Excretion of *Coxiella burnetii* in Cows with Secretion Disorder

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**INTRODUCTION**

Q fever is a zoonosis caused by an obligate intracellular microorganism, *Coxiella burnetii*. The disease is present throughout the world. The most important source for human infection is marked to be domestic animals. Dogs and cats are responsible for spread of disease primarily in urban areas. Ruminants are known to be the most important source of infection in humans [1,2]. In domestic animals, 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 [3]. The causative agent of Q fever - *Coxiella burnetii* is an immobile Gram negative bacterium, its life cycle is completed in phagosomes of infected cells [4]. It has a cell membrane similar to that of other Gram-negative bacteria. It is normally stained with Giemsa since they stain poorly with the Gram stain [4]. *Coxiella burnetii* is very virulent, so the infectious dose could be only one microorganism [5].

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 [6]. 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 and even during successive lactations [7], while secretion through feces and vaginal discharge lasts for a few weeks. Reservoirs of the agent are only partially known, but certainly include mammals, birds and arthropods, especially ticks. Although 40 species of ticks can be naturally infected with *Coxiella burnetii*, they obviously do not have a great importance for the infection of animals and humans [8]. However, the pathogen replicates in cells of the tick’s gut and it is excreted in large number through the feces. Leather and wool contaminated with feces of ticks can be a source of infection either through direct contact or after inhalation of dry feces. The farm animals - cattle, sheep and goats - are the most common source for human infections. Pets like dogs, cats or rabbits can also be source of infection with *Coxiella burnetii*. There is a report on the occurrence of disease in humans as a result of direct or indirect contact with cats during parturition [9]. In the case of dairy cows, occupational exposure of humans to infection should be highlighted. Most exposed are veterinarians, farmers, milkmen and workers in slaughterhouses and dairies. In the general population, categories at risk are smokers and immunocompromised persons [10]. Some authors [11] have also reported a significant association between seropositivity in humans and intake of non-pasteurized milk and milk products, whether people were in contact with animals or not.

While *Coxiella burnetii* is shed for extended periods in milk of dairy cattle, and has been shown to be immunogenic in dairy cattle, potential associations with clinical or subclinical mastitis only rarely have been examined [12]. A more recent report suggested that the prevalence of *Coxiella burnetii* infections was higher among dairy cattle with reproductive problems including mastitis [13]. For detection of *Coxiella burnetii* PCR method is highly sensitive and specific detection method that has been used previously to trace *Coxiella burnetii* in clinical samples. A PCR performed with primers based on a repetitive, transposonlike element (Trans-PCR) proved to be highly specific and sensitive, but extraction of DNA from milk samples took considerable effort and there was a high risk of contamination due to the numerous preparation steps [14]. The objective of this work was to explore the potential association between *Coxiella burnetii* detection in milk by PCR and elevated SCC in milk in successive lactation stages and also to recognize relation between immunoglobulin G in milk serum and PCR detection in milk. Findings of agent in milk does not always coincides with serological finding, so seronegative animals can also excrete *Coxiella burnetii* in milk [15].

**MATERIAL and METHODS**

Serological screening of blood serum samples for antibodies to *Coxiella burnetii* was performed on a farm with 200 holstein-friesian dairy cows by ELISA. Serum samples were tested for Q fever antibodies using the indirect ELISA kit (Idexx Switzerland, Switzerland), according to the protocol recommended by the manufacturer. Sera were prepared at 1:400 dilution, and specific antibodies were detected using a peroxidase-labeled anti-ruminant immunoglobulin G (IgG) conjugate. Results were expressed as a percentage of the optical density reading of the test sample (value), calculated as value = 100 × (S−N)/(P−N), where S, N, and P are the OD of the test sample, the negative control, and the positive control, respectively. Sera were considered to be ELISA positive if they had a value of 40% or more, suspect if the value was between 30% and 40%, and negative if the value was <30%.

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. In beginning of experiment all cows were in first lactation, and three of them newer got pregnant again. From another six, who got pregnant, one had abortion and five had normal calving.

From the experimental animals, milk samples during lactation, pregnancy and the postpartum period were collected during regular milking. With use of true-testers, from each cow two milk samples were taken. One sample was used for performing PCR and for determining of immunoglobulin G concentration in milk serum. Another sample was used for determination of somatic cell count.

In total 65 milk samples were taken during different stages of lactation. Along with milk samples, blood samples were also taken for determination of presence of *Coxiella burnetii* in serum.

After arriving in the laboratory, milk samples were placed in an incubator for 24-48 h. Incubation is 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. 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 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 kit (Bio-Rad, Munich, Germany) 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. Followed...
primers were used: Trans1: 5'-TGGTATTCTTGCCGATGAC-3';
Trans 2: 5'-GATCGTAACTGCTTAAT AAACCG-3'.

To determine the concentration of immunoglobulin G
in milk and blood serum immunodiffusion method with
RID plates was used. The RID plates with monospecific
antiserum to bovine immunoglobulin G were provided by
INEP (Belgrade, Serbia). Sample of milk serum was poured
in wells of RID plate, and after incubation for 48 h in room
temperature reading of results was done. Reading was
done by measuring the diameter of the precipitation ring.
Diameter was measured by RID meter with an accuracy of
0.1 mm. The value obtained using the following formula
calculates the concentration of immunoglobulin in the
tested serum.

The formula for the calculation is: 
$$C = \frac{(R^2 - b)}{a} \times 30,$$
where R is the radius of precipitation ring, b is a constant
whose value is 8.69, a is a constant with a value of 47.48.
The resulting value is the concentration of immuno-
globulin in the serum.

The correlation coefficient between IgG concentration
and presence of Coxiella burnetii in milk serum was
calculated using Statistica v. 7.5 software.

Research is approved by Ethics Commission to
safeguard the welfare of experimental animals of the
University of Novi Sad, number 01-153/7-3.

RESULTS

Processing of blood serum samples from 200 cows on
tested farm by ELISA test has shown 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).

In Table 2 presence of Coxiella burnetii in blood serum
of infected cows is shown. From Table 2 it can be seen
that during all lactation stages there was a small oscillation
in presence of agent in blood serum.

Concentration of immunoglobulin G in milk serum of
infected cows is shown in Table 3. Highest concentration was
in colostrums stage and significantly lower concentration
was measured in successive lactation stages.

Somatic cell count in cumulative milk samples was
measured during lactation stages and results are shown in
Table 4. It is evident that SCC in milk samples from infected
cows was increased during all lactation stages and some
samples had very high values.

The correlation coefficient between presence of
Coxiella burnetii genome in blood serum and excretion in
colostrums and milk during all lactation stages was 0.072.

The correlation coefficient between excretion of Coxiella
burnetii

<table>
<thead>
<tr>
<th>Table 1. Excretion of Coxiella burnetii through milk in different lactation stages</th>
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<td><strong>Stage of Lactation</strong></td>
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<tr>
<td>Number of samples</td>
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<td>C. burnetii excretion</td>
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<th>Table 2. Presence of Coxiella burnetii in blood serum in different lactation stages</th>
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<tr>
<td>Number of samples</td>
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<td>C. burnetii in blood serum</td>
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<tr>
<th>Table 3. Concentration of Immunoglobulin G in milk serum in different lactation stages</th>
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<td>-------------------------</td>
</tr>
<tr>
<td>Number of samples</td>
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<tr>
<td>IgG concentration g/L</td>
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burnetii through milk and of immunoglobulin G concentration in milk serum at each stage of lactation was 0.072.

**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. In animals, Coxiella burnetii is found in the reproductive system, both uterus and mammary glands, and may cause abortion or infertility [16].

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 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].

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 the next stage of lactation infected cows had increased number of somatic cells, with some samples having very high values. Barlow et al. and Radinović et al. had similar finding examining the milk from cows with Coxiella burnetii infection.

Infected cows shed Coxiella burnetii through milk during whole lactation with highest intensity in the second stage, while presence of pathogen in blood serum is similar in all lactation stages. Concentration of immunoglobulin G in milk serum corresponds to values in uninfected cows. Somatic cell count is increased in infected cows during whole lactation stages.

**ACKNOWLEDGEMENT**

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**REFERENCES**


**Table 4.** Somatic cell count in cumulative milk samples from infected cows

<table>
<thead>
<tr>
<th>Lactation Stage</th>
<th>First Stage 10-60 Days</th>
<th>Second Stage 60-180 Days</th>
<th>Third Stage Over 180 Days</th>
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<tbody>
<tr>
<td>SCC x 10⁵/mL</td>
<td>147</td>
<td>778</td>
<td>2277</td>
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<tr>
<td></td>
<td>1004</td>
<td>419</td>
<td>1092</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>361</td>
<td>-</td>
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<tr>
<td>Average</td>
<td>773±711</td>
<td>903±512</td>
<td>961±558</td>
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