# Prevalence of *Coxiella burnetii* Infection in Dairy Cattle with Reproductive Disorders

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ABSTRACT. The prevalence of *Coxiella burnetii* infection in 207 cattle with reproductive disorders was studied by using an indirect immunofluorescence (IF) test, nested polymerase chain reaction (PCR) and isolation. IF antibodies to phase I and phase II antigens of *C. burnetii* were found in 122 (58.9%) and 125 (60.4%) of the sera, respectively, and PCR-positives were found in 8 (3.9%) of the sera and in 51 (24.6%) of the milk samples. In addition, *C. burnetii* was isolated from 51 (24.6%) of the milk samples by inoculating laboratory mice. The results indicate that the IF test plus PCR are useful in the diagnosis of bovine coxiellosis. It is difficult to deny that dairy cattle with reproductive disorders would be one of the important reservoirs of *C. burnetii* responsible for infection in both animal and human populations in Japan. — KEY WORDS: bovine coxiellosis, *Coxiella burnetii*, nested polymerase chain reaction.

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Dairy cattle, in addition to sheep and goats, are considered to be the reservoirs of *Coxiella burnetii* responsible for the infection in animals and humans. Infected cattle shed enormous numbers of the organisms in their milk, birth fluid and placenta which are potential sources of the infection in animals and humans via inhalation of infectious aerosols or airborne dust [1–4, 11]. Although human Q fever is usually a clearly marked illness, bovine coxiellosis is rarely an overt disease, except in reproductive disorders (such as infertility, metritis and mastitis) in the females. An increase in the prevalence of *C. burnetii* infection in dairy cattle has been well documented, and its association with reproductive problems in these animals has been reported in Canada, the U.S.A., Cyprus, France, Hungary, Switzerland and West Germany [2, 10, 11].

In Japan, serological evidence of *Coxiella* infection in domestic and companion animals, wild animals and humans has been reported [5–9, 12, 16, 21–24]. In addition, several reports of isolation of *C. burnetii* from humans, cattle and ticks have been published [14, 15, 18, 19]. However, there are no records of the infection among dairy cattle with reproductive disorders. The purpose of this work was to investigate the prevalence of *C. burnetii* infection in cattle with reproductive disorders in central Japan.

Serum and raw milk samples were collected from 207 dairy cattle with reproductive disorders at Livestock Hygiene Service Centers of 4 Prefectures in central Japan (Chiba, Mie, Shizuoka and Gifu) from June to October, 1995. These cattle included 93 cases of infertility and 114 cases of metritis and mastitis. Follow-up samples were not obtained from the individuals.

The prevalence of antibodies to *C. burnetii* was determined by an indirect immunofluorescence (IF) test as described previously [7]. Twofold serum dilutions in phosphate-buffered saline (pH 7.2) from 1:32 to 1:4,096 were tested against fixed and purified antigens of Nine Mile phase I and phase II strains of *C. burnetii*. A fluorescein isothiocyanate-conjugated rabbit anti-bovine IgG (heavy and light chains) (Organon Teknika Co., Cappel Laboratories, U.S.A.) was used for determination of antibodies. Positive and negative controls were run with each test. Titers of 1:32 or more were considered positive.

The serum and milk samples were tested for the presence of the organism by using a 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 CAA TAA 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 [20]. Milk samples for the PCR were prepared as described elsewhere [13]. The nested PCR was performed as described previously [19].

Only the PCR-positive milk samples were used for isolation of *C. burnetii.* The procedure used for isolation of *C. burnetii* was similar to that described previously [18], with minor modifications as follows: (i) each sample was inoculated into 2 A/J mice, each animal receiving 1 m*l* intraperitoneally and (ii) 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, Gimenéz staining and PCR, respectively. Mice were considered to have the infection if they had an antibody titer of 1:32 or more, had the organisms and IF antigens, and/or were positive by the PCR. The IF test and Gimenéz staining were performed as described previously [18].

The prevalence of antibodies to *C. burnetii* in the 207 tested sera is shown in Table 1. Overall, the antibodies to phase I and phase II antigens were present in 122 (58.9%) and 125 (60.4%) of the sera, respectively. Of the 93 infertile cattle, 54 (58%) of the sera were found to have antibodies to phase I and phase II antigens. Of the 114 cattle with metritis and mastitis, 68 (59.7%) of the sera had antibodies to phase I antigens and 71 (62.3%) of the sera had antibodies

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Table 1. Detection rates of *Coxiella burnetii* using IF test, PCR and isolation from serum and milk samples from dairy cattle with reproductive disorders

| Clinical manifestation | No. of animals tested | Positive (%) by IF test | | Positive (%) by PCR | | Positive (%) by isolation |
|---|---|---|---|---|---|---|
| | | Phase I[a)] | Phase II[a)] | No. of sera | No. of milk | No. of milk[b)] |
| Infertility | 93 | 54 (58.0)[c)] | 54 (58.0) | 2 (2.2) | 19 (20.4) | 19 (20.4) |
| Metritis and mastitis | 114 | 68 (59.7) | 71 (62.3) | 6 (5.3) | 32 (28.0) | 32 (28.0) |
| Total | 207 | 122 (58.9) | 125 (60.4) | 8 (3.9) | 51 (24.6) | 51 (24.6) |

a) Against purified antigens of Nine Mile phase I and phase II. b) Only PCR-positive samples were used for isolation. c) Number of positive (positive %).

to phase II antigens. Many sera were positive at high-titer levels of over 1:512 to phase I and phase II antigens (data not shown).

The PCR-positives were found in 8 (3.9%) of the sera and 51 (24.6%) of the milk samples, and *C. burnetii* was isolated from 51 (24.6%) of the milk samples. The IF antibodies were found in 51 pairs of mouse sera, while the IF antigens, the organisms and DNA of *C. burnetii* were found only in 45 of 51 pairs of mouse spleen specimens. The antibody titers to phase II antigen ranged from 1: 64 to 1: 1,024.

In this study, we showed a high prevalence of *Coxiella* infection in dairy cattle with reproductive disorders and also demonstrated the usefulness of the IF test plus PCR in the diagnosis of bovine coxiellosis.

The prevalence of the infection in dairy cattle with reproductive disorders has been reported in several countries [11]. In Hungary, Rady *et al.* [17] reported that the seropositive rate was rather high among cattle with reproductive problems. In West Germany, Krauss *et al.* [10] reported that 40% of 1,193 selected serum samples from 84 herds with infertility problems had *Coxiella* antibodies, and 80% of the herds were positive, and in Canada, Cyprus, France, West Germany and Czechoslovakia, numerous abortions of cattle were attributed to *C. burnetii* infection [11].

This study shows that the IF test plus PCR is useful, and comparatively simple to perform, takes less time when a large number of samples are being tested, and also is a highly sensitive assay compared with the isolation for the diagnosis of bovine coxiellosis from serum and raw milk samples. This observation supports statements that anti-*C. burnetii* antibodies usually persist for a long time, but apart from the mammary gland, *C. burnetii* does not seem to persist for long in bovine tissue [1, 2, 11].

The prevalence of antibodies to *C. burnetii* in cattle with reproductive disorders seems to be higher than that in apparently healthy cattle (35.6% of the 424 sera collected from Chiba, Mie, Shizuoka and Gifu from 1989 to 1990 in a study by Hirai [6]). He also showed that *Coxiella* infection is widespread among dairy cattle. The present results suggest that *Coxiella* infection is highly associated with reproductive problems (infertility, metritis and mastitis) in cattle in Japan. The high prevalence of *Coxiella* infection in dairy cattle with reproductive problems showed that these infected cattle play an important role in maintaining the infection and in dispersing the pathogenic agent into the environment, where the resistant organism can remain viable over long periods of time through excretions, i.e., milk, colostrum, urine and birth fluid. Thus, such excretions are considered to be potential sources of the infection in animals and humans via inhalation of infectious aerosols or airborne dust [19, 21, 23].

The importance of bovine coxiellosis as a source of Q fever has been well documented [1–3, 11]. With such an understanding, control of bovine coxiellosis must be aimed at the root of the problem to prevent the spread of the infection in animal and human populations.

This study confirms statements that cattle are one of the important reservoirs of *C. burnetii* in Japan [6, 7, 18, 21, 24]. Clearly, further intensive studies on *Coxiella* infection among dairy cattle-farm workers and milk-processing workers and on the possible dangers of dairy products (milk, cheese and butter) will be needed to elucidate the epidemiology of Q fever in Japan.

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