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## SURVEY OF Q-FEVER AGGLUTININS IN BIRDS AND SMALL RODENTS IN NORTHERN CALIFORNIA, 1975-76 <sup>□</sup>

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**Abstract:** Serum samples from 15 species of rodents and 33 species of birds were tested for agglutinins against *Coxiella burnetii* by the microagglutination test. Of 759 rodents tested, 21 (3%) were seropositive. Antibody positive rodents included muskrats, *Ondatra zebethica*, (11%), *Rattus* spp. (10%), Beechey ground squirrels, *Otospermophilus beecheyi*, (6%), wood rats, *Neotoma fuscipes*, (5%), and *Peromyscus* spp. (2%). Of 583 birds tested, 118 (20%) were seropositive. This included white crowned sparrows, *Zonotrichia leucophrys*, gold crowned sparrows, *Z. atricapilla*, and English sparrows, *Passer domesticus*, (68% in the composite); coots, *Fulica americana*, (29%); blackbirds, *Euphagus cyanocephalus*, (33%); crows, *Corvus brachyrhynchos*, (29%); robins, *Turdus migratorius*, (16%); pigeons, *Columba fasciata*, (10%); and mallard ducks, *Anas platyrhynchos*, (7%). There was a tendency for the seropositive animals to have been collected in the vicinity of endemically infected livestock.

### INTRODUCTION

*Coxiella burnetii*, the rickettsia that causes Q-fever in man is maintained in two cycles in nature. The most evident cycle, because humans are in closer contact with it, is among domestic ungulates such as cattle,<sup>6,16,22-25,41</sup> sheep,<sup>20,33</sup> goats, camels, swine and buffaloes.<sup>21,29,39</sup> Heavy concentrations of *C. burnetii* are secreted in the milk, urine, and birth fluids of infected animals.<sup>3,22</sup> Because of their resistance to environmental conditions rickettsiae can thus be spread to new hosts via aerosols, contaminated airborne dust, and ingestion of raw milk.<sup>11,20,23,41,42</sup>

Like other rickettsiae, *C. burnetii* can undergo a wildlife cycle in arthropods and their vertebrate hosts. Since its early discovery in *Dermacentor andersoni*,<sup>10,26</sup> *C. burnetii* had been found in numerous other species of ticks.<sup>3,5,10,13</sup> In ticks the organism undergoes transstadial as well

as transovarial transmission.<sup>3,10</sup> Feces from infected ticks are laden with rickettsiae and inhalation of dried tick fecal particles is considered to be a possible mode of infection.<sup>12,26</sup> Although ticks have been implicated in cases of Q-fever,<sup>12</sup> they are considered to be an unusual mode of infection.<sup>12,14</sup>

The distribution of *C. burnetii* among man and animals appears to be practically worldwide.<sup>1-6,9-16,18-25,27-42</sup> This wide distribution of the organism in nature, yet with only occasional sporadic outbreaks in man, has led some investigators to believe that the organism has decreased greatly in virulence.<sup>7</sup> It has been speculated that such changes in rickettsial virulence may occur as a result of continued passage from animal to animal without an intermediate tick host.<sup>7,8</sup>

There have been several large-scale studies of complement-fixing (CF) an-

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tibodies among wild mammals. In the United States, for example, the prevalence of CF antibodies has been studied among several species of wild mammals in Utah,<sup>34-37</sup> western Montana,<sup>9</sup> southern Texas,<sup>30</sup> and on a 2020 ha sheep ranch in northern California.<sup>13-15</sup> Wild birds in selected areas of the world have been tested by either the CF or capillary tube agglutination test (CAT).<sup>1,2,15,38</sup> The prevalence of agglutinins, as measured by the micro-agglutination test (MA), has been reported recently for wild animals in southern Texas<sup>30</sup> and for wild carnivores in northern California.<sup>32</sup>

This report deals with the distribution of *C. burnetii* agglutinins in rodents and birds throughout northern California.

## MATERIALS AND METHODS

**Population Sampled.** Small rodents and birds were collected via live trapping or shooting from 26 locations in 15 counties throughout northern California (Figure 1). Some of the birds were collected using crow traps in cooperation with activities of county agricultural commissioners. Blood samples were obtained by cardiac puncture using a 1 ml. syringe and 23 ga. needle. The serum was kept frozen at -22 C until tested.

**Serology.** The sera were diluted from 1:2 to 1:16 on microtiter plates and tested for agglutinins against *C. burnetii* by the MA test.<sup>17</sup> If there was an agglutinating reaction at a dilution  $\geq 1:4$ , the animal was considered to have been infected with *C. burnetii*.

**Rickettsiae.** The *C. burnetii* used for preparation of the MA antigen was the Nine Mile strain in phase I. The strain was originally isolated from ticks and had undergone 306 guinea pig and 4 egg passages.

## RESULTS

**Rodents.** The prevalence of antibodies against *C. burnetii* among 15



FIGURE 1. Collection sites of wildlife species tested for agglutinating antibodies against *Coxiella burnetii* in California.

species of rodents is summarized in Table 1.

Of 759 individual rodents tested, 21 (2.8%) were seropositive. The highest prevalence of antibodies was found among muskrats (11%), rats (10%), Beechey ground squirrels (6%) and wood rats (5%). The most frequently trapped rodents, because of their almost ubiquitous distribution in nature, were the three species of *Peromyscus* common in California; *P. maniculatus*, *P. truei*, and *P. boylei*. Of 306 *Peromyscus* tested, only 5 (2%) were seropositive.

**Birds.** Of the 583 birds from 33 species that were tested for antibodies against *C. burnetii*, 118 (20%) were seropositive (Table 2). The highest rates of infection were among the "sparrows", i.e., gold crowned- (80%), white crowned- (57%), and English- (50%). These high prevalence levels were followed by those for coot (45%), Brewer's blackbird (33%), crow (29%), robin (16%), pigeon (10%), and mallard duck (7%).

TABLE 1. Distribution of agglutinating antibodies against *C. burnetii* in small rodents, Northern California, 1975-76.

Genus and species (common name)	Tested			Reciprocal of Titer*				
	No.	No. Pos.	(%)	0	2	4	8	≥16
<i>Peromyscus</i> spp. (deer and brush mouse)	306	5	(2)	293	8	3	2	-
<i>Neotoma fuscipes</i> (woodrat)	21	1	(5)	19	1	1	-	-
<i>Reithrodontomys megalotis</i> (western harvest mouse)	8	0	(0)	8	-	-	-	-
<i>Microtus</i> spp. (meadow mouse)	12	0	(0)	10	2	-	-	-
<i>Ondatra zebethica</i> (muskrat)	19	2	(11)	13	4	2	-	-
<i>Otospermophilus beecheyi</i> (Beechey ground squirrel)	112	7	(6)	98	7	1	4	2
<i>Callospermophilus lateralis</i> (gold-mantled ground squirrel)	34	0	(0)	33	1	-	-	-
<i>Eutamias</i> spp. (chipmunk)	85	1	(1)	78	6	1	-	-
<i>Sciurus griseus</i> (Western gray squirrel)	1	0	-	1	-	-	-	-
<i>Tamiasciurus douglasii</i> (Douglas squirrel)	3	2	-	-	1	2	-	-
<i>Perognathus parvus</i> (pocket mouse)	22	0	(0)	16	6	-	-	-
<i>Dipodomys heermanni</i> (kangaroo rat)	17	0	(0)	12	5	-	-	-
<i>Rattus</i> spp. (Norway and brown rat)	30	3	(10)	15	12	3	-	-
<i>Mus musculus</i> (house mouse)	83	0	(0)	76	7	-	-	-
<i>Erethizon dorsatum</i> (porcupine)	6	0	(0)	6	-	-	-	-
Totals	759	21	(3)	678	60	13	6	2

\*Reciprocal of highest serum dilution showing agglutination reaction; agglutination at dilutions ≥1:4 is considered a specific reaction; 0 = nonreactor.

Many of the high prevalence rates among birds were reflections of small sample sizes and the location of the collections. There was a tendency for the ground feeding birds that gleaned insects and seeds in livestock areas to have a higher prevalence of seropositives. For instance, most of the seropositive sparrows were collected around barns and pastures inhabited by sheep endemic for *Coxiella*. The Q-fever organism can often be isolated with ease from the dust, soil, and water of such habitats.<sup>41</sup>

It is interesting to note that among 82 linnets tested, none were seropositive. Several other avian species also provided no seroevidence of infection, but these findings were based on less than 20 birds for any species.

**Titers.** In general, the antibody titers of both small rodents and birds were in the low (1:4 to 1:8) range; 47% of the seropositive bird sera and 90% of the test positive rodent sera were in this range.

The greater proportion of higher titers (≥16) among some of the birds and in 2 of

TABLE 2. Distribution of agglutinating antibodies against *C. burnetti* in birds, Northern California, 1975-76.

Genus and species (common name)	Tested			Reciprocal of titer*				
	No.	Pos.	(%)	0	2	4	8	≥16
<i>Anas platyrhynchos</i> (mallard)	14	1	(7)	-	13	1	-	-
<i>A. cyanoptera</i> (cinnamon teal)	4	1	-	1	2	1	-	-
<i>A. acuta</i> (pintail)	5	0	-	1	4	-	-	-
<i>A. strepera</i> (grey duck)	1	1	-	-	-	1	-	-
<i>Olor columbianus</i> (whistling swan)	1	1	-	-	-	1	-	-
<i>Branta canadensis</i> (Canada goose)	1	0	-	-	1	-	-	-
<i>Columba fasciata</i> (band-tail pigeon)	31	3	(10)	20	8	3	-	-
<i>Zenaidura macroura</i> (mourning dove)	9	0	(0)	8	1	-	-	-
<i>Buteo jamaicensis</i> (red-tailed hawk)	1	1	-	-	-	-	1	-
<i>Cathartes aura</i> (vulture)	4	2	-	2	-	1	-	1
<i>Oreortyx pictus</i> (mountain quail)	6	0	-	4	2	-	-	-
<i>Phasianus colchicus</i> (ring-necked pheasant)	28	1	(4)	17	10	1	-	-
<i>Gallus domesticus</i> (chicken)	19	0	(0)	2	17	-	-	-
<i>Fulica americana</i> (American coot; mudhen)	33	15	(45)	7	11	13	2	-
<i>Aphelacoma californicus</i> (California jay)	6	1	-	5	-	1	-	-
<i>Corvus corax</i> (raven)	2	1	-	-	1	-	-	1
<i>C. brachyrhynchos</i> (crow)	41	12	(29)	15	14	7	2	3
<i>Cyanocitta stelleri</i> (Stellar's jay)	9	0	(0)	7	2	-	-	-
<i>Pica pica hudsoni</i> (magpie)	3	0	-	1	2	-	-	-
<i>Euphagus cyanocephalus</i> (Brewer's blackbird)	18	6	(33)	11	1	1	2	3
<i>Junco oreganus</i> (Oregon junco)	2	1	-	-	1	1	-	-
<i>Zonotrichia atricapilla</i> (gold-crowned sparrow)	10	8	(80)	2	-	-	-	8
<i>Z. leucophrys</i> (white-crowned sparrow)	48	34	(57)	9	5	6	-	28
<i>Sturnus vulgaris</i> (starling)	157	18	(11)	83	56	7	-	11
<i>Passer domesticus</i> (English sparrow)	14	7	(50)	5	2	1	-	6

TABLE 2. (continued)

Genus and species (common name)	Tested			Reciprocal of titer*				
	No.	Pos.	(%)	0	2	4	8	≥16
<i>Sturnella neglecta</i> (meadowlark)	2	1	-	1	-	-	1	-
<i>Turdus migratorius</i> (robin)	19	3	(16)	13	3	2	-	1
Totals	583**		(20.2)	309**	156	48	8	62

\*An agglutinating reaction  $\geq 3+$  at  $\geq 1:4$  serum dilution was considered positive; 0=nonreactor

\*\*Several species tested had no seroreactors, including: 82 *Carpodacus mexicanus* (linnet), 4 *Agelaius phoeniceus* (red-winged blackbird), 4 *Colaptes cafer* (red-shafted flicker), 3 *Pipilo fuscus* (brown towhee), and one each of *Psaltiriparus minimus* (common bushtit) and *Bubo virginianus* (great horned owl).

the Beechey ground squirrels probably reflect their place of collection, i.e. sheep pastures.

**Geographic Distribution.** Although there was serological evidence of *C. burnetii* among wildlife in all sections of Northern California (Figure 1), there was a tendency for the highest MA antibody prevalences and titers to be among wildlife species collected near high densities of livestock.

## DISCUSSION

The prevalence of agglutinating antibodies in rodents sampled on nearly a statewide basis in this study was 3% in comparison to 14% prevalence of CF antibodies among rodents on a 2020 ha sheep range in a previous study.<sup>11</sup> A comparative study of CF antibodies and agglutinins (by the CAT) in wildlife showed that the prevalence of CF antibodies was approximately three times higher among rodents than was the prevalence of agglutinins (15% versus 5%).<sup>14</sup> Inasmuch as the MA test selects for the IgM antibodies, which appear soon after infection and decline thereafter at a relatively rapid rate, the

lower antibody ratio and titers in the present study may be due to earlier infections that were no longer detectable by the MA test.

Many of the sera showed agglutination at the 1:2 dilution, including 60 of the 759 rodents tested and 156 of the 583 birds sampled. While there is a common tendency to consider this a "nonspecific" reaction, experience with recovering *C. burnetii* from field samples of livestock, wildlife, and arthropods by laboratory mouse inoculation, indicates that a 1:2 reaction in some species may be specific.<sup>6,15</sup> In view of the ubiquitous distribution of strains of *C. burnetii* with low pathogenicity, a titer of 1:2 could indeed be indicative of exposure to the organism.

Early studies in Australia presented evidence for a simple cycle of Q-fever maintained in bandicoots and their ticks.<sup>42</sup> Since that time, there have been numerous reports of the disease agent in wild vertebrates and their ectoparasites throughout the world.<sup>3-5</sup>

In the United States, *Coxiella* was first isolated from wild animals in Utah.<sup>3,4</sup> Subsequent studies found much additional evidence of infection, with an epizootic occurring in wild animals of

Utah in 1960.<sup>40</sup> Serologic evidence of infection by *Coxiella* has been found for the following common genera of wild rodents in the United States and Canada: *Citellus* (*Otospermophilus*; *Callospermophilus*), *Dipodomys*, *Eutamias*, *Microtus*, *Mus*, *Neotoma*, *Onychomys*, *Perognathus*, *Peromyscus*, *Reithrodontomys*, *Sciurus*, and *Thomomys*.<sup>3,9,13,14,24,35-37,40</sup>

The present serological survey has confirmed many of these, and helps to define the extent to which the organism is distributed in wild rodents.

Evidence of the susceptibility of birds to infection by *C. burnetii* is available from a wide range of geographic areas.<sup>1,2,4,15,24</sup> However, the potential hazards of Q-fever infection, due to exposure to birds is not very well defined, for among 149 bird ringers studied recently in Q-fever-free Finland, only one had a titer of 8.<sup>27</sup> In that same study, the first cases of Q-fever in Finland were described, and *all* had acquired the infection through a visit to a country where the disease is endemic.

The present study confirms findings from our earlier work in Northern California<sup>15</sup> that the following common genera of birds have serological evidence of Q-fever infection: *Anas*, *Aphelocoma*, *Buteo*, *Cathartes*, *Columba*, *Corvus*, *Euphagus*, *Turdus*, and *Zonotrichia*.

Furthermore, extensive testing of starlings indicated relatively high prevalence of agglutinating antibodies in this species.

Epidemiologic evidence indicates that *C. burnetii* may have diverged substantially from the usual vertebrate-arthropod life cycle in nature that has been commonly accepted as descriptive for rickettsiae. As the ever-expanding livestock populations encroach into wildlife habitats, the rickettsia appears to be adapting into an agent for which the primary mode of infection among

wildlife as well as livestock and man is via inhalation or ingestion. *C. burnetii* is reinforced in nature by ungulates including cattle, sheep, goats, and deer which have access to nearly every wildlife habitat. Large numbers of the organism are present in the placentas of infected animals and are expelled into the environment where the resistant organisms can remain viable over long periods of time. The infection can therefore be spread to a new host that forages in contaminated areas. This is especially evident in flocks of ground feeding birds which may have 100% prevalence when feeding near livestock. Similarly, evidence of infection among wildlife is more common among those that are near livestock than those in remote areas.

The present study confirms the relatively high prevalence of antibodies (albeit agglutinins) among birds commonly found around barns and domestic animal enclosures. Also, rodents in these same areas were more likely to have agglutinating antibodies against *C. burnetii* than were rodents remote from domestic animal contact. Although our study covered a period of almost two years' duration, sufficient rodents of any species were not tested during the entire course of the study to be able to determine seasonal changes in the prevalence of agglutinating antibodies. Such seasonal patterns, if they exist, would be useful in further defining the ecology-epidemiology of Q-fever at the wildlife-livestock interface. Small rodents, because of their limited home range and relatively short life spans, would seem to be almost ideal "sentinels" in defining and describing the extent to which sheep, cattle, and wild ungulates may be shedding and spreading the organism in the environment. Further studies of small rodents, possibly patterned after the extensive earlier studies in Utah<sup>35-37,40</sup> and in California<sup>14</sup> could provide important information on the current status of Q-fever at the wildlife livestock interface.

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