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Q-fever Antibodies in Birds¹

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Abstract

Serum samples were obtained from 307 birds collected on a sheep range (Hopland Field Station) in northern California. Forty (13%) of these birds had agglutinating antibodies to *Coxiella burnetii*. At a nearby dairy farm, sera from 49 of 129 (38%) birds tested were positive for Q fever antibodies. In both areas the birds with the highest antibody prevalence were the carrion-eating birds (crows, ravens and turkey-vultures) and those birds (Brewer's and red-winged blackbirds, golden-crowned sparrows and pigeons) that live and feed in close proximity to infected livestock. The extent to which migratory birds are involved in the ecology of this zoonosis is uncertain. Immigrant birds may have been exposed to Q fever prior to their arrival in the area; however, emigrating birds have the potential to disperse the rickettsiae from such areas where livestock are infected with *C. burnetii*.

Introduction

Coxiella burnetii, the rickettsia that causes Q fever in man, has been demonstrated in domestic and wild birds as well as in a wide variety of mammals and ectoparasites.^{2,5,15,16} Among birds the organism has been isolated from the kidney of a pigeon, the spleen and liver of wild birds, and the ectoparasites (*Ornithomyia biloba*) of swallows.¹⁶ Rickettsiae have also been recovered from the spleen, kidney and stools of experimentally infected hens.¹⁶ Human infections of Q fever have occurred from eating raw eggs from infected hens.¹⁸ Serological evidence of Q fever is highest in birds living on farms inhabited by infected domestic stock and is lowest in birds living independently of human activities.¹⁶

In an ecological study of *C. burnetii* among the livestock and wildlife of Hopland Field Station⁴ parturient ewes were found to be an important reservoir host of enzootic Q fever in the study area. The possibility of birds spreading the Q fever organism in their range because of involvement in the ecology of this rickettsia should not be overlooked. Infection via the respiratory system from intratracheal exposure to aerosols containing the rickettsiae has been demonstrated in sheep,¹ and birds have been experimentally infected through the trachea and orally.¹²

The results of studies of *C. burnetii* in wild birds during a three year investigation of Q fever in an area shared by livestock and wildlife are reported here.

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Study Area

The University of California field station is four miles east of Hopland, Mendocino County, California. Elevation of the 5,000 acre field station ranges from 500 feet at the Russian River on the west boundary of the station, to about 3,000 feet. Cover is typical of this part of state, consisting of a grass and oak woodland association intermixed with patches of chaparral. Zonally, it is predominantly Upper Sonoran with occasional traces of humid Transitional vegetation such as Douglas fir (*Pseudotsuga menziesii*) and madrone (*Arbutus menziesii*).

Hopland Field Station is a multipurpose agricultural and range experimental field station extensively utilized for research with sheep and wildlife. Approximately 1,200 sheep are maintained in an area with native birds and mammals.

Birds collected at the feedlot on a dairy farm at Talmage, 10 miles north of Hopland, were also tested for Q fever antibodies. Cattle are the dominant species of domestic animals on the feedlot, but sheep, hogs and horses are also present.

Materials and Methods

Blood samples were collected from birds that had been trapped or shot. Trapped birds were anesthetized with ether, and blood from the jugular vein was collected in test tubes. Blood samples were allowed to coagulate at ambient temperature or were placed in warm water to enhance coagulation. Clots were removed from the tubes and residual blood cells separated from the serum by centrifugation. Serum samples were frozen and stored at -22°C until tested. The period of storage ranged from a few days up to a maximum of 6 months; most serum samples were tested within 2 months after collection. All bird sera were tested with capillary tube agglutination tests (CAT) by the method of Luoto⁷ as described for birds.¹² Two hundred ninety

four serum samples were tested using the direct complement fixation (CF) test.⁸ For comparison of tests, 71 of the sera tested by CAT were also tested using the microtiter system of the indirect complement fixation (ICF) test.^{6,11} The Nine Mile strain of CF antigen (Lederle) and a CAT antigen obtained from CDC* were used for these tests.

Portions of spleens from birds were homogenized in a 10% saline suspension and 0.5 ml was injected intraperitoneally into Swiss mice or hamsters in attempts to isolate *C. burnetii*. A specific antibody response using serum collected from these laboratory animals by cardiac puncture 30 days after injection was an indication the bird's spleen harbored *C. burnetii*.

Results

Three hundred seven birds of 31 species were collected from the Hopland Field Station. Forty (13%) of these birds had agglutinating serum antibodies for Q fever rickettsiae (Table 1). Similar data were collected from 11 species of birds of 7 families found on the dairy feedlot (Talmage). Forty-nine of 129 (38%) sera tested from birds on the feedlot had antibodies to *C. burnetii*.

An analysis of the prevalence of agglutinating antibodies to *C. burnetii* relative to migration¹⁶ and food habits¹⁷ is summarized in Table 2. Species that include

carrion and vertebrates in their diet had the highest prevalence (33-67%) of agglutinating antibodies to *C. burnetii*. Species whose diet includes principally plants (seeds) and invertebrates (insects and worms) had antibody prevalence of 20%. The lowest prevalence was observed among the species whose diet includes mainly seeds and plants (5% prevalence), and among birds that subsist on insects (none of 4 tested had antibodies).

*Center for Disease Control, Atlanta, Georgia 30333.

TABLE 1. Results of serologic tests for agglutinating antibodies of *Coxiella burnetii* in birds collected from Hopland Field Station and a dairy feedlot in Talmage, Mendocino County, California, from October, 1964 through March, 1967.

Family — Species	Common Name	Food ¹	Res. ²	Hopland ³		Talmage ³	
				No. Pos./Total	% ⁴	No. Pos./Total	% ⁴
<i>Anatidae</i>							
<i>Anas platyrhynchos</i>	Mallard	P	M R	1/12	8	—	—
<i>Cathartidae</i>							
<i>Cathartes aura</i>	Turkey Vulture	C V	SV	9/16	56	—	—
<i>Accipitridae</i>							
<i>Accipiter striatus</i>	Sharp-shinned Hawk	V I	WV	0/3	—	0/1	—
<i>A. cooperii</i>	Cooper's Hawk	V I	WV	0/2	—	—	—
<i>Buteo jamaicensis</i>	Red-tailed Hawk	C V I	R	1/3	—	—	—
<i>Falconidae</i>							
<i>Falco sparverius</i>	Sparrow Hawk	V I	R	1/1	—	—	—
<i>Phasianidae</i>							
<i>Lophortyx californicus</i>	California Quail	P	R	0/29	0	—	—
<i>Columbidae</i>							
<i>Columba livia</i>	Domestic Pigeon	P	R	2/6	33	1/5	20
<i>Zenaidura macroura</i>	Mourning Dove	P	M R	0/21	0	—	—
<i>Picidae</i>							
<i>Colaptes cafer</i>	Red-shafted Flicker	I P	R WV	0/7	0	0/2	—
<i>Melanerpes formicivorus</i>	Acorn Woodpecker	I P	R	0/3	—	—	—
<i>Corvidae</i>							
<i>Aphelocoma coerulescens</i>	Scrub Jay	I P	R	2/29	7	—	—
<i>Corvus corax</i>	Common Raven	C V	R	2/3	—	—	—
<i>Corvus brachyrhynchos</i>	Common Crow	C V I	R	—	—	7/12	58

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TABLE 1. continued

Family — Species	Common Name	Food ^①	Res. ^②	Hopland ^③		Talmage ^③	
				No. Pos./Total	% ^③	No. Pos./Total	% ^③
<i>Mimidae</i>							
<i>Toxostoma redivivum</i>	California Thrasher	I P	R	1/8	13	—	—
<i>Turdidae</i>							
<i>Turdus migratorius</i>	Robin	P I	R WV	2/43	5	—	—
<i>Sialia mexicana</i>	Western Bluebird	I	R	0/2	—	—	—
<i>Icteridae</i>							
<i>Agelaius phoeniceus</i>	Red-winged Blackbird	P I	SV	7/24	29	27/72	38
<i>Euphagus cyanocephalus</i>	Brewer's Blackbird	P I	SV	0/3	—	13/19	68
<i>Molothrus ater</i>	Brown-headed Cowbird	P I	SV	—	—	1/2	—
<i>Fringillidae</i>							
<i>Carpodacus mexicanus</i>	House Finch	P I	SV	0/2	—	—	—
<i>Pipilo erythrophthalmus</i>	Rufous-sided Towhee	I P	R	1/7	14	—	—
<i>P. fuscus</i>	Brown Towhee	I P	R	3/22	14	—	—
<i>Zonotrichia leucophrys</i>	White-crowned Sparrow	P I	WV	0/2	—	0/13	0
<i>Z. atricapilla</i>	Golden-crowned Sparrow	P I	WV	8/58	14	—	—
<i>Passerella iliaca</i>	Fox Sparrow	P I	WV	0/4	—	—	—
TOTALS (including species listed below)		31 species	40/307 — 13% Pos.	11 species	49/129 = 38% Pos.		

① Food habits; P = Plants (vegetation, seeds or fruit), C = carrion, V = vertebrates (birds and small mammals), I = invertebrates (insects, grubs and worms).

② R = Resident; M = migratory (Spring and Fall); SV = summer visitor (May through Sept.), WV = winter visitor (Oct. through Feb.) MR = migratory birds with resident populations.

③ One bird of each of the following species was tested but was negative: At Hopland: Mountain Quail (*Oreortyx pictus* P-R³), American coot (*Fulica americana* P-WV), Band-tailed Pigeon (*Columba fasciata*, P-R), Pygmy Owl (*Glaucidium gnoma*, IV-R), Black Phoebe (*Sayornis nigricans*, I-R), Olive-sided Flycatcher (*Nuttallornis borealis*, I-SV), and Steller's Jay (*Cyanocitta stelleri*, P-I-R). At Talmage: Bantam Rooster (*Gallus domesticus*, IP-R), Western Meadowlark (*Sturnella neglecta*, IP-R), and Tricolored Blackbird (*Agelaius tricolor*, IP-R).

④ 5 or more samples only.

TABLE 2. The prevalence of agglutinating antibodies to *Coxiella burnetii* in birds by food habits and residency.

Residency	FOOD									
	Carion and Vertebrates		Carion, Vertebrates, and Invertebrates		Invertebrates and Plants		Plants		TOTAL	
	Ratio ¹	% ²	Ratio	%	Ratio	%	Ratio	%	Ratio	%
Summer Visitor ³	9/16	56	—	—	48/122	39	—	—	57/138	41
Resident ⁴	2/3	—	8/15	53	7/73	10	4/64	6	21/155	14
Winter Visitor ⁵	—	—	—	—	10/129	8	0/1	—	10/130	8
TOTAL	11/19	57	8/15	53	65/324	20	4/65	6	88/423	21

¹ Number positive/number tested² Percent indicated when 5 or more birds were tested³ Includes species that are summer visitors with resident populations⁴ Includes migratory birds with resident populations⁵ Includes species that are winter visitors with resident individuals

The species were grouped by their migratory or residential habits to determine if a seasonal factor was involved in their exposure to this agent. The three groups were a) species that are essentially summer visitors, but may include some individuals that are resident all year, b) resident species and those that migrate through northern California in the spring and fall (e.g. ducks and doves) but also have resident populations, and c) the winter visitors including the species with resident individuals. The turkey vultures are essentially a resident species but are absent from the Hopland area from late December to early February and therefore were classed as summer visitors.

The highest prevalence of agglutinating antibodies to *C. burnetii* was in the species of birds that were summer visitors (41%). The resident birds had a prevalence of 14% and the winter visitors had a prevalence of 8%. However, strict interpretation of exposure to Q fever by seasonal migration and residency is complicated by the diets (e.g., carrion or plants) and local habitats (e.g., barnyards, pastures or woodlands) used by various species.

Sera from 71 birds collected from January to November, 1965, were tested by the indirect complement fixation (ICF) test and the CAT (Table 3). Using the CAT test, 35% (25/71) of the sera were positive, and using the ICF test, 24% (17/71) of the sera were positive. Fourteen percent (10/71) were antibody positive by both techniques, and 55% (39/71) were negative by both techniques.

TABLE 3. A comparison between the capillary tube agglutination (CAT) test and the indirect complement fixation (ICF) test for Q fever antibodies in bird serum.

ICF	CAT		
	Pos.*	Neg.	Total
Pos.	10	7	17
Neg.	15	39	54
TOTAL	25	46	71

* Positive at 1:4 for Indirect CF or undiluted serum for CAT.

Six of 294 sera tested by the direct CF test were antibody positive and 10 were anticomplementary. The ability for avian serum to react in the direct CF test appears to depend upon individuals within certain avian species. The serum from 2 of 16 turkey vultures, 3 of 33 robins and 1 of 22 red-winged blackbirds were positive in the direct CF test. One serum

sample from a turkey vulture and one from a red-winged blackbird also gave positive results in the indirect CF test and the CAT.

Our attempts to isolate the rickettsia from spleen tissues of 149 birds (19 species) by intraperitoneal inoculation of laboratory animals were unsuccessful.

Discussion

Food habits, residence and seasonal migration were salient factors influencing the exposure of birds to *C. burnetii* and contributed to the differences in antibody prevalence among the avian species in this study. The high prevalence of serum antibodies to *C. burnetii* among the species that eat carrion (vultures, ravens and crows) can be attributed to their feeding on dead sheep, placentas from postparturient ewes, dead animals at the roadside or other forms of contaminated carrion. The carnivorous species (red-tailed hawks and sparrow hawks) that feed on small birds and mammals probably are exposed to the rickettsiae while feeding on infected prey.

Species of birds that eat plants, grain, insects and grubs seem more likely to be exposed to *C. burnetii* as a result of where they feed rather than what they eat. Brewer's and red-winged blackbirds and brown-headed cowbirds frequent stockyards, pastures and barns, feeding and roosting among livestock where contamination of the air and soil occurs with this resistant organism, especially during lambing and calving. These birds are exposed to infectious aerosols or dust and contaminated food in the area. Domestic pigeons are another example of exposure by habitat. Although pigeons, doves and quail are all granivorous, 27% of the pigeons had antibodies but none of the doves or quail tested had detectable antibodies to this agent. The pigeons roosted in barns and around livestock buildings whereas the other species were found in the open or in bushy thickets. Birds that feed principally on insects appear to avoid exposure to *C. burnetii* by foraging in the underbrush or in the areas away from concentrations of this

organism. Infection among these species probably occurs from incidental airborne exposure to rickettsia of animal origin.

The 38% prevalence of antibodies to *C. burnetii* in birds collected on the dairy feedlot (Talmage) compared to the 13% prevalence at the Hopland sheep range reflects in part differences in the concentration and kind of livestock at these locations, and differences in food habits in the species of birds that were collected from each area.

Seasonal migration or characteristics of residence are also factors affecting the exposure to *C. burnetii* among birds. Large numbers of *C. burnetii* are released during the parturition of livestock, and in urine and feces following parturition.¹⁷ This shedding of rickettsia produces a persistent focus of infection for wildlife.⁴ Consequently, summer visitor and resident birds that are in the area immediately following the spring lambing season (late December through February) and during calving (summer) have a greater risk of infection than the migratory or winter visitor birds. On the other hand migratory birds have the potential of becoming infected in one portion of their range and transporting the organism over wide areas. This possibility needs further investigation.

The CAT appears to be the test of choice for the detection of antibodies to *C. burnetii* in bird serum. It has been demonstrated that avian serum is incompatible with mammalian complement⁸ which may account for the apparent lack of antibodies to this agent in birds during earlier studies in this country.¹⁴ Although avian serum occasionally gave

positive results (6/294) in the direct CF test, the indirect CF test or modifications of the CF test using avian complement^{9,10} should be tried.

The extent to which birds transmit the Q fever organism among man and animals remains obscure. However, serolo-

gical evidence of a high rate of infection in certain groups of birds and the ability for *C. burnetii* to be passed in avian stools and eggs indicate that birds could be a source of Q fever infection. Further investigations should be directed into this aspect of the ecology of Q fever.

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