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EXCRETION OF *COXIELLA BURNETII* THROUGH MILK OF COWS AS RISK TO HUMAN HEALTH

M. RADINOVIĆ, S. BOBOŠ, I. DAVIDOV, M. PAJIĆ, M. ERDELJAN, M. RADINOVIĆ & A. GALFI

Faculty of Agriculture, University of Novi Sad, Serbia

Summary

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Coxiella burnetii is the causative agent of very important zoonoses. Transmission of infection to humans occurs mainly by domestic ruminants that excrete cthe ausative agent with faeces, urine, lochia, placenta and milk. Of all these routes, the longest lasting is the excretion through milk, which can be over several lactations. In this paper, the dynamics of excretion of *C. burnetii* through milk in seropositive cows and the level of immunoglobulin G in milk serum were examined. Correlation between these two parameters was also examined. A PCR method was used for determination of *C. burnetii* genome and RID plates – for the concentration of immunoglobulin. The excretion of the causative agent through milk differed between different stages of lactation, being the lowest in the first phase (16.6%) and the highest in the second phase (80.0%) when the lowest concentration of immunoglobulin G in milk serum (4.0 g) was detected.

Key words: *Coxiella burnetii*, cows, immunoglobulin G, milk

INTRODUCTION

Q fever is one of the most important rickettsial diseases with a special significance due to its zoonotic character. 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 (Vidić *et al.*, 2008). The causative agent of Q fever – *Coxiella burnetii* – is an immobile Gram negative bacterium, whose life cycle is completed in phagosomes of infected cells (Woldehiwet, 2004). It has a cell membrane similar to that of other Gram-negative bacteria, but does not Gram stain well, instead better stains by Gimze (Gimenez, 1964). *C. burnetii* is very virulent, so the infectious dose could be only one microorganism (McQuiston *et al.*, 2002).

The pathogenesis of this disease is characterised with primary replication in the lymph nodes, followed by stage of bacteriaemia and after that, localisation of agent in predilection organs: primarily in mammary gland and uterus in pregnant animals (Babudieri, 1959). Localisation 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 (Muskens *et al.*,

2011), while secretion through faeces and vaginal discharge lasts for 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 *C. burnetii*, they obviously do not have a great importance for the infection of animals and humans (Maurin & Raoult, 1999). However, the cause replicates in cells of the tick gut and is excreted in large numbers through the faeces. Leather and wool contaminated with faeces of ticks can be a source of infection either through direct contact or after inhalation of dry faeces. The most commonly identified source of human infection are farm animals – cattle, sheep and goats. Pets like dogs, cats or rabbits can also be source of infection with *C. burnetii*. There is a report on the occurrence of disease in humans as a result of direct or indirect contact with cats during parturition (Marrie & Raoult, 2002). In the case of dairy cows as a source of infection for humans, we should highlight occupational exposure to infection: most exposed are veterinarians, farmers, milkmen and workers in slaughterhouses and dairies. In the general population, particular categories at risk from those that are not occupationally exposed are smokers and immunocompromised persons (Van der Hoek *et al.*, 2010). Some authors (Cerf & Condron, 2006) also reported a significant association between seropositivity in humans and drinking non-pasteurised milk products whether people were in contact with animals or not.

The aim of the study was to examine the dynamics of excretion of *Coxiella burnetii* through cows' milk and to establish the phase of lactation with highest percentage of positive samples. Also, the aim was to determine the relationship between IgG concentration in milk serum and the excretion of the agent.

MATERIALS AND METHODS

Serological screening of blood serum samples for antibodies to *Coxiella burnetii* was performed on a farm of 200 Holstein-Friesian dairy cows by ELISA. Commercial ELISA kits Chekit Q fever manufactured by IDEXX Laboratories were used. Based on the results of the ELISA tests, an experimental group of cows serologically positive for *C. 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. From the experimental animals, milk samples during lactation, pregnancy and the postpartum period were collected during regular milking.

Upon maturity in the laboratory, samples were placed in an incubator for 24–48 hours. Incubation is carried out at a temperature of 38 ºC to form coagulum and milk serum. The PCR method.was used to determine the presence of *C. burnetii* genome in milk serum samples. Used primers were as followed: Trans1: 5'-TGGTATTCTTGCCGATGAC-3'; Trans 2: 5'-GATCGTAACTGCTTAAT AAACCG-3'. In total, 65 milk serum samples were taken from all stages of lactation: 4 samples in colostral phase, 8 samples in first, 20 samples in second and 33 samples in third phase of lactation.

To determine the concentration of immunoglobulin in the milk serum RID plates manufactured by INEP Belgrade were used. The RID plates contained monospecific antiserum to bovine IgG, sodium azide and merthiolate as preservatives. The milk serum sample was poured in RID plate wells, and after

incubation for 48 hours at room temperature, reading of results was done by measuring the diameter of the precipitation ring. Diameters were measured by RID meter with an accuracy of 0.1 mm. The value was obtained using the following formula for calculation of the concentration of immunoglobulin in the tested serum:

$$C = [(R^2 – b)/a] \times 30,$$

where R – the radius of the precipitation ring, b – a constant equal to 8.69, a – a constant equal to 47.48. The resulting value is the concentration of immunoglobulin 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. In order to more clearly present the results of the work we have defined stages of lactation based on three features displayed during the lactation period. The first phase is the phase in which increasing of milk production and stabilisation of somatic cells in milk takes place. In the second phase, full-lactation milk production is steady and somatic cell numbers stable. The third phase is the phase of late lactation when the decline in milk production leads to increase in the number of cells in milk. To understand the results of the analysis of milk serum more clearly, a colostral phase is also defined comprising the first ten days of lactation. During this period, the mammary gland secretion is colostrum.

RESULTS

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

From seropositive cows, 65 samples of milk serum were collected by lactation phases. 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 phase when 80% of milk serum samples were prositive for *C. burnetii*. In the colostral phase, there was a high percentage of *C. burnetii* excretion through milk (50% of positive milk serum samples). The lowest percentage of excretion through milk was in the first phase of lactation.

Immunoglobulin G concentrations in the tested milk serum samples are shown in Table 1. The highest concentration of immunoglobulin G was in colostral phase when it measured 153.1 g/L, which fell off sharply after the first two phases of lactation when the concentration of IgG was 4.0 g/L. In the third phase of lactation, it increased again up to 8.4 g/L.

The correlation coefficient between excretion of *C. burnetii* through milk and

Table 1. The excretion of *Coxiella burnetii* in cows' milk per lactation phases

Phase of lactation	Colostral phase	I phase	II phase	III phase
Number of samples	4	8	20	33
Excretion of *C. burnetii*	50%	16.6%	80%	40.6%
IgG concentration (g/L)	153.1	5.0	4.0	8.4

of immunoglobulin G concentration in milk serum at each stage of lactation was 0.072.

DISCUSSION

Coxiella burnetii is the causative agent of a very important and widespread disease in humans and animals. From the time when the disease first appeared, its nature and etiology were almost simultaneously explained at both ends of the world – in Australia and North America. Greatest importance in the spread of infection to humans and other animals have cattle, sheep and goats (Vidić *et al.*, 2008). Infected animals of these species excrete bacteria through milk, lochia and foetal membranes (To *et al.*, 1998). Excretion via urine and faeces is also present. The main route of excretion is through milk. Dairy cows infected with the causative agent can excrete *C. burnetii* over many months and even years. According to some authors excretion in cows' milk lasts a lifetime (Miljković, 1958). The results of our study indicate intermittent secretion through milk as shown in Table 1, with oscillations in the presence of the pathogen during certain phases of lactation.

Although *C. burnetii* is the most heat-resistant organism of public heath significance, pasteurisation of milk is a process for its inactivation (Cerf & Condron, 2006) but we should not rule out the fact that a certain amount of milk reaches the consumer directly without heat treatment. Also, the production of some types of cheese does not include heat treatment of milk at higher temperature. According to our results, the lowest percentage of excretion of *Coxiella burnetii* was in the first phase of lactation, amounting to 16.6%. With the transition to the next phase of lactation, a striking increase in the percentage of excretion was noticed, and in the second phase it was 80% to decrease again in the third phase to 40.6%. Although the most intense excretion of *Coxiella bur*netii was in the second phase, the greatest risk for human infection is in the postpartum period, when an infectious aerosol is formed near the animals as a result of excretion through the placenta and lochia (Vidić *et al.*, 2008). Observing immunoglobulin G concentration in serum of dairy cows we noticed a highly pronounced variability (Table 1). Colostrum samples had very high concentrations of immunoglobulin values and the average value exceeded 150 g/L. This finding is fully consistent with literature data (Boboš & Vidić, 2005) which suggests that in the colostral phase concentration of immunoglobulin G can exceed 100 g/L. In the second phase we observed a very strong reduction of serum immunoglobulin with average value of 5 g/L. Now we do not speak of mammary gland secretion but of milk, and the information relating to the concentration of immunoglobulin G also corresponds to the values expected in milk. With lactation course, the concentration of immunoglobulins further declined during the second phase to an average concentration of 4.0 g/L. During the firther course of lactation and with decrease of milk production, IgG in the third phase increased again to 8.4 g/L.

We believe that the increase in the concentration of immunoglobulins is just a consequence of reducing the amount of milk in the third stage of lactation and the beginning of the process of mammary gland involution. Although IgG concentration varied during lactation phases there was a relationship between this parameter and *Coxiella burnetii* excretion over

the entire lactation as seen from the calculated positive correlation coefficient.

CONCLUSION

The excretion of *Coxiella burnetii* has been proven through all stages of lactation in serologically positive cows. All cows in the trial excreted pathogens through milk. The highest intensity of excretion was in the second stage. Immunoglobulin concentration in milk serum varied considerably during lactation and the lowest concentrations were during the second phase when the animals excreted pathogens through milk at the highest extent. During all lactation phases, the correlation between *Coxiella burnetii* excretion and concentration of immunoglobulins in milk serum was positive.

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Correspondence:

Dr Miodrag Radinović
Faculty of Agriculture,
Novi Sad, Serbia
e-mail: radinovic@polj.uns.ac.rs