



Transmission of *Coxiella burnetii* to Calves from Infected Cows

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ABSTRACT

Background: *Coxiella burnetii* is the causative agent of a very important disease with zoonotic potential. Infected cows represent risk for spreading of infection to humans and to other animals on farm and also to their offspring. There is possibility for calves from infected cows to be infected nearly after parturition or during intrauterine life. Studies have shown that *Coxiella burnetii* initially infects the placenta and subsequent spread to the fetus may occur either by haematogenous or by the amniotic-oral route providing congenital infection. The main objective of the present study is to determine the presence of *Coxiella burnetii* genome in milk serum of infected cows and blood serum of calves.

Materials, Methods & Results: A total of 200 blood serums from dairy cows were tested for presence of antibodies to *Coxiella burnetii* and nine of those were found positive. These animals compiled experimental group. From animals in experimental group milk samples during lactation, pregnancy and the postpartum period were collected. Samples were used for performing PCR test for determination of *Coxiella burnetii* presence in milk serum. On calving of each cow blood samples were taken from calves during first 24 hours after calving, from jugular vein. These blood samples were also used for PCR test to determine the presence of *Coxiella burnetii*. Milk serum analysis showed presence of *Coxiella burnetii* genome in serum, indicating on intermittent excretion. During lactation, the excretion of bacteria was greatest in the second stage when 80% of milk serum samples were positive for *Coxiella burnetii*. In the colostrums stage, there was a high percentage of *Coxiella burnetii* excretion through milk (50% of positive milk serum samples). The lowest percentage of excretion through milk was in the first stage of lactation. Analyzing blood serum samples from calves, taken on first day at calving using PCR method, all serums were positive for presence of *Coxiella burnetii* genome.

Discussion: In animals, *Coxiella burnetii* is found in the reproductive system, both uterus and mammary glands, and may cause abortion or infertility. 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 of pathogenic agent to environment. The lowest percentage of excretion of *Coxiella burnetii* was in the first stage of lactation, amounting to 16.6%. With the transition to the next stage of lactation, a striking increase in the percentage of excretion was noticed. In the second stage it was 80%. In the third stage there was decrease to 40.6%. In colostrums stage percentage of excretion through milk was 50%. Similar results claims that excretion of *Coxiella burnetii* through milk starts after eight to twelve weeks of lactation in most cows. This period coincides with second stage of lactation as we divide it. Blood serums taken from calves were proven positive on *Coxiella burnetii* which indicates on intrauterine infection as described in the literature. Intrauterine infection takes place after placenta infection when bacteria penetrate the placenta and contaminate the amniotic fluid and gets aspirated or swallowed by fetus. Besides this, haematogenous spread can also occur.

Keywords: *Coxiella burnetii*, transmission, cow, calves.

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INTRODUCTION

Coxiella burnetii is the causative agent of Q fever, zoonosis that is present throughout the world [3]. The most important source for human infection is marked to be domestic animals [12,15]. Q fever mostly passes as a latent disease and the commonest clinical symptoms are abortions and reduced fertility. In addition to these symptoms related to the reproductive tract, occurrence of pneumonia, mastitis and polyarthritis have also been observed [13,18].

The pathogenesis of this disease is characterized with primary replication in the lymph nodes, followed by stage of bacteremia and after that, localization of agent in predilection organs, primarily in mammary gland and uterus in pregnant animals [11,12]. Localization of pathogens in the mammary gland is critical for long-term secretion through milk, so the cows can excrete the agents through milk more than a year [7,12]. Presence of bacteria in uterus causes a latent infection that remains localized in the placenta or spreads to the fetus [1]. This situation is characterised by normal offspring that may or may not be congenitally infected and vaginal excretion of organisms during parturition and in the postpartum period. An active infection that may remain limited to the placenta, or may spread to the fetus by the haematogenous or the amniotic-oral route will most likely compromise the fetus and cause abortion, premature delivery, stillbirth and weak offspring and normal but congenitally infected offspring also can be found.

The objective of this work was to explore possibility of intrauterine infection of calves originating from seropositive cows and also to examine excretion of *Coxiella burnetii* through colostrums and milk.

MATERIALS AND METHODS

Experimental animals and sample collection

Serological screening of blood serum samples for antibodies to *Coxiella burnetii* was performed on a farm with 200 holstein-friesian dairy cows using ELISA method. Commercial ELISA kits (Chekit Q fever)¹. Based on the results of the ELISA tests, an experimental group of cows, serologically positive for *Coxiella burnetii* was formed. In total, the experiment included nine dairy cows. The cows were in good body condition and showed no clinical signs of disease. There was only one abortion that occurred

during experiment, one cow could not get pregnant and another seven cows gave birth to clinically healthy calves.

From the experimental animals, milk samples during lactation, pregnancy and the postpartum period were collected during regular milking using true-testers. Before taking milk samples teat ends were disinfected using 70% alcohol. Samples were used for performing PCR test for determination of *Coxiella burnetii* presence in milk serum. On calving of each cow blood samples were taken from calves during first 24 h after calving, from jugular vein. These blood samples were also used for PCR test to determine the presence of *Coxiella burnetii*.

Performing of PCR test

After arriving in the laboratory, milk samples were placed in an incubator for 24-48 h. Incubation was carried out at a temperature of 38°C to form coagulum and milk serum.

Blood samples were stored in room temperature for 48 h to segregate the serum. After that serum was pour off and stored in freezer until performing PCR test.

The PCR method was used to determine the presence of *Coxiella burnetii* genome in milk and blood serum samples. For serum samples, a 200-µL of sample volume was used. Cells were lysed with proteinase K (final concentration, 200 µg/mL) at 56°C overnight. DNA was prepared with a Prep-A-Gene purification kit² by using 10 µL of silica matrix. DNA was eluted from the silica matrix by adding 100 µL of Prep-A-Gene elution buffer. To increase the yield, DNA was eluted at 56°C for 5 min and centrifuged again. One microliter of supernatant containing DNA was used for amplification. Used primers were as followed:

Trans1: 5'-TGGTATTCTTGCCGATGAC-3';

Trans 2: 5'-GATCGTAACTGCTTAATAAACCG-3'.

RESULTS

Processing of blood serum samples from 200 cows on tested farm by ELISA test has shown presence of antibodies for *Coxiella burnetii* in 9 cows. These animals accounted for 4.5% of total herd.

From seropositive cows, 65 samples of milk serum were collected by successive lactation stages. The results of the analysis of these samples using the

PCR method are shown in Table 1. During lactation, the excretion of bacteria was greatest in the second stage when 80% of milk serum samples were positive for *Coxiella burnetii*. In the colostrums stage, there was a high percentage of *Coxiella burnetii* excretion through milk (50% of positive milk serum samples).

The lowest percentage of excretion through milk was in the first stage of lactation (Table 1).

Analyzing blood serum samples from calves, taken on first day at calving using PCR method, all serums were positive for presence of *Coxiella burnetii* genome (Table 2).

Table 1. Excretion of *Coxiella burnetii* through milk in different lactation stages.

Stage of lactation	Colostrum stage First 10 days	First stage 10-60 days	Second stage 60-180 days	Third stage Over 180 days
Number of samples	4	8	20	33
<i>C. burnetii</i>	50%	16.6%	80%	40.6%

Table 2. Presence of *Coxiella burnetii* genome in blood serum of calves.

ID number of calf	6956	6989	2729	6952	6981	2710	2721
<i>C. burnetii</i>	positive	positive	positive	positive	positive	positive	positive

DISCUSSION

Q fever disease caused by *Coxiella burnetii*, is an important zoonosis found worldwide. In humans, it causes a variety of diseases such as acute flu-like illness, pneumonia, hepatitis, and chronic endocarditis [3,4]. In animals, *Coxiella burnetii* is found in the reproductive system, both uterus and mammary glands, and may cause abortion or infertility [19,20]. 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 of pathogenic agent to environment. Thus, such excretions (milk, colostrums, urine, and birth fluid) are considered to be potential sources of infection in animals and humans via inhalation of infectious aerosols or airborne dust [13,21].

According to our results (Table 1), the lowest percentage of excretion of *Coxiella burnetii* was in the first stage of lactation, amounting to 16.6%. With the transition to the next stage of lactation, a striking increase in the percentage of excretion was noticed. In the second stage it was 80%. In the third stage there was decrease to 40.6%. In colostrums stage percentage of excretion through milk was 50%. Similar results were published by Rodolakis et al. [16] who claim that excretion of *Coxiella burnetii* through milk starts after eight to twelve weeks of lactation in most cows. This

period coincides with second stage of lactation as we divide it. Also shedding of *Coxiella burnetii* through milk is often associated with presence of subclinical mastitis [4].

Blood serums taken from calves were proven positive on *Coxiella burnetii* (Table 2) this indicates on intrauterine infection as described by Agerholm [1]. Intrauterine infection is aftermath of placenta infection, which can remain confined to placenta or bacteria can penetrate the placenta and contaminate the amniotic fluid so after aspirated or swallowed by fetus [14]. Besides this, haematogenous spread through umbilical vessels can also occur leading to finding of bacteria in multiple tissues. Fetus can produce antibodies to *Coxiella burnetii*, so IgM antibodies can be found in fetal blood serum, which can be helpful in assessment of significance of *Coxiella burnetii* as cause of abortion.

Presence of *Coxiella burnetii* genome in blood serum is consequence of lysis of infected cells by antibody-dependent system. So bacteria became free in blood serum [7].

CONCLUSION

Dairy cows represent an important reservoir of *Coxiella burnetii*, spreading the agent into environment, in food chain and to their offspring via intrauterine infection.

MANUFACTURERS

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²Bio-Rad Laboratories GmbH. Munich, Germany.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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