

Abortion Caused by *Coxiella burnetii* in a Cow and Goat in Korea

Kyung-Hyun Lee¹, Hyun-Kyoung Lee¹, Kang-Hyun Baek¹, Jae-Gu Oem¹, Ha Young Kim¹, Lucienvan Keulen², Jae-Won Byun¹, Byung-Jae So¹ and Eun-Jin Choi^{1*}

¹Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, 175 Anyang-ro, Manan-gu, Anyang 430-757, Korea

²Central Veterinary Institute, Wageningen University and Research Centre, PO Box 65,8200 AB Lelystad, The Netherlands

*Corresponding author: Eun-Jin Choi, Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, 175 Anyang-ro, Manan-gu, Anyang 430-757, Korea, Tel: +82-54-912-0461; Fax: +82-31-467-0465; E-mail: mylovehyun@korea.kr

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Abstract

Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium. Q fever is usually subclinical, but is occasionally associated with abortion in ruminants. Two cases of abortion, one in a goat and the other in a cow, were reported to the Animal and Plant Quarantine Agency for diagnostic testing. Histologically, suppurative placentitis was apparent in both cases and more severe in the goat than in the cow. *C. burnetii* antigens were detected by polymerase chain reaction of DNA from various tissue extracts and antibodies were detected in the sera in both cases. To our knowledge, this is the first report of bovine and caprine abortion caused by *C. burnetii* in South Korea.

Keywords: *Coxiella burnetii*; Bovine; Caprine; Korea; Abortion

Introduction

Coxiella burnetii, an obligate intracellular Gram-negative bacterium, is the pathogen that causes Q fever in humans and animals. This bacterium causes a variety of symptoms, including acute flu-like symptoms, pneumonia, hepatitis and chronic endocarditis in humans [1]. *C. burnetii* infection in animals is generally asymptomatic, but can become symptomatic in pregnancy. Metritis, abortion, stillbirth, and delivery of weak offspring are the most frequent clinical signs of the disease [2]. In symptomatic cases, *C. burnetii* is excreted via feces, vaginal mucus, milk, and birth products. In pregnant goats, the clinical manifestation of *C. burnetii* infection is abortion or stillbirth in the final stage of gestation [3-5]. Routine diagnosis of Q fever in cattle is usually based on a specific enzyme-linked immunosorbent assay or complement fixation test [4]. Culturing the bacterium is difficult and requires a Biosafety Level 3 laboratory. Several safe and useful polymerase chain reaction (PCR) methods are currently available for detecting *C. burnetii* infection in milk, feces and vaginal mucus, and in aborted bovine fetuses and placental tissue [1,6,7]. *C. burnetii* is found in trophoblasts and mononuclear cells in an infected placenta [8]. *C. burnetii* is present worldwide, with the exception of New Zealand [6]. However, abortion caused by *C. burnetii* in cattle and goats has not been reported in Korea. In this study, we describe the first known cases of bovine and caprine abortion caused by *C. burnetii* in this country.

Histories

An aborted fetus and placenta from a Holstein cow were submitted to the Animal and Plant Quarantine Agency on July 15, 2013. The cow was from a farm that housed approximately 150 cows in Hawseong, Gyeonggi-do, Korea. The cows were vaccinated against foot and mouth disease in early 2013. Abortion samples, including the fetus, placenta, and blood from a Korean black goat were subsequently submitted on May 14, 2014. The goat was one of approximately 1,000 goats raised on a farm in Imsil, Jeonrabuk-do, Korea. Abortion at various gestational

ages was detected in 30 goats on this farm between March and May 2014; all goats were treated with penicillin after abortion from March 2014 onwards, but the incidence was not reduced.

Post-mortem findings

Necropsy was performed on both fetuses and placentas. All tissues were fixed in 10% buffered formalin and processed normally. Tissue sections 4- μ m-thick were obtained from multiple organs for hematoxylin and eosin staining and immunohistochemistry. All organs were fixed in formalin, embedded in paraffin, sectioned at 4- μ m thickness, and stained with hematoxylin and eosin for microscopic examination. Immunohistochemistry was performed using the ABC method. Antigen retrieval was performed in citrate solution (pH 6.0) and anti-*C. burnetii* (MAB 313) was used as the primary antibody. For microbiology, the fetus and placenta were inoculated onto *Brucella* and blood agar, and then incubated for 3 days in a 5% CO₂ incubator. Multiplex PCR was carried out to detect bacterial agents such as *Neospora canis*, *Leptospira* spp, *Campylobacter fetus*, *Listeria monocytogenes*, and *C. burnetii*. PCR was also used to detect viral pathogens such as bovine herpesvirus types 1 and 5, bovine viral diarrhoea virus, Aino virus, Chuzan virus, Ibaraki virus, bovine ephemeral fever virus, and Akabane virus. Both organs were examined for bacterial and viral pathogens.

The crown-rump lengths in the cow and goat were 35 cm and 30 cm, respectively. Autolysis was observed in the fetus and placenta in both cases. The placental cotyledons were hemorrhagic in the caprine case.

Mild suppurative placentitis was found in the bovine placenta and severe suppurative placentitis and mineralization were found in the caprine placenta (Figure 1). It was difficult to identify a histologic lesion that would have caused autolysis in the bovine placenta; however, the goat placenta contained multifocal to confluent areas of mineralization and diffuse suppurative placentitis with heavy neutrophil infiltration and extensive necrosis of the cotyledonary villi and intercotyledonary epithelium.

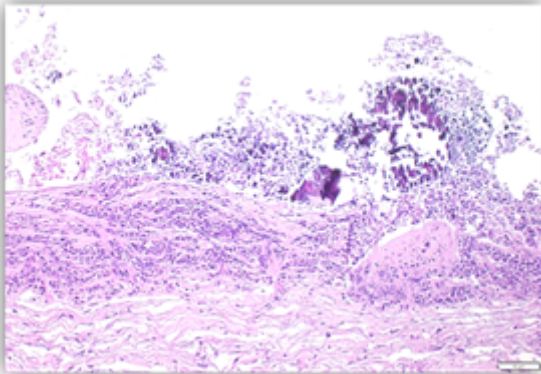


Figure 1: Severe infiltration of polymorphonuclear leukocytes and mononuclear cells in the chorionic stroma with foci of mineralization in the placenta of a goat with *Coxiella burnetii* infection. Bar=50 µm.

No lesions were observed in the fetal organs. The bovine and caprine placentas were positive for *C. burnetii* on PCR testing. Immunohistochemistry revealed *C. burnetii* antigen in the caprine placenta (Figure 2). The bovine fetus was negative by PCR because of autolysis; however, positive reactions were observed in all internal organs of the caprine fetus. No positive reaction to other viral or bacterial agents was detected. A strong Q fever-positive reaction was detected in the serum of both dams.

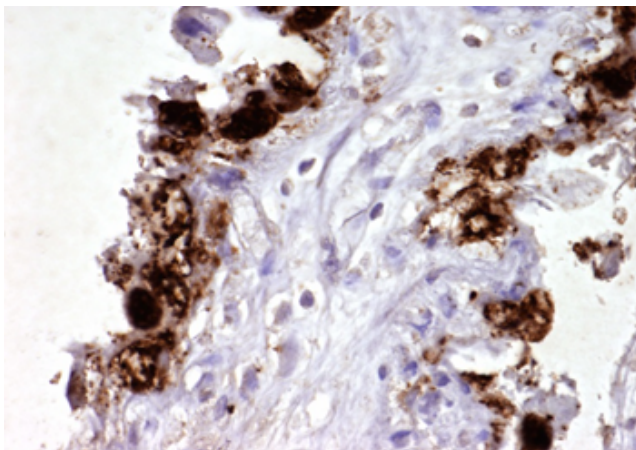


Figure 2: Strong positive reaction (brown) of trophoblasts in the placenta of a goat with *Coxiella burnetii* infection. Bar=50 µm.

Discussion

Both cases described here were diagnosed as abortion caused by *C. burnetii* on the basis of histopathologic features and a positive PCR test. The life cycle, route of infection, and pathogenesis of *C. burnetii* are under experimental investigation. The major routes of infection in humans are inhalation of contaminated aerosol following parturition

of an infected animal and ingestion of contaminated raw milk or milk products [8]. A minor route of infection is through the skin [7].

No gross placental lesions were present in the caprine case reported here, but such lesions have been described in previous cases of bovine coxiellosis [9]. Microscopically, the placentas showed a range of microscopic lesions varying from severe hemorrhage and necrosis to mildly increased numbers of mononuclear cells within a diffusely edematous chorionic stroma. These findings are similar to those described in a previous report on inflammatory changes in the placentas of four cows with mild *C. burnetii* infection [8]. Further, there has been a report of more severe placental lesions in caprine coxiellosis [3].

The seropositivity of *C. burnetii* in Korean dairy cattle and native Korean goats has been reported [9]. *C. burnetii* seropositivity has also been confirmed in Korean dairy farmers and health professionals, including veterinarians working in local clinics and laboratories [10]. Considering the high rate of Q fever infection observed among cattle and goats and previous reports of humans with high-risk occupations, such as herders, veterinarians and abattoir workers, contracting this disease, the risk of transmission of *C. burnetii* from livestock to humans is presumed to be high [10].

This report includes the first case of an antigen-positive placental lesion with abnormal pathology in Korea. Further studies of placentas in ruminants are needed to clarify the distribution and prevalence of *C. burnetii*. Clinicians should consider *C. burnetii* in the differential diagnosis in cases of abortion in animals that have been vaccinated and treated with antibiotics.

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Authors' Contributions

KHL, HKL, and KHB performed the pathologic analysis. EJC and BJS designed the general experiments. HYK and JWB performed the microbiologic analysis. LVK performed the immune histochemical analysis. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version of the manuscript.

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