

## Detection of *Coxiella burnetii* in Cattle by PCR

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**Abstract:** This study is aimed to detect *Coxiella burnetii* in cattle by Polymerase Chain Reaction (PCR). A total of 138 cattle blood samples were collected from 8 farms and then examined in laboratory conditions. The examination of *Coxiella burnetii* was carried out by PCR with specific primers. In this study, a total of 6 (4.3%) cattle serum samples were found PCR positive for *Coxiella burnetii*. This result proves that cattle are an important reservoir of *Coxiella burnetii* infection.

**Key Words:** *Coxiella burnetii*, cattle, PCR

### Sığırlarda *Coxiella burnetii*'nin Polimeraz Zincir Reaksiyonu ile Tanımlanması

**Özet:** Bu araştırmada, sığırlarda *Coxiella burnetii*'nin Polimeraz Zincir Reaksiyonu (PZR) kullanılarak tanımlanması amaçlanmıştır. Toplam 138 sığır kan örneği çiftliklerden toplandı ve laboratuvar koşullarında araştırıldı. *Coxiella burnetii* varlığı, bütün örneklerde spesifik primerler ile hazırlanan PZR kullanılarak incelendi. Bu araştırmada, toplam 6 (% 4,3) sığır serum örneği, *Coxiella burnetii* için PZR pozitif tespit edildi. Bu sonuç, sığırların *Coxiella burnetii* enfeksiyonu için önemli bir konakçı olduğunu göstermektedir.

**Anahtar Sözcükler:** *Coxiella burnetii*, sığır, PZR

### Introduction

Q fever is a zoonosis caused by an obligate intracellular bacterium, *Coxiella burnetii*. This disease is endemic worldwide (1,2) except in New Zealand (3).

*Coxiella burnetii* infections have been reported in humans, farm animals, pets, wild animals, and arthropods (2). Animals are often naturally infected but usually do not show typical symptoms of *Coxiella burnetii* infection (1,3).

Ticks are considered to be the natural primary reservoir of *Coxiella burnetii* and responsible for the spread of the infection in wild animals and for transmission to domestic animals (2). Cattle, sheep and goats are the main sources of human infection (3). Infected animals shed highly stable bacteria in urine, faeces, milk, and through placental and birth fluids. Infection via inhalation of aerosolized organisms or ingestion of raw milk or fresh dairy products has been reported in humans and animals (4).

In humans, Q fever is most often asymptomatic, but acute disease (mainly a limited flu-like illness, pneumonia or hepatitis) or chronic disease (chronic fatigue syndrome or endocarditis) can occur (1). Acute Q fever is a flu-like illness, which is self-limiting or easily treated with antibiotics when an appropriate diagnosis is made. Chronic Q fever is a severe disease that requires prolonged antibiotic therapy because the infection can result in endocarditis (5) or granulomatous hepatitis (6). In addition, the *Coxiella burnetii* infection can lead to abortions, stillbirth, or pre-mature deliveries in pregnant women (5).

In humans, infection results mainly from inhalation of contaminated aerosols from amniotic fluid, placenta, or contaminated wool, but the disease may also be acquired by the digestive route (4). At greatest risk are persons in contact with farm animals (veterinarians, farm workers, butchers), as well as laboratory personnel who work with infected animals (7).

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In cattle, Q fever is mainly associated with reproductive disorders (abortion, metritis, and infertility) (8). Cattle are often naturally infected but usually do not show typical symptoms of *Coxiella burnetii* infection. Clinical signs of *Coxiella burnetii* infection are abortion in sheep and goats, and reproductive disorders in cattle. *Coxiella burnetii* can be isolated from the blood, sera, lungs, spleen, and liver of infected animals in the acute phase of the disease. The uterus and mammary glands are the primary sites of infection in the chronic phase of *Coxiella burnetii*. Shedding of *Coxiella burnetii* into the environment occurs mainly during parturition by birth products, particularly the placenta of sheep. In addition, shedding of *Coxiella burnetii* in milk by infected dairy cattle is well documented (1,3).

*Coxiella burnetii* is highly infectious; only one organism is required to produce infection under experimental conditions (9). *Coxiella burnetii* is currently considered a potential warfare agent and is classified as a category B biological agent by the Center for Diseases Control and Prevention.

Routine diagnosis of Q fever is usually based on the detection of specific antibodies by complement fixation, and immunofluorescence and enzyme-linked immunosorbent assay (ELISA) tests. Isolation of *Coxiella burnetii* is hazardous, difficult and time-consuming, and requires confined biosafety level 3 laboratories due to the zoonotic nature of the microorganism (10).

Rapid differentiation of *Coxiella burnetii* in clinical specimens is very important for the control of Q fever, because prompt antibiotic therapy may lead to a better prognosis for individuals (11).

In contrast, PCR is a safe and useful method for detection and diagnosis of *Coxiella burnetii*. Recently, several PCR-based methods have been developed targeting the isocitrate dehydrogenase gene (12), the superoxide dismutase gene (13) and a transposon-like repetitive region (14).

At present, the polymerase chain reaction (PCR) technique has become a useful tool to detect *Coxiella burnetii* in biological samples (15). A PCR assay performed with primers based on a repetitive, transposon-like element (Trans PCR) (14) has proved to be highly specific and sensitive for the laboratory diagnosis of *Coxiella burnetii* infections, as it detects even very few copies of a specific DNA sequence.

The aim of present study was to detect *Coxiella burnetii* in the cattle for Q fever disease by PCR.

## Materials and Methods

### The study group

Cattle blood samples were collected from 8 dairy farms located in Aydin province, Turkey. A total of 782 dairy herds were present in these farms and the blood samples were collected from the dairy herds with abortion history and repeat breeding signs in the past. Due to the difference among the farms regarding the suspicious animals, the numbers of collected blood samples varied from farm to farm.

A total of 138 (17.6%) blood samples were collected from the suspected animals. The numbers of collected blood samples were 15, 16, 17, 18, and 20; from farms 1 and 2, farm 7, farms 3 and 6, farm 4, and farms 5 and 8, respectively.

Collected blood samples were brought in ice-pack containers to the Department of Microbiology laboratories, Faculty of Veterinary Medicine, Adnan Menderes University. Sera of the blood samples were separated and kept at -20 °C until analysis.

### DNA Extraction

A volume of 200 µl sera was extracted by DNA extraction kit (Fermentas) as recommended by manufacturer.

### PCR Primers

A pair of primers (Trans1: 5'-TGGTATTCTTGCCGATGAC-3', Trans 2: 5'-GATCGTAACTGCTTAATAAACCG-3') derived from the transposon-like repetitive region of the *Coxiella burnetii* genome was used to detect the agent (14).

### PCR assay

The PCR was performed in a thermocycler (Eppendorf – Mastercycler personal) in a total reaction volume of 50 µl, containing 5 µl of 10 × PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM potassium chloride, 0.1 per cent Triton X-100), 5 µl 25 mM magnesium chloride, 250 µM of each deoxynucleotide triphosphate, 2 U of Taq DNA polymerase (MBI Fermentas), 1 µM of each primer and 5 µl of template DNA. The PCR amplification was carried out under the reaction conditions described by Berri et al. (15). The 'touchdown' PCR assay was

performed under the following conditions: 5 cycles consisting of denaturation at 94 °C for 30 s, annealing at  $66 \pm 61$  °C (the temperature was decreased by 1 °C between consecutive steps) for 1 min and the extension at 72 °C for 1 min and then 40 cycles consisting of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s and extension at 72 °C for 1 min.

The reference strain of *Coxiella burnetii* Nine Mile Strain Phase I was used as positive control in the PCR; for negative control *Escherichia coli* was also used to ensure that contamination did not take place during the assays.

#### Detection of the amplification product

The 10 µl amplified products were detected by staining with 0.5 µg/ml ethidium bromide after electrophoresis at 70 V for 1 h in 1.5 per cent agarose gels. PCR products of 687 base pairs were considered indicative for identification as *Coxiella burnetii*.

#### Results

A total of 6 (4.3%) samples were found PCR positive for *Coxiella burnetii* obtained from the blood of 138 suspected animals

Amplification revealed a band at approximately 687 bp, which was in agreement with the expected size for identification as *Coxiella burnetii*. Control experiments performed with *Coxiella burnetii* Nine Mile Strain Phase I as a positive control and negative controls of *Escherichia*

*coli* and distilled water were also used to ensure that contamination did not take place during the assays.

Some PCR amplification-products are shown in Figure.

#### Discussion

Q fever disease, caused by *Coxiella burnetii*, is an important zoonoses found worldwide. In humans, it causes a variety of diseases such as acute flu-like illness, pneumonia, hepatitis, and chronic endocarditis. In animals, *Coxiella burnetii* is found in the reproductive system, both uterus and mammary glands, and may cause abortion or infertility.

In Europe, acute Q fever cases in humans are more frequently reported in spring and early summer. They may occur at all ages, but they are more frequent in men than in women. Q fever is usually benign, but mortality occurs in 1% to 11% of patients with chronic Q fever (16).

Since the clinical presentation is very pleomorphic and nonspecific, the incidence of Q fever among humans is probably underestimated, and diagnosis particularly relies upon the physician's awareness of the symptoms of Q fever, and the presence of a reliable diagnostic laboratory. In southern France, 5% to 8% of cases of endocarditis are due to *Coxiella burnetii*, and the prevalence of acute Q fever is 50 cases per 100,000

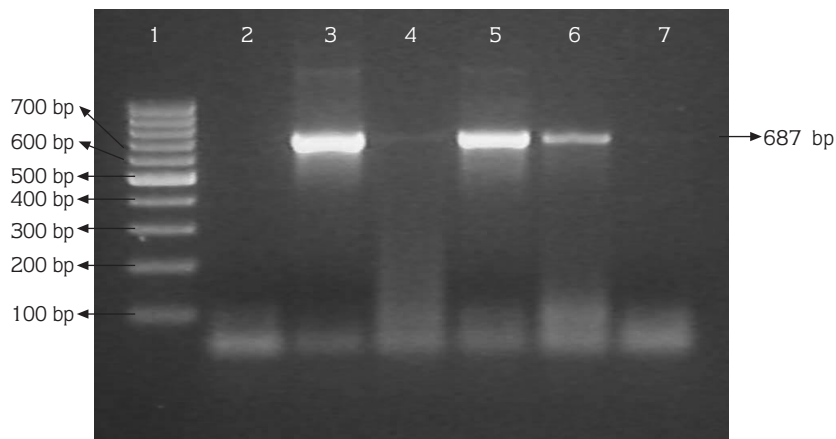


Figure. PCR assay for *Coxiella burnetii* (specific amplification of the 687 bp fragment from the total DNA of *Coxiella burnetii*). Lane 1: 1-kb plus DNA ladder (MBI Fermentas); Lane 2: *Escherichia coli* (negative control); Lane 3: *Coxiella burnetii* Nine Mile Strain Phase I (positive control); Lanes 4 to 7: *Coxiella burnetii* DNA.

inhabitants (17). Large outbreaks of Q fever have also been reported in the Basque region in Spain (18), and also in Great Britain (19).

Q fever disease is highly associated with reproductive problems (infertility, metritis, and mastitis) in cattle. The high prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive problems showed that these infected cattle play an important role in maintaining the infection and in disseminating the pathogenic agent to the environment. Thus, such excretions (milk, colostrum, urine, and birth fluid) are considered to be potential sources of the infection in animals and humans via inhalation of infectious aerosols or airborne dust (20).

In the USA, the animal studies show wide variation in seroprevalence, with goats having a significantly higher average seroprevalence (41.6%) compared to sheep (16.5%) or cattle (3.4%). Evidence of antibody to *Coxiella burnetii* was reported among various wild-animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds. This investigation suggests that *Coxiella burnetii* is enzootic among ruminants and wild animals throughout much of the United States and that there is widespread human exposure to this pathogen (21).

Abortions constitute an important economic burden for the livestock industry due to deaths and decreased milk production. The incidence of infectious abortions is very high throughout Western Turkey, which is caused by Brucellosis, Listeriosis, or Coxiellosis.

Although animal Coxiellosis is believed to be a tick borne disease, almost all the abortions were observed during the colder months when ticks are not active. A likely explanation for this is that even if ticks are an important reservoir in the environment, *Coxiella burnetii* is widely present among domestic animals. Cattle frequently shed *Coxiella burnetii* in milk (22) and small ruminants maintain carrier status for a long period after abortion, and thus are an important source of infection for both humans and other animals (23). Abortion is the clinical manifestation of a widespread occurrence of *Coxiella burnetii* in the animal population and is mainly concentrated during the reproductive season of small ruminants (24)

In Germany, some researchers showed that in herds suffering from abortions a seroprevalence of  $> \text{ or } = 20\%$  means an additional risk of infection for farmers (25).

In Bosnia and Herzegovina, researchers reported that specific *Coxiella burnetii* antibodies were detected in 249 (35.2%) individuals, whereof 75 (30.12%) female individuals and 174 (69.9%) male individuals (26).

One investigation findings suggest that Japanese veterinarians (the positive rate of IgG antibody 13.5%) have a higher risk of infection by *Coxiella burnetii* compared to blood donors (3.6%,  $P < 0.001$ ) and medical workers (5.1%,  $P < 0.001$ ) of the Japanese population (27).

People who are exposed to parturient farm animals are at the highest risk of acquiring the disease. Prevalences ranging from 7% to 45% have been reported in people in high-risk occupations such as veterinarians, farmers, and abattoir and laboratory workers (28-30).

In our study, a total of 6 (4.3%) cattle sera samples were found PCR positive for *Coxiella burnetii*. This result demonstrated that cattle can play a role of transmission to humans and these results were found very important for the spread of the disease.

Serum is one of the easiest samples to obtain and, when sampled early in the evolution of a systemic disease, is likely to contain DNA copies of systemic pathogens (31). Therefore, it is important to be able to use this sample for direct diagnosis. The diagnosis must be considered in the case of an unexplained fever, especially if the fever recurred following contact with possibly contaminated mammals. The best tests for diagnosis are those permitting the direct detection of bacteria. They include shell vial cell culture, PCR amplification, and immunodetection with tissue biopsy specimens. All these techniques require a level 3 biosafety laboratory and trained personnel due to the extreme infectivity of *Coxiella burnetii*.

In this investigation, a *Coxiella burnetii* infection on dairy herd farms was detected by PCR analysis. Isolation of *Coxiella burnetii* is not performed routinely for diagnostic purposes in veterinary medicine because it is a tedious procedure. Cultural recovery of *Coxiella burnetii* in embryonated eggs or by cell culture is time consuming, hazardous, expensive, and requires extensive laboratory support. PCR method is commonly employed for research and clinical purposes and could also be used as a sensitive, specific, and rapid test for the diagnosis of Q fever and easy to perform and safe for laboratory personnel. The

Trans-PCR assay was demonstrated to be far more sensitive and specific for the Q fever disease. In fact, PCR based upon this gene is useful for the direct detection of *Coxiella* DNA in clinical samples (15).

As a result, this study confirms that cattle are important reservoirs of *Coxiella burnetii* and contributed further insights into this important disease in western Turkey. The detection of *Coxiella burnetii* by PCR has a

significant application for assessing the absence of Q fever disease ruminants and minimizing the potential risks of Q fever outbreaks.

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