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 Q-fever. 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.

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'-GGG GGT GAT GGT ACC ACA ACA-3'; Q3, 5'-GGG 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 ltpB 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 ml 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, Gimenez 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 Gimenez 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.
Table 1. Detection rates of Coxiella burnetii using IF test, PCR and isolation from serum and milk samples from dairy cattle with reproductive disorders

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>No. of animals tested</th>
<th>Positive (%) by IF test</th>
<th>Positive (%) by PCR</th>
<th>Positive (%) by isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase I(a)</td>
<td>Phase II(b)</td>
<td>No. of sera</td>
</tr>
<tr>
<td>Infertility</td>
<td>93</td>
<td>54 (58.0)</td>
<td>54 (58.0)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Mastitis and metritis</td>
<td>114</td>
<td>68 (59.7)</td>
<td>71 (62.3)</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>122 (58.9)</td>
<td>125 (60.4)</td>
<td>8 (3.9)</td>
</tr>
</tbody>
</table>

(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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かと思われた。

生理学
ウシ卵胞液中でのトロンピン形成に対するマグネシウムによる制御——山田 学・平橋佳代・井上美一・堀内啓俊・酒井淳一 1 部生物資源学
(2) 畜産科学) (Livestock Science) ................................. 837-842
ホルスタイン種乳牛から得た卵胞液中のカルシウム (Ca) とマグネシウム (Mg) の定量および両者のトロンピン形成における役割について血液成分と比較検討した。総 Ca 量は卵胞の発育に伴い増加したが、総 Mg 量は卵胞液の貯留初期の卵胞の初期発育初期に著しく高く、卵胞の発育に伴い漸減した。in vitro で卵胞液に Mg を添加したところ、卵胞の直径が 2 ㎜以下の卵胞から採取した卵胞液のプロトロンピン時間 (PT) は短縮したが、3 ㎜以上の直径の卵胞から採取した卵胞液では影響が認められなかった。しかし、いずれの発育時期の卵胞液においても、ラッセル蛇毒によって活性化した第 X 因子活性に対しては Mg の追加は作用の抑制を示した。以上の結果から、Ca 濃度の低い卵胞腔形成初期に Mg がトロンピン形成を緩やかに促進していることが明らかとなった。

公衆衛生学
繁殖障害の乳牛における Coxiella burnetii の浸染状態（短報）——To, Ho, Htwe, Khin Khin, 加古奈緒美, 金弘秋, 坂口淑女, 玉野克哉（岐阜大学農学部家畜微生物学講座）................................. 859-861
繁殖障害の乳牛における Coxiella burnetii 感染状況を 207 例の血清および生乳を用いた間接蛍光 (IF) 抗体法、ネスタドポリメラーゼ連鎖応答 (PCR) および分離により検討した。IF 抗体は、C. burnetii の 1 相に対し 122 例 (58.9 %) が、2 相に対し 125 例 (60.4 %) が陽性を示した。PCR では血清 8 例 (3.9 %) から、また生乳の 51 例 (24.6 %) から遺伝子が検出された。C. burnetii は、血清 51 例 (24.6 %) から分離された。以上の結果は、乳牛のクロシエラ症の発症和 IF と PCR が有用であること。また、繁殖障害を伴う乳牛が動物およびヒトの重要な感染源の一つになる可能性を示唆している。

タイ国の人口食及び食品から検出されたサルモネラの血清型（短報）——Boonmar, Sumalee, Bangtrakulnonth, Aroon 1, Ponruanongwong, Srisra 1, Marrim, Nopharat 2, 金子賢一 2, 小川益男 2（医歯学部、Kasetsart University, Thailand, 1 WHO International Salmonella & Shigella Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand, 2 東京農工大学農学部獸医学科）................................. 877-880
タイ国の人口及各種食品等から検出されたサルモネラの分布を明らかにする目的で、1993 年から 1999 年にタイ国の 4,916 株を分離、検査および生検を分離されたサルモネラ合計 27,497 株について血清型別を行った。人由来株は 72 血清型に、非人由来株は 82 血清型に型定された。得られた成績に基づいて、それぞれの食品がサルモネラ症を媒介する可能性について考察を加えた。

臨床繁殖学
着床障害時の子宮由来蛋白による着床障害胚の栄養脳外胚葉における DNA 合成再開の阻止——片桐成二・高橋芳彦・金川弘司・石原 美・月明, Young S. 1 (北海道大学院獣医学研究科繁殖学教室, 1 Department of Obstetrics and Gynaecology, The University of British Columbia, Canada)................................. 791-794
着床障害時に DNA 合成を含む胚の代謝が抑制されていることはマウスを含む多くの種で報告されているが、その機序は明らかにされていない。本試験は、マウスの着床障害時に特異的に出現する子宮由来蛋白 (DiAP) 70K の胚の DNA 合成抑制効果について、[3H]thymidine の取り込みを指標として検討した。マウスの着床障害は、妊娠 3 日目に卵巣を摘出し、フェルステロン注射により誘起した。DiAP70K は、50 μg/m で着床