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Source: Journal of Wildlife Diseases, 34(2) : 310-316

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-34.2.310>

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COXIELLOSIS IN DOMESTIC AND WILD BIRDS FROM JAPAN

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ABSTRACT: Serological evidence of infection with *Coxiella burnetii* was found in 41 (2%) of 1,951 domestic birds and in 167 (19%) of 863 wild birds from 17 and 5 prefectures in Japan, respectively, by microagglutination (MA) test. The bacteriological evidence of the infection was found in 17 (41%) of 41 domestic birds and 37 (22%) of 167 wild birds by the nested polymerase chain reaction (PCR). In addition, *C. burnetii* was isolated from five each of serum, spleen and fecal specimens from five jungle crows (*Corvus macrorhynchos*) (whose sera were positive by both the MA test and PCR) by inoculating laboratory mice. Domestic quail (*Coturnix coturnix japonica*) (3%), domestic muscovy ducks (*Cairina moschata*) (3%), domestic chickens (2%), domestic mallards (*Anas platyrhynchos domesticus*) (2%), carrion crows (*Corvus corone*) (37%), jungle crows (35%), and wild rock doves (*Columba livia*) (6%) showed serologic evidence of experience with *C. burnetii*. There was a tendency for a high prevalence among birds living and/or feeding in close proximity to infected livestock. This suggests that these birds are one of the less important links in maintaining the whole cycle of *C. burnetii* infection.

Key words: *Coxiella burnetii*, domestic birds, microagglutination test, nested polymerase chain reaction, Q fever, survey, wild birds.

INTRODUCTION

Q fever is caused by *Coxiella burnetii*, an organism widely distributed in nature and responsible for the infection in arthropods, domestic and wild animals, and humans. Infected cattle, sheep, and goats are the known primary reservoirs for humans. The placentas of these animals may be heavily infected and at the time of parturition aerosols are created. When inhaled, these may result in a variety of clinical responses in humans, including a self-limited febrile illness, atypical pneumonia, hepatitis, or rarely endocarditis (Lang, 1990).

Coxiella burnetii in domestic and wild birds has been reported. Babudieri and Moscovici (1952) found that rock doves (*Columba livia*) and geese (*Anser anser domesticus*) may be naturally infected with *C. burnetii* in Italy. Syrucek and Raska (1956) demonstrated natural infections in certain domestic and wild birds in Czechoslovakia. Zhmaeva et al. (1955) isolated the

organism from nestling field sparrows (*Passer montanus pallidus*) in Russia.

Serological evidence of *C. burnetii* in domestic animals was reported by Omori et al. (1952), and Kitaoka (1954) in Japan. In previous studies (Htwe et al., 1992 a, b; Ejercito et al., 1993; To et al., 1995, 1996; Nagaoka et al., 1996), we showed the serological and bacteriological evidence of this infection in several species of animals as well as in human populations in Japan.

Because a high prevalence of antibodies to *C. burnetii* was detected in a number of wild mammals in Japan, we suspect a broad sylvatic reservoir. This study was initiated to determine if *C. burnetii* also is reservoirized in domestic and wild birds in Japan.

MATERIALS AND METHODS

Individual serum samples were aseptically collected from domestic and wild birds in 18 prefectures of Japan (33° to 45°N, 130° to 145°E) (Fig. 1). The domestic birds included domestic chickens, quail (*Coturnix coturnix ja-*

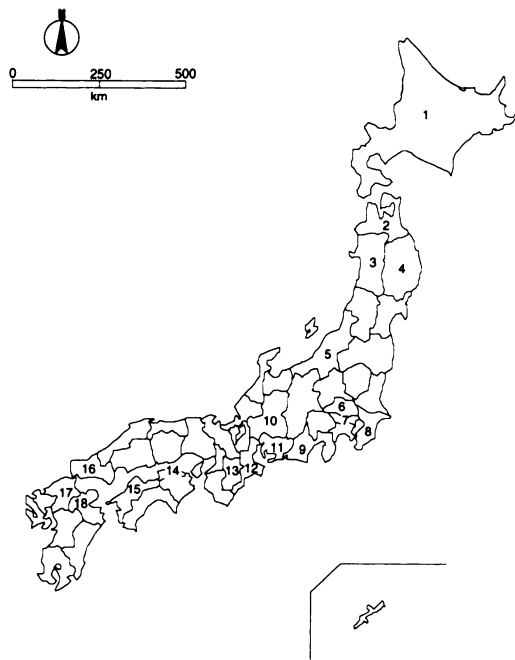


FIGURE 1. A map of Japan showing localities where bird samples were collected for testing for *Coxiella burnetii*: 1, Hokkaido; 2, Aomori; 3, Akita; 4, Iwate; 5, Niigata; 6, Saitama; 7, Tokyo; 8, Chiba; 9, Shizuoka; 10, Aichi; 11, Gifu; 12, Mie; 13, Nara; 14, Kagawa; 15, Ehime; 16, Yamaguchi; 17, Fukuoka; 18, Oita.

ponica), domestic mallards (*Anas platyrhynchos domesticus*), and domestic muscovy ducks (*Cairina moschata*). Wild birds included carrion crows (*Corvus corone*), jungle crows (*Corvus macrorhynchos*), rock doves (*Columba livia*), wild mallards (*Anas platyrhynchos*), and whooper swans (*Cygnus cygnus*). Blood samples were obtained by jugular veinpuncture. Serum, spleen and stool samples from 41 jungle crows were collected for isolation of *C. burnetii* (Table 1). The samples were frozen and stored at -30°C until tested.

All sera were tested with the microagglutination (MA) test by the method of Fiset et al. (1969). Phase II *C. burnetii* antigen of Nine Mile strain produced from highly purified suspension of phase I *C. burnetii* by extraction with trichloroacetic acid (TCA) (referred to as TCA-phase II antigen) was obtained from Jan Kazar (Slovak Academy of Sciences, Slovak Republic) was used for the MA test. In the dilution used, this TCA-phase II antigen was stable and was agglutinated not only by homologous sera, but also by sera of guinea pigs and mice infected with other prototype strains or Japanese strains. At the same time, this antigen re-

acted very well with the sera obtained from Q fever patients and animals with coxiellosis but did not react with the indirect immunofluorescence (IF) test-negative sera from humans and animals. Chicken and jungle crow sera positive and negative by IF and MA tests, respectively, were used as controls. Two each of positive and negative controls were run with each test. An antibody titer of 1:8 was considered positive. The positive samples at the endpoint titer of 1:8 were proven by rerunning one more time.

The MA test-positive sera, spleen tissue, and fecal samples were tested for *C. burnetii* by using the nested polymerase chain reaction (PCR) with two pairs of oligonucleotide primers (Q5, 5'-GCG GGT GAT GGT ACC ACA ACA-3'; Q3, 5'-GGC AAT CAC CAATAA GGG CCG-3'; and Q6, 5'-TT GCT GGA ATG AAC CCC A-3'; Q4, 5'-TC AAG CTC CGC ACT CAT G-3') derived from the *C. burnetii* *htpB* gene (1,658 bp) of a 62-kDa antigenic polypeptide (Vodkin and Williams, 1988). The nested PCR was performed as described previously (To et al., 1996).

The PCR-positive serum, spleen and fecal samples from carrion and jungle crows trapped in Mie and Gifu Prefectures were used for isolation of *C. burnetii*. The isolation procedure was similar to that described previously (To et al., 1996), with minor modifications including (1) spleen and fecal samples were prepared as 10 and 20% suspensions (w/v) in sucrose-phosphate-glutamate, respectively, except that the sera were inoculated without further preparation; (2) each sample was inoculated into two A/J mice (Nihon Small Laboratory Animal Center, Shizuoka, Japan), each animal receiving 0.1 ml of serum, 0.5 ml of the spleen suspension or 0.1 ml of stool suspension intraperitoneally; and (3) sera and spleens of mice of the second passage were collected on the 14th day after inoculation and tested for the presence of antibody to phase II antigen, the antigen, and *C. burnetii* by an IF test, Giménez staining, and PCR, respectively. The IF test and Giménez staining were performed as described elsewhere (To et al., 1996). Mice which had an antibody titer $\geq 1:32$, had the organisms and IF antigens, and/or were positive by the nested PCR were considered to have evidence of the infection.

Differences in prevalence of antibodies among species were determined by the chi-square test for 2×2 contingency tables. Significance was inferred at $P \leq 0.05$.

RESULTS

Prevalence of *C. burnetii* among the domestic and wild birds is summarized in Ta-

TABLE 1. Results of microagglutination test, nested polymerase chain reaction, and isolation of *Coxiella burnetii* from domestic and wild birds in Japan.

Original sample	Common name	Geographical source	Sampling year	Total positive sample/total sample tested					
				MA test	%	Nested PCR	Isolation		
Domestic bird									
Serum	Chicken	17 ^a	1995	3/100	3	1	NT ^c		
		17	1995–96	26/1,000	3	11			
		9	1996	1/96	1	1			
		1–6, 8, 11–18 ^b	1996	2/393	1	0			
	Quail	10	1992–95	1/74	1	0			
		9	1992–95	4/100	4	2			
		17	1993–95	3/158	2	1			
	Domestic mallard	2	1994–95	0/11	0	0			
	Muscovy duck	3	1994–95	0/9	0	0			
		8	1994–95	1/10	10	1			
		Wild bird							
Serum	Rock dove	1	1982, 86	4/100	4	0	NT		
		12	1991–92	5/51	10	2			
		11	1991–92	3/50	6	1			
	Whooper swan	2	1994	0/10	0	0			
		12	1991–92	0/101	0	0			
	Wild mallard	11	1991–92	0/120	0	0			
		Carrion crow	1	1992–94	4/42	10		0	
	7		1992–94	27/50	54	3			
	12		1991–93	18/41	44	5			
	Jungle crow	11	1991–93	15/40	38	5			
		1	1992–94	6/45	13	0			
		7	1992–94	32/60	53	3			
		12	1991–93	14/50	28	6			
		11	1991–93	25/62	40	7			
	Serum	Jungle crow	11–12	1991–92	14/41	34		5/14	5/5
	Spleen	Jungle crow	11–12	1991–92	NT			5/41	5/5
	Stools	Jungle crow	11–12	1991–92	NT			5/41	5/5

^a (1–18) number of locations where samples collected as illustrated in Fig. 1.

^b 15 locations included 1 (25 samples), 2 (16), 3 (24), 4 (99), 5 (32), 6 (5), 8 (6), 11 (83), 12 (23), 13 (10), 14 (17), 15 (13), 16 (16), 17 (12) and 18 (11).

^c NT: not test.

ble 1. Overall, agglutination antibody to the phase II *C. burnetii* antigen was present in 208 (8%) of 2,814 sera. Of these, 54 (26%) sera were positive by the PCR. In addition, *C. burnetii* was isolated from serum, spleen and fecal samples of five jungle crows.

Of 1,951 domestic birds tested, 41 (2%) had the antibodies to *C. burnetii*. Antibody positive birds included quail (1 to 4%), muscovy ducks (0 to 10%), domestic chickens (1 to 3%) and domestic mallards (2%). The highest prevalences of antibodies were found among muscovy ducks orig-

inating from Chiba Prefecture (10%), quail originating from Shizuoka Prefecture (4%), and chickens from Fukuoka Prefecture (3%). A low prevalence (1%) was observed from chickens in Shizuoka and 15 other prefectures, and from quail at Aichi Prefecture. Antibodies to *C. burnetii* were not found in muscovy ducks from Aomori and Akita prefectures.

By the PCR, we detected *C. burnetii* in 17 (41%) of 41 domestic birds. The highest PCR-positive frequencies were found among quail from Shizuoka Prefecture (50%), and chickens from Fukuoka Pre-

TABLE 2. Distribution of agglutination antibodies to *Coxiella burnetii* in sera of domestic and wild birds from Japan.

Species	Total number tested	Titers of agglutination antibodies to <i>Coxiella burnetii</i>								
		2	4	8	16	32	64	128	256	≥512
Chicken	1,589	1,339	218	32	27	27	21	0	0	0
Quail	174	142	27	5	0	0	0	0	0	0
Domestic mallard	158	110	45	3	0	0	0	0	0	0
Muscovy duck	30	14	15	1	0	0	0	0	0	0
Carrion crow	173	102	81	64	50	50	43	35	25	14
Jungle crow	258	115	97	91	79	72	61	52	47	21
Rock dove	201	131	58	12	8	5	1	0	0	0
Wild mallard	221	189	32	0	0	0	0	0	0	0
Whooper swan	10	5	5	0	0	0	0	0	0	0
Total	2,814	2,147	578	208	164	154	126	87	72	35

fecture (41%). The organism was undetected in chickens from 15 prefectures and quail from Aichi Prefecture.

Of 863 wild birds, 167 (19%) were found to be seropositive for antibodies to *C. burnetii*. Antibody positive wilds included carrion crows (37%), jungle crows (35%) and rock doves (6%). The highest antibody prevalence was observed in carrion and jungle crows from suburbs of Tokyo (54 and 53%, respectively), Mie Prefecture (44 and 28%, respectively), and Gifu Prefecture (38 and 40%, respectively). Lower prevalences occurred in carrion and jungle crows from Hokkaido Prefecture (10 and 13%, respectively), and rock doves from Mie Prefecture (10%). Antibodies were not detected in wild mallard ducks from Gifu and Mie Prefectures, and from whooper swans from Aomori Prefecture. The prevalence of antibodies against *C. burnetii* was significantly higher in carrion and jungle crows than in the other species ($P < 0.001$). Difference in the seroprevalence between carrion and jungle crows was not statistically significant ($P > 0.05$).

The bacteriological evidence for the presence of *C. burnetii* in wild birds was found in 37 (22%) of 167 sera tested by using the PCR. The highest PCR-positive frequencies were found in the carrion and jungle crows from the prefecture of Mie

(28 and 43%, respectively), Gifu (33 and 28%, respectively), and Tokyo (11 and 10%, respectively). The organism was not detected in the carrion and jungle crows or rock doves from Hokkaido Prefecture. In addition, in the isolation experiment the IF antibodies were found in 15 pairs of mouse sera, while the IF antigens, the organisms, and DNA of *C. burnetii* were found only in 14 of 15 pairs of mouse spleen samples. The antibody titers to phase II antigen ranged from 1:64 to 1:512.

Distribution of agglutination antibodies against the phase II *C. burnetii* in domestic and wild birds is listed in Table 2. Among domestic birds, a high titer (1:16 or more) was observed in some chickens. Among wild birds, the greater proportion of higher titers (1:128 or more) was found only in the carrion and jungle crows.

There was serological evidence of *C. burnetii* infection among the birds in the north (Hokkaido Prefecture), central (Tokyo, Chiba, Shizuoka, Aichi, Mie and Gifu Prefectures) and south (Fukuoka Prefecture) of Japan. The bacteriological evidence was found only in samples collected in central and southern Japan. The absence of serological or bacteriological evidence in some prefectures may be due partly to small sample sizes and the location of the collections.

DISCUSSION

Our main objective for this study was to determine what species of domestic and wild birds in Japan were naturally infected with *C. burnetii*. We used the MA test to screen for the prevalence of antibodies to *C. burnetii*, and the nested PCR to detect the presence of the organism. The isolation experiment was used to determine shedding of *C. burnetii* into the environment. The MA test offers several advantages for screening potential host for *C. burnetii*, the most important of which are economy of time in performing and reading the test and economy of reagents. It has the same sensitivity and specificity as capillary agglutination (CA) test and agglutination suspension (Fiset et al., 1969). Recently, nested PCR has been used for detection of the organism in clinical samples, which seems to be the most promising direct technique because of its high sensitivity and specificity compared with those of isolation (Mallavia et al., 1990). Also, it is comparatively simple to perform and takes less time when a large number of samples are being tested (Willems et al., 1993; To et al., 1996).

The choice of the cutoff value of serological tests for *C. burnetii* depends upon the source and purity of the antigen, and the amount of background antigen stimulation in the host population to be studied (Marrie and Raoult, 1997). The cutoff value of MA test for *C. burnetii* varies from laboratory to laboratory. The positive cutoff value was suggested at 1:2 for phase I and trichloroacetic (TCA)-phase II antigens by Fiset et al. (1969), 1:8 for phase I antigen by Enright et al. (1971), 1:4 for TCA-phase II antigen by Biberstein et al. (1974), 1:8 for phase I antigen by Rarotra et al. (1978), and 1:4 for phase I antigen by Riemann et al. (1979). In the present study the TCA-phase II antigen of *C. burnetii* strain was stable and was agglutinated only by sera containing antibodies to *C. burnetii*. The negative serum controls showed partial agglutination at dilutions of

1:2 and 1:4 and a tight, regular button at dilutions of $\geq 1:8$, whereas the positive controls showed a film covering the bottom of the cup at dilutions of $\geq 1:2$ during the study. Therefore, we determined the titer of $\geq 1:8$ to be the positive cutoff value for the MA test.

Our results for prevalence of agglutination antibodies to phase II antigen were lower at 2% for domestic birds and higher at 19% for wild birds compared to the results obtained by Syrucek and Raska (1956) at 12 and 8%, respectively, in Czechoslovakia (complement-fixation test); by Fiset and Barber (recorded in a review of Marmion and Stoker, 1958) at 12% in domestic chickens in England (indirect complement-fixation test); by Enright et al. (1971) at 13% in wild birds in California (USA) (capillary agglutination (CA) test); by Rarotra et al. (1978) at 13% in domestic chickens in India (CA test); and by Riemann et al. (1979) at 20% in wild birds in California (MA test). The reasons for these discrepancies could be explained by differences in spatial, temporal, host, strain, and many other factors determining the prevalence of Q fever and animal coxiellosis, as well as possible differences among laboratories and testing procedure.

In our study, the bacteriological evidence of infection as determined by both the PCR and isolation from serum, spleen and stool samples suggested that these species of birds, especially the crows, are one of the less important links in maintaining the whole cycle of *C. burnetii* infection in nature. *Coxiella burnetii* has been isolated previously from blood, lung, liver, spleen, kidney and feces of birds infected experimentally or naturally (Babudieri and Moscovici, 1952; Syrucek and Raska, 1956; Zhmaeva et al., 1955; Schmatz et al., 1977 a, b).

Varying prevalences of *C. burnetii* across species of domestic birds from different areas in Japan could be reflective of small sample sizes or of actual differences in the prevalence of *C. burnetii* in these geographical areas. This coupled with dif-

ferences in food habits, residence versus seasonal migration, and other factors might explain discrepancies in the prevalence of infection among wild species. Syrucek and Raska (1956), Marmion and Stocker (1958), Enright et al. (1971), Rarotra et al. (1978), and Riemann et al. (1979) also showed that the prevalence of infection among birds living in close proximity to infected livestock was higher than these same hosts from other areas, where the infection was rare. High prevalence of the infection in domestic animals and humans has been well documented in central (Htwe et al., 1992 a, b; To et al., 1995, 1996; Nagaoka et al., 1996) and south (Oda and Yoshiie, 1989; Yoshiie et al., 1991; Yuasa et al., 1996) Japan.

Carion and jungle crows that are found commonly around barns and domestic animal enclosures and often are pecking and scraping animal waste or decaying flesh of animal carcasses seem much more likely to be exposed to *C. burnetii* than most other species. This helps explain the high prevalence of antibodies to *C. burnetii* in common crows (*Corvus brachyrhynchos*) in California as shown by Enright et al. (1971), and Riemann et al. (1979).

This study demonstrated that there was serological and bacteriological evidence of *C. burnetii* in birds from many areas of Japan, and there was an increased tendency toward high prevalences among bird species living and/or feeding in close proximity to infected livestock. Further, these findings provide suggestive evidence that birds are indicators of foci of infection existing in Japan. Now, studies on the prevalence of infection among species of wild rodents are necessary to further elucidate the epidemiology of Q fever in Japan.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in Aid for Developmental Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 07306015).

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Received for publication 28 April 1997.