JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1979, p. 322-325
0095-1137/79/09-0322/04$02.00/0

Vol. 10, No. 3

Natural Infection of Dogs on Cape Cod with *Rickettsia rickettsii*

WILLIAM C. FENG,[1] EDWARD S. MURRAY,[1]† GAIL E. ROSENBERG,[1] JUDITH M. SPIELMAN,[1] AND JOSEPH L. WANER[2]*

Departments of Microbiology[1] and Tropical Public Health,[2] Harvard School of Public Health, Boston, Massachusetts 02115

Received for publication 18 June 1979

Four isolates of rickettsiae from sick dogs on Cape Cod, Mass., were serologically identical to isolates of *Rickettsia rickettsii* from human patients with Rocky Mountain spotted fever. The antigenic analysis used the indirect fluorescent-antibody test and antisera prepared in mice to each of the isolates and to reference strains of *R. rickettsii* and *Rickettsia montana*. Serological responses of infected dogs were specific for *R. rickettsii*, although antibodies to *R. montana* were also detected in the sera of most of the canines.

Rocky Mountain spotted fever (RMSF), an acute febrile infection of humans caused by *Rickettsia rickettsii*, is endemic on Cape Cod, Mass. The dog tick *Dermacentor variabilis* is common on Cape Cod and believed to be the principal vector of RMSF. Dogs respond clinically (1, 3) to experimental infection with *R. rickettsii*, and ticks may become infected if they feed on dogs during the peak period of rickettsemia (7). There is serological evidence that rickettsial exposure of dogs may be common in endemic areas (8, 9); one report described a possible case of RMSF in a dog with an acute illness 2 weeks before RMSF appeared in the owner (8). This communication reports the isolation of *R. rickettsii* from sick dogs and humans on Cape Cod and documents specific serological responses to the infection.

MATERIALS AND METHODS

Rickettsial isolations. Acute-phase sera were obtained from canine and human patients at the time of clinical presentation of RMSF; covalescent-phase sera were obtained 4 to 6 weeks later. The canine and human patients were not members of the same households. Routine procedures were followed. In brief, approximately 10 ml of whole blood was drawn and held at 4°C (less than 24 h) until the serum was separated and stored at −20°C for subsequent serological studies. The clot was rinsed twice with phosphate-buffered saline (PBS), placed in a 10-ml syringe, and disrupted by injection into a 20-ml vial; an equal volume of phosphate-glutamate-sucrose was added to make a 50% suspension. A sample was cultured on blood agar for bacterial contaminants; the remainder was frozen in a 95% alcohol-dry ice bath and then stored at −80°C. Isolation of rickettsiae was attempted in guinea pigs and/or embryonated eggs. The suspension was thawed at 37°C and held on ice. Two male guinea pigs each were inoculated intraperitoneally with 1 ml of the suspension. Temperatures were taken twice each day. If fever developed, animals were bled at the height of the fever, usually on day 4 to 6 post-inoculation; all animals were bled 28 days after inoculation. Blood was taken by cardiac puncture and allowed to clot. The clot was processed as before, and the serum was stored at −20°C for serology.

Five-day-old embryonated eggs were inoculated with 0.4 to 0.5 ml of the clot suspension prepared from dogs, humans, or guinea pigs. The eggs were incubated at 35.5°C and candled twice each day. Embryos that died between day 4 and 7 were considered infected. Smears were made of each yolk sac and stained with Giménez stain; rickettsiae were identified by their characteristic morphology and by a direct fluorescent-antibody test with group-specific, guinea pig anti-*R. rickettsii* immunoglobulin conjugated with fluorescein isothiocyanate (supplied by Willy Burgdorfer). The yolk sacs were placed in vials containing glass beads, phosphate-glutamate-sucrose was added to make a 50% suspension, and the vials were frozen with 95% alcohol and dry ice and stored at −80°C.

Identification of rickettsiae isolates. Six-week-old, male, white Swiss mice were used to produce strain-specific antisera to the isolates (6). A 0.2-ml amount of a 2% infected yolk sac suspension in PBS was inoculated via the tail vein; 7 days later, the mice were similarly reinoculated and were bled 3 days later. The Sheila Smith strain of *R. rickettsii* and a strain of *R. montana* obtained from Willy Burgdorfer were used as reference strains.

Indirect fluorescent-antibody test. Rickettsial isolates were passaged in eggs until a satisfactory concentration was obtained for use as slide preparations of antigen. A 50% suspension of yolk sac in PBS was then homogenized in an Omni-mixer (Ivan Sorvall Inc., Newtown, Conn.) and centrifuged at 250 × *g* to remove lipids. The suspension was diluted 1:5 with PBS to give approximately 10^3 organisms per 40× field

† Deceased.

for the fluorescent-antibody test. Antigen (0.025 ml) was placed on a slide, air dried, and fixed in acetone for 10 min. Slides were stored at −80°C until used. Sera were diluted in PBS containing a 2.5% suspension of normal yolk sac, and fourfold dilutions ranging from 5 to 5,120 were tested. Antigen on slides was exposed to 0.025 ml of antiserum for 1 h at 37°C in a humid chamber and washed for 10 min with stirring in 200 ml of PBS containing 0.001% Evans blue. The slides were rinsed with distilled water and dried. After incubation with the appropriate fluorescein isothiocyanate conjugated anti-immunoglobulin (immunoglobulin M and immunoglobulin G) for 30 min at 37°C in a humid chamber, the slides were washed once in PBS for 10 min with stirring followed by one rapid wash with distilled water and dried. Slides were mounted with 7A mounting fluid (Difco Laboratories) and glass cover slips and viewed at 40×. The fluorescence reaction was rated 1 to 4 depending on the intensity of the brightness and the number of organisms stained; reactions rated equal to or greater than 2 were considered positive. If sequential fourfold dilutions were rated as 3 and 1, respectively, the titer of the serum was judged to be the intermediate twofold dilution. Reactivity at a dilution of 1:40 or greater was considered positive.

RESULTS

Serological identification of rickettsial isolates with strain-specific antisera. Antisera produced in mice to rickettsiae isolated from dogs or human patients reacted comparably with all isolates and with the reference strain of *R. rickettsii* (Table 1); the antisera were not reactive with the reference strain of *R. montana* at a dilution greater than 1:5. Antisera prepared to the reference strains of *R. rickettsii* and *R. montana* showed negligible cross-reactivity. Antiserum to the reference strain of *R. rickettsii* reacted at dilutions ranging from 320 to 640 with the dog or human isolates, whereas the antiserum prepared to *R. montana* did not react with the isolates at a dilution greater than 1:5 (Table 1).

Reactivity to *R. rickettsii* and *R. montana* antigens of acute- and convalescent-phase sera of canine and human patients from whom *R. rickettsii* were isolated. Acute-phase sera were obtained from canine or human patients at the time of the clinical presentation of RMSF; convalescent-phase sera were obtained 4 to 6 weeks later. Table 2 presents the titers obtained with three pairs of human sera and three pairs of canine sera obtained from subjects from whom rickettsial organisms were isolated and subsequently identified as *R. rickettsii* with murine, strain-specific antisera; an additional acute serum from a human patient is included. Two pairs of canine sera and one from a human patient showed increases of antibody titer to *R. rickettsii* from <5 to 1,280 or greater. The titers of the convalescent-phase sera of the remaining pairs of sera were three- to fivefold greater than the respective acute-phase sera. Six of the seven acute-phase sera from canines and human patients were not reactive for *R. montana* (<5), whereas the titers of the convalescent-phase sera ranged from 20 to 640; in each subject, however, the titer to *R. montana* was significantly lower than was the titer to *R. rickettsii*.

Reactivity to *R. rickettsii* and *R. montana* antigens of acute- and convalescent-phase sera from canines with clinical evidence of

TABLE 1. *Antigenic analysis of rickettsial organisms isolated from canines and humans with antisera prepared in mice to the isolates*

Source of isolates	Isolate no.	Titer of antisera prepared to rickettsial isolates obtained from canines and humans										
		Antiserum no. to isolates from canines				Antiserum no. prepared to isolates from humans					Antiserum to reference rickettsial strain	
		1	2	3	4	1	2	3	4	5	*R. rickettsii*[a]	*R. montana*[b]
Canine	1	1,280	640	2,560	320	640	2,560	1,280	2,560	2,560	640	5
	2	1,280	640	5,120	320	640	2,560	1,280	1,280	1,280	640	5
	3	2,560	640	5,120	640	640	2,560	1,280	1,280	2,560	640	5
	4	2,560	320	2,560	320	640	2,560	1,280	2,560	2,560	320	5
Human	1	2,560	640	2,560	640	640	5,120	1,280	1,280	1,280	640	5
	2	1,280	320	2,560	640	640	5,120	1,280	2,560	2,560	640	5
	3	1,280	320	5,120	640	640	2,560	1,280	2,560	2,560	640	5
	4	2,560	640	5,120	320	640	2,560	1,280	2,560	2,560	640	5
	5	1,280	320	5,120	640	640	2,560	1,280	1,280	1,280	640	5
Reference strains												
R. rickettsii		1,280	320	5,120	640	640	2,560	1,280	1,280	1,280	320	5
R. montana		5	10	20	10	5	20	10	20	20	20	320

[a] *R. rickettsii* Sheila Smith strain.
[b] *R. montana* strain M/5-6B e.p. no. 7 from Willy Burgdorfer, Rocky Mountain Laboratory, Hamilton, Mont.

TABLE 2. *Antibody titers to R. rickettsii and R. montana of acute and convalescent sera obtained from canine and human patients*

Source of sera	Serum no.	Rickettsial antigens			
		R. rickettsii		*R. montana*	
		Acute	Convalescent	Acute	Convalescent
Canine	1	80	2,560	40	640
	2	<5	1,280	<5	160
	3	<5	1,280	<5	80
Human	1	10	80	<5	20
	2	320	NT[a]	<5	NT
	3	80	1,280	<5	80
	4	<5	5,120	<5	320

[a] NT, Not tested; serum not available.

TABLE 3. *Antibody titers to R. rickettsii and R. montana of acute and convalescent sera obtained from canines with clinical evidence of RMSF*

Canine no.	Rickettsial antigens			
	R. rickettsii		*R. montana*	
	Acute	Convalescent	Acute	Convalescent
1	80	1,280	320	1,280
2	5	640	<5	160
3	20	640	<5	80
4	<5	1,280	<5	640
5	<5	2,560	<5	1,280
6	<5	320	<5	<5
7	<5	1,280	<5	80
8	40	640	<5	80
9	40	1,280	<5	320
10	20	1,280	80	640

RMSF. An additional 10 pairs of sera (acute and convalescent) were obtained from 10 canines with clinical presentations of RMSF but from whom rickettsiae were not isolated. Four of the dogs showed increases of antibody titer to *R. rickettsii* from undetectable levels (<5), and three of these responded similarly to *R. montana*. The remainder showed fourfold or greater rises in titer to *R. rickettsii* from preexisting levels of reactivity; in each instance a rise in titer to *R. montana* was also observed but never of a magnitude greater than that observed with *R. rickettsii* antigen (Table 3).

DISCUSSION

Canines have been shown under experimental conditions to be susceptible to infection of *R. rickettsii*, and they may experience clinical symptoms and a serological response (1, 3, 7). In this communication we have shown that canines may also be infected in nature with *R. rickettsii*. The clinical features of the disease in canines will be described in a separate report.

Ticks which frequently feed on humans carry various rickettsiae which differ antigenically and in their pathogenicity for male guinea pigs (2, 5, 6). The pathogenicity of these rickettsiae for humans and dogs, however, is virtually unknown. One of these rickettsiae, *R. montana*, occurs in dog ticks on Cape Cod (unpublished observations) and is serologically related to *R. rickettsii* but differs in that it is nonpathogenic for male guinea pigs (2, 4). Our results indicate, however, that *R. rickettsii* is the etiological agent of spotted fever disease in dogs on Cape Cod. *R. rickettsii* was the only rickettsiae isolated from sick dogs, and each dog observed with a clinical diagnosis of spotted fever had an elevated and relatively specific antibody response to *R. rickettsii*. Similar antibody titers to *R. rickettsii* and *R. montana* in some canines probably reflect greater exposure of canines to ticks infected with *R. montana*. Due to the antigenic relatedness of these organisms, primary infections with *R. rickettsii* of canines previously exposed to *R. montana* would evoke anemestic responses to *R. montana*. Dogs on Cape Cod could possibly be exposed to a rickettsiae other than *R. rickettsii* or *R. montana* that might be responsible for the disease. Preliminary results of our tick survey of rickettsial infection on Cape Cod, however, indicated that *R. montana* is the most prevalent rickettsiae.

Dogs may be involved in the transmission of RMSF as carriers by bringing infectious ticks close to humans. Epidemiological studies have shown that human patients often own a dog with evidence of rickettsial exposure (9); dogs have also been reported to become sick at the same time as their owners (8). Because dogs have a greater exposure to ticks than humans and are susceptible to *R. rickettsii* infections, a greater incidence of spotted fever in dogs and more serologically reactive dogs than humans could be expected. The dog, therefore, may be a good indicator of the prevalence and location of foci of RMSF.

LITERATURE CITED

1. **Badger, L. F.** 1933. Rocky Mountain spotted fever: susceptibility of the dog and sheep to the virus. Public Health Rep. **48**:791-795.
2. **Bell, E. J., G. M. Kohls, H. G. Stenner, and D. B. Lackman.** 1963. Nonpathogenic rickettsias related to the spotted fever group isolated from ticks, *Dermacentor variabilis* and *Dermacentor andersoni* from Eastern Montana. J. Immunol. **90**:770-781.
3. **Keenan, K. P., W. C. Buhles, Jr., D. L. Huxsoll, R. G. Williams, P. K. Hildebrandt, J. M. Campbell, and E. H. Stephenson.** 1977. Pathogenesis of infection with *Rickettsia rickettsii* in the dog: a disease model for Rocky Mountain spotted fever. J. Infect. Dis. **135**:911-917.
4. **Lackman, D. B., E. J. Bell, H. G. Stenner, and E. G. Pickens.** 1965. The Rocky Mountain spotted fever group of rickettsias. Health Lab. Sci. **2**:135-141.

5. **Parker, R. R., G. M. Kohls, G. W. Cox, and G. E. Davis.** 1939. Observation on an infectious agent from *Amblyomma maculatum*. Public Health Rep. **54**:1482–1484.
6. **Philip, R. N., E. A. Casper, W. Burgdorfer, R. K. Gerloff, L. E. Hughes, and E. J. Bell.** 1978. Serological typing of rickettsiae of the spotted fever group by microimmunofluorescence. J. Immunol. **121**:1961–1968.
7. **Price, W. H.** 1954. The epidemiology of Rocky Mountain spotted fever. II. Studies on the biological survival mechanisms of *Rickettsia rickettsii*. Am. J. Hyg. **60**: 292–319.
8. **Sexton, D. J., W. Burgdorfer, L. Thomas, and B. R. Norment.** 1976. Rocky Mountain spotted fever in Mississippi. Survey for spotted fever antibodies in dogs and for spotted fever group rickettsiae in the dog ticks. Am. J. Epidemiol. **103**:192–197.
9. **Shepard, C. C., and N. H. Topping.** 1946. Rocky Mountain spotted fever: a study of complement fixation in the serum of certain dogs. J. Infect. Dis. **78**:63–68.