a.m., 80° F, Wet	8/26/88													

#1A

Location Site:

Conditions

11:00 am, 86°F
Wet

12:45 pm, 90°F
Hot

Date

8/5/88

8/6/88

Specimen #

1

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3

4

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6

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8

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11

12

13

14

15

Flagging

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Human

Dog

Life Stage

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A

A

Sex

M

F

M

F

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M

D. variabilis

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A. americanum

Ixodidae

Hemolymph Test

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Unidentified Extracellular Bacteria

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Bacillary Organism

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Location Site: #1 A

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
11:00 a.m. 90°F, Hot	8/8/88	16	+			A	M	+			-	+	-	
		17	+			A	F	+			-	+	-	
		18	+			A	M	+				-	+	-
		19	+			A	M	+				-	+	-
		20	+			A	F	+				-	+	-
		21	+			A	M	+				-	-	-
		22	+			A	F		+			-	-	-
		23	+			A	M	+				-	+	-
		24	+			A	M	+				-	+	-
		25	+			A	F	+				-	+	-
11:30 a.m., 80°F	8/9/88	26	+			A	M	+			-	+	-	
		27	+			A	M	+			-	+	-	
		28	+			A	M	+				-	+	-
		29	+			A	M	+				-	+	-
		30	+			A	F	+				-	+	-

Location Site: #2

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
4:00 p.m. 75°F, Cool	4/30/88	1	+			A	M	+			-	-	-	
		2	+			A	M	+			-	-	-	
2:30 p.m., 73°F, Shady	4/30/88	3	+			A	M	+			-	-	-	
		4	+			A	F	+			-	+	-	
		5	+			A	M	+				-	-	-
		6	+			A	M	+				-	-	-
		7	+			A	M	+				-	-	-
		8	+			A	F	+				-	+	-
		9	+			A	M	+				-	+	-
		10	+			A	M	+				-	-	-
		11	+			A	M	+				-	+	-
		12	+			A	M	+				-	+	-
		13	+			A	F	+				-	+	-
		14	+			A	F	+				-	+	-
		15	+			A	F	+				-	+	-

Location Site: #2

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
4:00 p.m., 80° Clear, Shady	5/27/88	16	+			A	M	+			-	-	-	
		17	+			A	M	+			-	-	-	
		18	+			A	M	+				-	-	-
		19	+			A	M	+				-	+	-
		20	+			A	M			+		-	+	-
		21	+			A	M			+		-	-	-
		22	+			A	M			+		-	+	-
		23	+			A	M		+			-	+	-
		24	+			A	F		+			-	+	-
		25	+			A	F			+		-	-	-
		26	+			A	F		+			-	-	-
		27	+			A	F		+			-	-	-
		28	+			A	M		+			-	+	-
29	+			A	F		+			-	-	-		
30	+			A	M		+			-	-	-		

Location Site: #2

Conditions

3:00 p.m.,
80° F, Sunny

Date

5/28/88

Specimen #

31

32

33

34

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36

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42

43

44

45

Flagging

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Human

Dog

Life Stage

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Sex

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D. variabilis

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A. americanum

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Ixodidae

Hemolymph Test

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Unidentified Extracellular Bacteria

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Bacillary Organism

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#2

Location Site: _____

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<i>D. variabilis</i>	<i>A. americanum</i>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
1:30 p.m., 94°F, Hot	5/28/88	61	+			A	F	+			-	+	-	
		62	+			A	F	+			-	+	-	
		63	+			A	F	+				-	-	
		64	+			A	F	+				-	+	
		65	+			A	F	+				-	+	
		66	+			A	F	+				-	+	
		67	+			A	F			+		-	-	
	5/31/88	68	+				A	F	+			-	-	-
		69	+				A	F	+			-	-	-
		70	+				N			+		-	+	-
		71	+				A	M	+			-	-	-
		72	+				A	F	+			-	-	-
		73	+				A	M	+			-	+	-
		74	+				A	F	+			-	+	-
		75	+				A	F		+		-	-	-

Location Site: #2

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
	5/31/88	76	+			A	F	+			-	+	-
		77	+			A	M	+			-	+	-
		78	+			A	F	+			-	-	-
		79	+			A	F	+			-	-	-
		80	+			A	M	+			-	-	-
		81	+			A	M		+		-	-	-
		82	+			A	M	+			-	-	-
		83	+			A	M	+			-	-	-
		84	+			A	F	+			-	-	-
		85	+			A	M	+			-	+	-
		86	+			A	F	+			-	+	-
		87	+			A	F	+			-	+	-
		88	+			A	M	+			-	-	-
		89	+			A	F	+			-	+	-
		90	+			A	F	+			-	+	-

Location Site: #2

Conditions

11:00 a.m.
78°F, Sunny
Clear

Date

6/11/88

Specimen #

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

Flagging

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Human

Dog

Life Stage

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Sex

F

F

F

F

M

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M

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F

F

M

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F

D. variabilis

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A. americanum

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Ixodidae

Hemolymph Test

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Unidentified
Extracellular
Bacteria

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Bacillary Organism

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Location Site: #2

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<u>D. variabilis</u>	<u>A. americanum</u>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism
9:15a.m., 78°F Sunny	6/11/88	106	+			A	F		+		-	+	-
		107	+			A	F	+			-	-	-
		108	+			A	F	+				-	-
		109	+			A	F	+				-	-
		110	+			A	M	+				-	-
		111	+			A	F	+				-	-
		112	+			A	F	+				+	-
		113	+			A	F			+		+	-
		114	+			A	M	+				-	+
		115	+			A	F	+				-	+
		116	+			A	F	+				-	+
		117	+			A	F	+				-	+
118	+			A	F	+				-	+		
119	+			A	M	+				-	+		
120	+			A	F				+		+		

Location Site: _____

Conditions	Date	Specimen #	Flagging	Human	Dog	Life Stage	Sex	<i>D. variabilis</i>	<i>A. americanum</i>	Ixodidae	Hemolymph Test	Unidentified Extracellular Bacteria	Bacillary Organism	
11:30 a.m., 80°F, Cloudy	7/15/88	136	+			A	M	+			-	+	-	
		137	+			A	F	+			-	+	-	
		138	+			A	F	+				-	+	-
		139	+			A	M	+				-	+	-
10:30 a.m., 92°F, Hot	7/15/88	140	+			A	M	+			-	+	-	
		141	+			A	F	+			-	+	-	
		142	+			A	F	+				-	+	-
		143	+			A	M	+				-	+	-
11:15 a.m., Hot, 92°F	7/18/88	144	+			A	F		+		-	+	-	
		145	+			A	M	+			-	+	-	
		146	+			A	F	+				-	+	-
10:00 a.m., 88°F, Warm, Windy.	7/21/88	147	+			A	M	+			-	+	-	
		148	+			A	M	+			-	+	-	
		149	+			A	F	+				-	+	-

APPENDIX II

Presented here is the original research proposal for this work. Unfortunately due to restrictions found in the federal guidelines, these experiments could not be attempted. The fluorescent antibody stain for spotted fever group rickettsiae could not be obtained from Centers for Disease Control in Atlanta. A similar problem occurred in getting Peromyscus antibodies for the IFA test for R. rickettsii and R. montana antibodies in the field mice captured in this study. So instead of an immunity demonstration, the present study focused on a survey of rickettsial infection at the Three test sites in Hanover County, Virginia.

The goal of this research project is to ascertain whether an infection of Rickettsia montana in Microtus pennsylvanicus, the meadow vole and Peromyscus polionotus, the white footed mouse will impart an immunity to an infection of Rickettsia rickettsii, the etiological agent for Rocky Mountain Spotted Fever. Rickettsia montana is highly pathogenic to meadow voles and could increase the likelihood of immunity against R. rickettsii.

The incidence of Rocky Mountain Spotted Fever in humans has decreased in the West over the past few decades to a very low frequency (Burgdorfer, 1977). Infection in the tick is passed transovarially for only eight generations

and new rickettsiae must be obtained from rickettsemic hosts like the meadow vole or the white footed mouse. If these hosts are becoming immune to R. rickettsii, the ticks will not be reinfected and the pathway of transmission will be broken.

Three test sites have been selected in Hanover County, Virginia, an area known for Rocky Mountain Spotted Fever. The last reported case was in 1984 (Dept. of Epidemiology, Virginia).

The experimental design is in three parts. First is to collect ticks in these areas to determine the frequency of infection by R. montana and R. rickettsii. Collection will be by flagging and removal from host animals. The hemolymph test will be used to show the presence of rickettsiae and a fluorescein isothiocyanate labelled antibody stain will be used to identify the rickettsiae as part of the Spotted Fever Group. This method is unable to identify species.

The second part of the experimental design is to collect meadow voles and white footed mice, obtain blood samples and determine the frequency of infection by these two rickettsiae. Specimens will be collected in Sherman life traps, anesthetized with ether, and blood drawn from the orbital sinus. After marking for identification, animals will be released to their natural environment. Blood samples will undergo the indirect

fluorescent antibody or IFA test that will identify the presence of antibodies to R. montana and R. rickettsii. This is a species specific test.

The last part of the experiment is to conduct an immunity challenge test. Laboratory mice will be infected with the M/5-6 strain of R. montana. After two weeks or the establishment of an antibody titer, the mice will be infected with the "Sawtooth-2" strain of R. rickettsii. Injections will be intraperitoneally and $.1 \text{ cm}^3$ of an infectious yolk sac suspension diluted with brain heart infusion broth will be used. Temperatures will be taken daily and other signs of clinical infection observed. Titers will be measured with the IFA test. If the infection of R. montana has given an immunity against infection of R. rickettsii, the mice should not have severe clinical symptoms. If immunity is not present, the mice will probably die from the infection of R. rickettsii since this is a very virulent strain.

Vita

Peggy Ann Keefe was born in Chillicothe, Ohio, April 7, 1959. She attended grammar school in Orlando, Florida and high school in Salt Lake City, Utah. A Bachelor of Science degree in biology was received from the University of Utah in 1982. From 1983 to 1986, Miss Keefe was employed at Native Plants, Inc. where she was responsible for producing chemically induced mutations in commercial strawberries and grapes. She completed requirements for a Master of Science degree in biology at the University of Richmond in August, 1989. While at the University of Richmond, she was a member of Beta Beta Beta and an associate member of Sigma Xi. Miss Keefe is currently engaged in small cell lung cancer research at the Veterans Administration Medical Center, Richmond, Virginia.