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THE BEHAVIOR OF Q FEVER RICKETTSIAE ISOLATED [1] FROM WILD ANIMALS IN NORTHERN CALIFORNIA

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Abstract: The biological properties of strains of *Coxiella burnetti* from 9 species of wild animals and 2 species of ticks collected at Hopland, in Northern California, were compared to the properties reported for the highly infectious wildlife strains isolated in Western Montana, and the comparatively avirulent strains isolated from rodents in Utah. The Hopland strains are though to be similar to the Utah strains because they were usually more infectious for hamsters and induced higher antibody responses in this host than in guinea pigs or mice. The Hopland strains did not cause a febrile response and were difficult to transfer in guinea pigs. Although a slight splenomegaly was evident in inoculated mice and hamsters, there was no exudate around the spleen nor granuloma at the site of injection, as induced by typical virulent strains of *C. burnetii*. The Q fever rickettsiae isolated from deer and coyotes were the most infectious of the Hopland strains. They induced higher antibody responses in guinea pigs during the primary isolation and were more easily transferred through laboratory hosts.

Both the Utah and Hopland wildlife strains were isolated from animals collected in areas where livestock were present. It is not known whether the infectivity of certain strains of *C. burnetti* is influenced by host-parasite equilibrium in an animal population chronically exposed to the organism.

INTRODUCTION

The strains of *Coxiella burnetii* previously isolated from wild animals in two contrasting ecological environments had different properties of infectivity and pathogenicity. The comparatively avirulent strains isolated from rodents in the Great Salt Lake desert of Utah were more infective for hamsters than for guinea pigs or mice. The Utah strains induced antibody titers in hamsters that were 30 to 3000 times higher than those in guinea pigs, seldom caused a febrile response, and were difficult to passage in guinea pigs.[7,8]

Strains of *C. burnetii* isolated from rodents collected in a remote area of the Bitterroot Valley of western Montana were uniformly infectious and pathogenic for guinea pigs and mice.[2] The isolants produced a febrile response in guinea pigs after a 3-day incubation period. Antibody responses were similar in guinea pigs, mice, and hamsters.

During ecological studies of Q fever in an area in Northern California inhabited by livestock and wildlife,[3,4] isolations of *C. burnetii* were made from 9 species of wild mammals and 2 species of ticks collected in 1965-66. This report concerns observations on the biological properties of these strains of rickettsiae.

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MATERIALS AND METHODS

The Hopland Field Station comprises 5,000 acres in Northern California owned by the University of California and maintained primarily for research on range management, and with sheep and wildlife. Wild animals were trapped or shot, and blood samples, spleen, kidney, liver, and, when present, placental material and fetal tissues, were collected. Ectoparasites were collected from the animals, and free-living ticks (family *Argasidae)* were attracted to CO_2 traps with dry ice. The sera were frozen and kept at —20 C, and the tissues and ectoparasites were kept at —63 C, until used.

Portions of thawed tissues from 1 to 6 animals of the same species were pooled, and 10% to 20% suspensions were prepared in saline or Snyder's solution.[2] The remaining portions of the tissues were refrozen and stored. The ectoparasites were washed in three changes of sterile saline, and 50 to 270 of a particular classification collected from the same host species at the same time of year, and at the same geographical location, were pooled. The larger tick pools consisted of unengorged ticks, whereas the smaller pools contained females engorged with blood. Ectoparasite inocula were prepared as described above for the tissues, except 200 U of penicillin and 250 μg of streptomycin were included to control bacterial contamination, and the suspension was filtered through a double layer of sterile gauze to remove particles of the exoskeleton.

The tissue suspensions were centrifuged at low speed for 5 min. The supernatant fluid was collected and then inoculated intraperitoneally (IP), 0.5 ml into mice and hamsters and 1.0 ml into guinea pigs. The presence of *C. burnetii* was indicated if the initial passage host was seropositive 30 days after inoculation. Following such an indication, tissues from each original wildlife host were inoculated separately into laboratory animals to determine which wild animal was infected. Positive tissues were passaged through two or more species of laboratory animals, and embryonated hen's eggs, to compare differences in infectivity.

The sera were tested with the microtiter system of the complement fixation (CF) test described by Lennette,[6] using the phase II Nine-Mile strain of antigen (Lederle Laboratories). The sera were screened at a 1:8 dilution, and those reacting positively were titrated to the endpoint. Anticomplementary serum was treated with fresh guinea pig complement: one drop (0.025 ml) of serum was incubated with 1 drop of complement at 37 C for 30 min. Six drops of buffer were added to make a 1:8 dilution of serum which was then inactivated at 60 C for 30 min. and tested for CF antibodies.

The biological properties of the Hopland strains of *C. burnetii* from wild animals and ticks were evaluated by: 1) antibody response of laboratory animals during the primary transfer, 2) relative infectivity in hamsters, mice, guinea pigs, or embryonated hen's eggs, 3) capacity to be passaged in laboratory hosts, 4) presence (and degree) of splenomegaly, 5) amount of rickettsiae present in yolk sac or spleen smears, and 6) febrile response in guinea pigs. Not all of these criteria were evaluated for each individual isolant.

RESULTS

The results of the original isolation passage are shown in Table 1. The inoculum contained tissues from several animals, of which only one was usually infected. However, in each of the pools 1 through 3, *C. burnetii* was isolated from both deer sampled. The pools from which isolations were made usually contained at least one sample from a serologically positive animal, but some isolations were made from animals that had no detectable CF antibodies to *C. burnetii.*

Seven isolations were made from samples collected from 46 deer in 1966. Most isolations were from spleen or placental tissues of 4- to 10-year-old does, but isolations were also made from the spleens of a male fawn and a female fawn.

Tissue samples from deer DA640 were diluted into 1%, 10%, and 20% suspensions and inoculated IP into ham-

TABLE 1. Isolations of **Coxiella burnetii** from wild mammals and ticks, Hopland, California, 1965-66.

Original Host						1st Passage	
		Pool					
Species	Common Name	No.	Animals [1]	Tissue [2]	Max. Titer [3]	Host [4]	Max. Titer
Odocoileus hemionus columbianus	Deer	1	2*	S,P	0	GP	128
		2	2*	S,P	0	GP	256
		3	2*	L,K,S	64	GP	128
		4	2	L,K,S	8	GP	8
Canis latrans	Coyote	5	3	L,K,S	0	M	8
		6	3	S,U	64	H	256
		7	1	S	32	M	8
Urocyon cinereoargenteus	Gray fox	8	3	S	8	M	8
		9	2	S	32	GP	8
Peromyscus maniculatus	Deer mouse	10	4	S	0	H	8
		11	5	S	16	H	16
		12	6	S	0	H	16
		13	4	S	16	GP	8
Citellus beecheyi	Ground squirrel	14	4	S	0	GP	8
Sylvilagus bachmani	Brush rabbit	15	3	S	32	GP	8
Lepus californicus	Jack rabbit	16	4	S	32	GP	8
		17	4	S	8	GP	8
		18	4	S	16	GP	8
Spilogale gracilis	Spotted skunk	19	4	S	16	M	8
Mephitis mephitis	Striped skunk	20	3	S	8	GP	8
Ornithodoros coriaceus	Pajaroello tick	21	116	T	—	H	16
Dermacentor occidentalis	Pacific Coast tick	22	263	T	—	GP	8

[1] Number of wild animals or ticks tested in each tissue pool.
[2] L = liver, K = kidney, S = spleen, U = uterus, P = placenta, T = tick.
[3] Reciprocal of complement fixation antibody titer for the original host animal with the highest titer in the group.
[4] H = hamsrs, M = mce, GP = guinea pigs, 2 GP, 4 M, or 4 H used per passage group.
* *C. burnetii* isolated from both deer in the group.

sters, mice, and guinea pigs (Table 2). The 1% dilution induced CF antibody titers ranging from 1:128 to 1:1024 in hamsters, but 1:8 was the maximum titer in mice. The 10% dilution induced maximum titers of 1:512 in hamsters and 1:64 in mice, but failed to induce an antibody response in guinea pigs. However, guinea pigs responded to the 20% dilution of this inoculum by developing a maximum CF titer of 1:256. A 20% suspension of spleen and placental tissues from deer DA638 was infectious for hamsters and mice, but not for guinea pigs (Table 2). When this inoculum was passaged through eggs, a 10% yolk sac suspension induced a CF titer of 1:128 in guinea pigs.

The 3 isolations from coyotes were from an adult male, an adult female, and a 3 month old female. The original passage of these three strains resulted in a CF titer of 1:8 in mice and 1:256 in hamsters (Table 1). A 10% spleen suspension from coyote QF1422 induced CF titers of 1:256 in hamsters and 1:8 in mice (Table 2). The second passage of mouse spleen induced an antibody response of 1:512 in hamsters.

Mice were the most frequently collected animals, but *C. burnetii* was found only in 4 of the 121 deer mice *(Peromyscus maniculatus)* examined in 1966. No isolations were made from 52 brush mice *(Peromuscus truei)* examined in 1965 and 1966, although these species had CF antibodies to the rickettsiae.[4] Deer mouse strain QF1157 was infectious for hamsters on the first passage, but transfer of splenic tissue from these hamsters to mice gave negative serologic results (Table 2).

The strains of *C. burnetii* from 2 foxes, 3 jackrabbits, 1 brush rabbit, 1 ground squirrel, 1 striped skunk, and 1 spotted skunk induced low antibody responses in mice and guinea pigs on the first passage. These strains were difficult to transfer in laboratory animals and yolk sac cultures. However, there was sporadic evidence of infection at various passage levels, as indicated by specific antibodies in laboratory animals, small numbers of rickettsiae in spleen or yolk

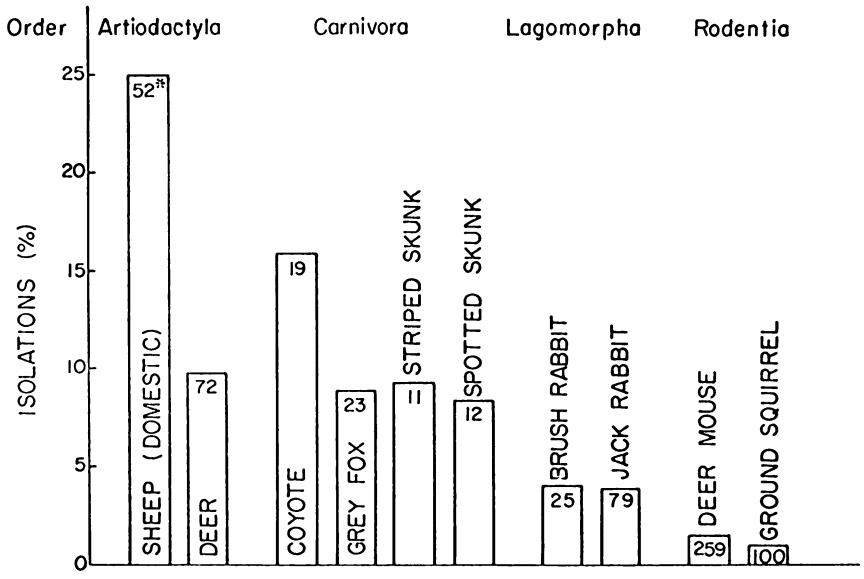

FIGURE 1. Isolation of **Coxiella burnetii** from domestic sheep and wild mammals
*Number of animals sampled.

TABLE 2. Antibody Response in Hamsters, Mice, and Guinea Pigs as Induced by Various Hopland Strains of **C. burnetii**.

		First Passage						Later Passages[2]			
		Inoculum		Antibody titer in laboratory hosts							
Strain		Tissue[3]	Suspension (%)	Original Host	Hamster	Mouse	Guinea Pig	Passage No.	Inoculum[3]	Host[3]	Antibody Response[1]
Deer	(DA640)	S,P	1	64	1024	8	ND	2	M S	M	512
			10		512	64	0	2	H S	H	256
			20		ND	ND	256	2	H S	H	8
Deer	(DA637)	S	20	0	128	128	0	2	H S	H	8
Deer	(DA638)	S,P	20	ND	16	8	0	2	YS[4]	GP	128
Coyote	(QF1422)	S	10	64	256	8	ND	2	M S	M	512
Deer Mouse	(QF1157)	S	20	16	16	ND	ND	2	H S	M	0
Ornithodoros coriaceus		T	20	—	16	0	ND	2	M S	M	8

[1] Maximum antibody titer in group of animals used for isolation of rickettsiae, expressed as reciprocal of highest serum dilution positive in the CF test. 0 = neg. at 1:8, ND = not done; Groups of 2 GP, 4 H, or 4 M were used.

[2] 10% spleen suspension used for 2nd and 3rd passages.

[3] S = spleen, P = placenta, T = ticks, M = mouse, H = hamster, GP = guinea pig, YS = yolk sac.

[4] Original tissues passaged once through eggs.

sac smears, and typical hemorrhagic deaths of egg embryos. Blind passaging when the strain appeared to be lost sometimes gave positive results. However, no further effort was made to adapt these srains to laboratory hosts.

Differences in the percentages of isolations of *C. burnetii* among the various species may indicate differences in rates of infection among orders of animals (Fig. 1). Among Artiodactyla, 25% of the sheep, and 10% of the deer were found to harbor the rickettsiae. *C. burnetii* was also found in up to 16% of the Carnivora, 4% of the Lagomorpha, and up to 2% of the Rodentia.

Coxiella burnetii organisms were isolated from ticks *(Dermacentor occidentalis)* collected from a male and two female deer necropsied in April 1965. Sera from the deer were negative for antibodies to *C. burnetii,* and rickettsiae were not isolated from the spleen nor placental tissues of the two female deer. The original tick suspension induced a low antibody response in guinea pigs, but no response in mice. However, transfer of splenic tissues from these mice into embryonated eggs resulted in typical *C. burnetii* growth in the yolk sac.

To test for "dormant" rickettsiae that may become more infectious after a blood meal,[1,5] *D. occidentalis* from several deer were fed on guinea pigs. When the female ticks were fully engorged with blood, they were prepared as described for ticks obtained from wild animals, and tissue suspensions were passaged into mice. Samples of spleen from both the host guinea pigs and the mice inoculated with tick material were negative for specific antibodies.

A strain of *C. burnetii* isolated from *Ornithodoros coriaceus,* a free-living, multiple-host tick, induced a 1:16 antibody response in hamsters, but no response in mice during the first passage. However, the third blind spleen passage in mice resulted in a 1:8 antibody response in this host (Table 2).

The temperature of guinea pigs inoculated with various passages of the Hopland strains ranged from 37.2 C to 39.4 C, somewhat below the 40 C to 41.7 C temperatures observed in guinea pigs inoculated with the Montana rodent strains.[2]

DISCUSSION

A majority of the strains isolated from Hopland wild mammals and ticks appears to be of relatively low virulence, similar to the Utah strains. Hopland isolants induced little, if any, febrile response or noticeable splenomegaly in guinea pigs, and were more infectious for hamsters than for mice or guinea pigs. A slight splenomegaly without exudate was observed in hamsters and mice, and there was no lesion at the site of inoculation. Rickettsiae were sparse or absent in stained smears of spleen or yolk sac.

Repeat inoculations of frozen material from rodents collected in Utah, and stored after inducing antibodies to Q fever in guinea pigs, infected hamsters but not guinea pigs. Serial passage through guinea pigs resulted in such irregular febrile and antibody responses that the strains appeared to be lost, until later transfers became seropositive.[7] The original frozen and stored material from Hopland deer was infectious for guinea pigs and mice, but usually induced a higher antibody response in hamsters. However, the capacity of *C. burnetii* organisms in deer and coyote tissues that had been stored, thawed, and refrozen, to be infectious for mice and guinea pigs, and the capacity of these rickettsia to be transferred in laboratory animals and embryonated eggs, indicate that these strains may have been more infective than the Utah rodent isolants. On the other hand, the lower antibody response in guinea pigs, and the lack of pathologic reactions, indicate that they were not as virulent as the Montana rodent strains.

The Utah and Montana studies were based on strains which were adapted through several passages in embryonated hen's eggs.[2,7] However, we worked primarily with the original host tissues and the first few animal passages to simulate more closely the activity of the phase I organism in nature. Establishing these Hopland strains in embryonated eggs may increase their pathogenicity to that of more virulent strains.

The pattern of isolations of rickettsia among orders (Fig. 1) indicates a possible relationship between food habits and exposure to the disease agent. Sheep, and possibly deer, seem to maintain the infection by intraspecific exposure, and are a likely major source of infection for other species. The Lagomorpha and Rodentia may become infected by exposure (contamination) in their physical environment. The Carnivora prey and feed on animals in the other three orders, and are possibly infected from eating animals that are harboring the organism in their tissues.

Differences in virulence among various strains of *C. burnetii* have been discussed by Brezina *et al.*[1] Strains that have undergone a series of passages through the same laboratory host (the Henzerling-strain in mice, and the Nine Mile and Dyer strains in eggs) decreased in virulence. Their studies also verified that strains of low virulence were more infectious for hamsters than for mice or guinea pigs. They also found that passaging the Henzerling strain through ticks *(Dermacentor pictus)* increased its virulence.

The similarity between the virulence of the Hopland strains and the Utah strains may be evidence that the pathogenicity of *C. burnetii* in wildlife is influenced by the presence of livestock in the area.[1,2] The Hopland and Utah areas were both accessible to livestock, whereas the more virulent isolants from Montana were from animals collected in an area inaccessible to livestock. The apparent difference in infectivity between strains of *C. burnetii* in nature may be related to the level of antibody protection in the resident animal population of an area where Q fever is endemic, to a long series of passages in the same host species, or to differences in modes of transmission (by ectoparasites or environmental factors). The comparatively low virulence in some of the Hopland strains may be a subtle reflection of transmission in livestock and wildlife populations which are relatively adapted to Q fever rickettsia.

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