Full Length Research Paper

Presence of *Coxiella burnetii* in blood serum and concentration of IgG in infected cows

Miodrag Radinović*, Stanko Boboš, Ivana Davidov, Mihajlo Erdeljan and Marija Pajić

Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad,
Trg D.Obradovića 8, 21000 Novi Sad, Serbia.

Infection with *Coxiella burnetii* in cows usually runs without typical symptoms. If clinical symptoms occur, they are mainly related to reproduction disturbance such as abortion, reproduction disorders, and low vital offspring. The aim of the paper was to determine the relation between IgG concentration and the presence of *C. burnetii* in serum of seropositive cows. Experiment was conducted on group of cows serologically positive to *C. burnetii*. For serological examination, enzyme-linked immunosorbent assay (ELISA) method was used. PCR method was used to prove the presence of *C. burnetii* in serum. Radial immunodiffusion was used to determine concentration of IgG in serum. Presence of *C. burnetii* in serum was from 48.6 to 66.6% in different stages of lactation and the concentration of IgG was from 76.1 to 95.4 g/L in different stages of lactation. In the control group of cows from same farm, the concentration of IgG in blood serum was from 35.7 to 42.2 g/L.

Key words: Cow, *Coxiella burnetii*, serum, IgG.

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 of human infection is marked to be domestic animals such as dogs and cats who are responsible for the disease primarily in urban areas and ruminants that are the most important source of infection in humans (Arricau-Bouvery and Rodolakis, 2005; Vidic et al., 2008). *C. burnetii* is highly infectious; some authors claim that only one organism is required to produce infection under experimental conditions (Hatchette et al., 2000). In humans, Q fever is most often asymptomatic, but acute disease, mainly a limited flu-like illness, pneumonia or hepatitis or chronic disease can occur (Van Der Hoek et al., 2010). 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 or

granulomatous hepatitis (Raoult and Marrie, 1995).

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 *C. burnetii* is hazardous, difficult and time-consuming, and requires confined biosafety level 3 laboratories due to the highly infectious nature of this zoonotic microorganism (Field et al., 2000).

Domestic animals are an important part of the natural cycle of infection, which primarily affects sheep, cattle and goats. The disease often runs asymptomatic in them and the only symptom that may indicate the occurrence of this infection is abortion (Kazar, 2005). Although there are reports of respiratory illness caused with *C. burnetii*, the only symptom in experimentally infected animals, primarily sheep and goats and rarely in cattle is abortion. In animals that have had abortions *retentio secundinarum* occurs and reduced fertility (Guatteo et al, 2007).

Chronically, infected cows have increased values of IgG concentration in blood serum (Boboš and Vidić, 2005; Radinović et al., 2011) and this study was conducted to test the influence of increased values of IgG

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*Corresponding author. E-mail: radinovic@polj.uns.ac.rs. Tel: +381/21-485-3464.

on presence of *C. burnetii* in blood serum of infected cows. The aim was also to compare IgG concentrations in serum from infected and non-infected cows. For detection of *C. burnetii* presence in blood serum, PCR method is proven to be adequate. A PCR assay performed with primers based on a repetitive, transposon-like element (Trans PCR) has proved to be highly specific and sensitive for the laboratory diagnosis of *C. burnetii* infections, as it detects even very few copies of a specific DNA sequence (Kirkan et al., 2008).

MATERIALS AND METHODS

On a dairy farm in Vojvodina region, blood samples were obtained by venipuncture on jugular vein from totally 200 cows, using vacutainers. After sampling, blood samples were stored at room temperature for 12 h in order to single out the serum, and then centrifuged. A total of 200 samples of blood serum were examined. The sera were examined using the Q-fever CHEKIT ELISA test kit "IDEXX Laboratories" by the manufacturer's instructions. Out of the initial 200 cow blood samples, nine bovines were found positive for Q fever; these nine animals formed the experimental group. Another 10 animals from the same farm with negative findings formed the control group.

From cows in the experimental and control groups, blood samples were taken during different phases of production during 18 months. Blood samples were taken by jugular vein venipuncture. For blood sampling, we used vacutainers manufactured by BD Vacutainer Systems, Preanalytical Solutions UK. After sampling, blood samples were stored at room temperature in order to segregate the serum.

Serums were overflowed and stored at -20°C. A total of 78 serums from nine cows in experimental group were examined plus 82 serums from ten cows in control group.

For measuring immunoglobulin G concentration in serums, radial immunodiffusion using RID plates, manufactured by "INEP" Zemun was provided. Serum samples were diluted with saline in ratio 1/30 and then poured into pools of RID plate. Each plate had eight pools for eight different samples. Plates were then stored at room temperature for incubation during 48 h. After incubation, diameter of rings was measured and then used for calculation of the IgG concentration. In order to calculate the concentration of IgG, the following formula was used: Concentration $=(R^2-b)/a$ Where, R is the diameter of the ring; a is a constant equals 47.48; b is a constant equals 8.69.For detection of *C. burnetii* in blood serum, PCR method was used provided by "Qiagen".

DNA extraction

A volume of 200 µl sera was extracted by DNA extraction kit (Qiagen) as recommended by the 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 *C. burnetii* genome was used to detect the agent.

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 % Triton X-100), 5 µl 25 mM magnesium chloride, 250 µM of each deoxynucleotide triphosphate, 2 U of Taq DNA polymerase , 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. (2000). The `touchdown' PCR assay was performed under the following conditions: 5 cycles consisting of denaturation at 94°C for 30 s, annealing at 66 ± 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 *C. burnetii* Nine Mile Strain Phase I was used as positive control in the PCR; for negative control, *Escherichia coli* was also used to demonstrate that contamination did not take place during the assays (Figure 1).

Detection of the amplification product

Amplified products (10 µl) were detected by staining with 0.5 µg/ml ethidium bromide after electrophoresis at 70 V for 1 h in 1.5 % agarose gels. PCR products of 687 base pairs were considered indicative for identification as *C. burnetii*.

Statistical analysis

Correlation coefficient between IgG concentration and presence of *C. burnetii* in serum was calculated using program "*Statistica version 7.5.*".

In order to show results more clearly, phases of lactation were determined. First phase of lactation was the period from 10 to 60 days post partum, second phase was period from 60 to 180 days post partum and the third phase was period after 180 days. The duration of third phase was different and depended on time of conception.

In addition to these lactation phases, there are two more phases in production cycles. There is colostrums phase, which takes place in the first ten days after calving and during this phase, udder secret is not milk but colostrums and is not used for human consumption. Another phase of production cycle is dry period which includes the last two months of gestation.

RESULTS

A total of 78 sera from nine ELISA positive cows were examined plus 82 serums from ten cows in the control group.

Concentration of immunoglobulin G in the control group was in the range from 73.1 to 95.4 g/L (Table 1). Average concentration was 81.3 g/L. In the control group of cows, IgG concentration was in the range from 35.7 to 42.2 g/L (Table 2) and average concentration was 40.0 g/L.

The lowest concentration of immunoglobulin G was in the colostrums phase when it counted 73.1 g/L and the highest was during the third phase of lactation when it counted 95.4 g/L.

In control group of cows highest concentration of IgG was in first lactation phase (41.8 g/L) and lowest was in dry period (35.7 g/L) (Table 2).

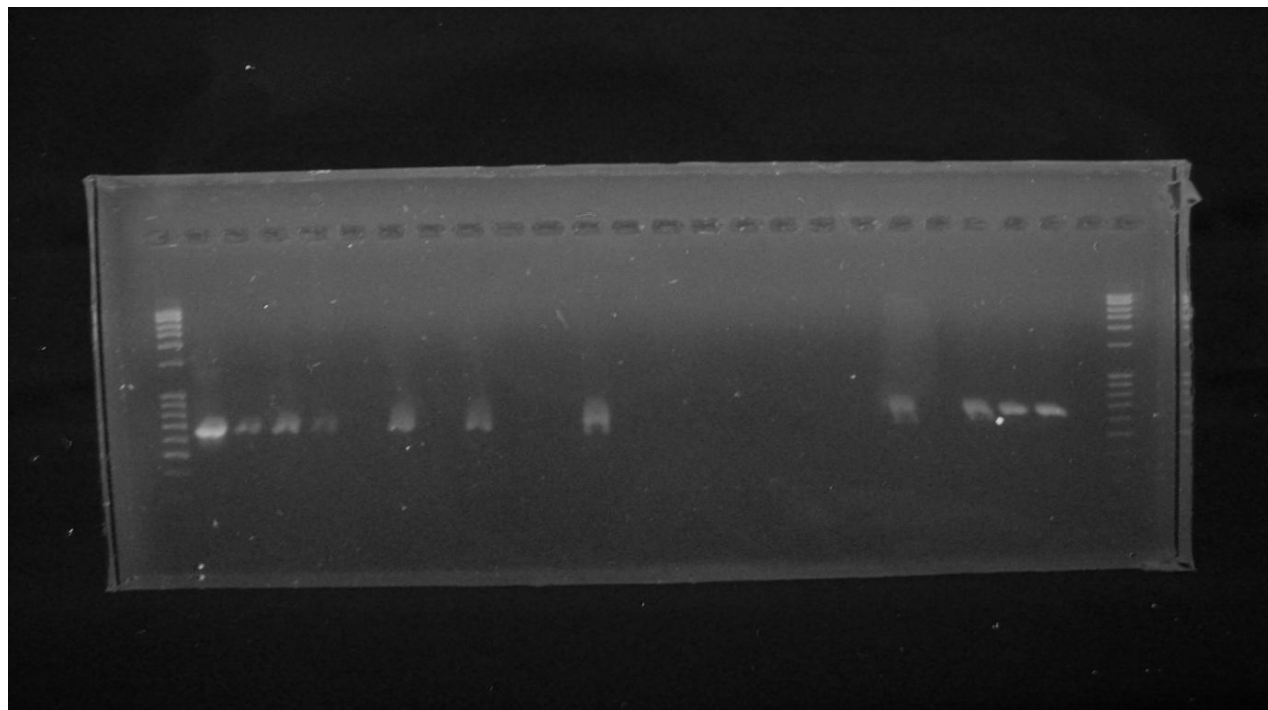The presence of *C. burnetii* in blood serum was

Figure 1. Example of PCR assay for *Coxiella burnetii.*

Table 1. Concentration of immunoglobulin G in serum from cow in experimental group.

Phases of production	Number of sample	IgG concentration (g/L)
Colostrums phase	6	73.1±17.5
First phase	7	80.2±16.3
Second phase	21	82.0±22.1
Third phase	38	95.4±20.8
Dry period	6	76.1±12.6

Table 2. Concentration of immunoglobulin G in serum from cow in control group.

Phases of production	Number of sample	IgG concentration (g/L)
Colostrums phase	7	36.2±6.3
First phase	10	41.8±6.0
Second phase	25	39.5±4.8
Third phase	32	40.9±5.2
Dry period	8	35.7±5.4

examined in 78 blood serums obtained from nine positive cows during different phases of production and 44 (56.4%) were positive.

Presence of *C. burnetii* in the blood serum in different phases of lactation is shown in Table 3 showed small variation during production phases; the lowest value was in the third phase (48.6%) and the highest value was during the first and second phase (66.6%).
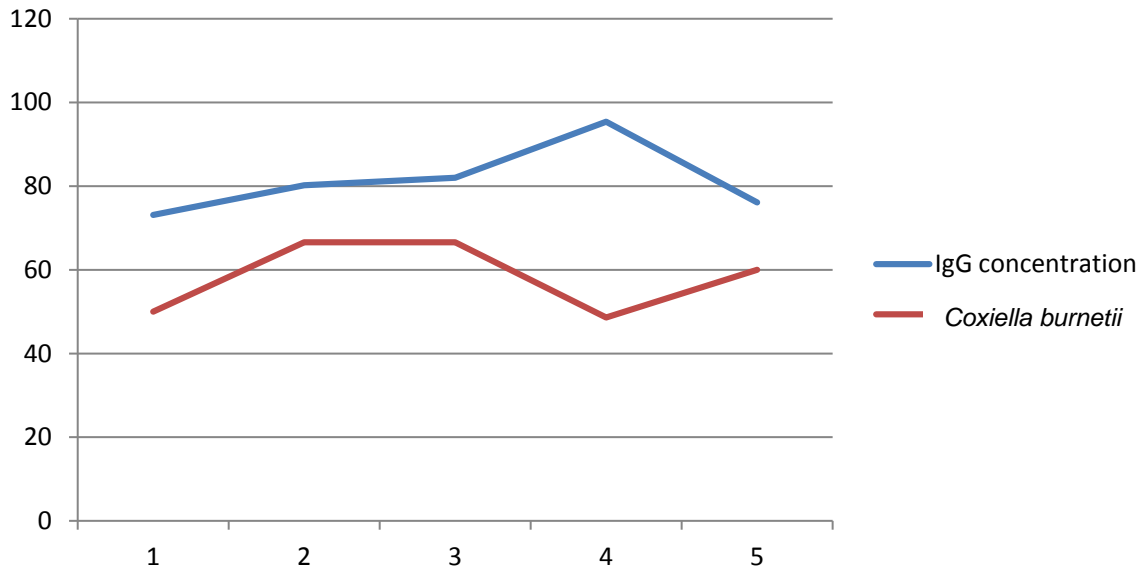
Relation between IgG concentration and presence of *C. burnetii* in blood serum from cows in experimental group is shown in Figure 2.

Correlation coefficient between IgG concentration and presence of *C. burnetii* in blood serum from cows in experimental group was -0.27. So, negative correlation

Table 3. Presence of *C. burnetii* in blood serum in different phases of lactation.

Phases of production	Number of sample	Presence of *C. burnetii* in blood serum (%)
Colostrums phase	6	50
First phase	7	66.6
Second phase	21	66.6
Third phase	38	48.6
Dry period	6	60

Figure 2. Relation between IgG concentration and presence of *C. burnetii* in blood serum of infected cows in five stages of production cycle.

was calculated.

DISCUSSION

Q fever disease, caused by *C. 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, *C. burnetii* is found in the reproductive system, both uterus and mammary glands, and may cause abortion or infertility (Kirkan et al., 2008).

The high prevalence of *C. 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 (To et al., 1998).

Concentration of immunoglobulin G in cows infected with *C. burnetii* (Table 1) was higher than the concentration in non infected cows (Table 2). The highest value of IgG concentration was in the third phase of lactation (95.4 g/L) and the lowest concentration was in colostrums phase (73.1 g/L). The reason for the decrease of concentration in colostrums phase was the lowering of immunoglobulin from blood in colostrums (Leyton et al., 2007).

The presence of *C. burnetii* in blood serum of infected cows was in the range from 48.6 to 66.6% and average presence was 56.4% (Table 3). If we calculate average presence of *C. burnetii* in blood serum on whole herd of200 cows where research was conducted, it was 2.5%. Other authors (Kirkan et al., 2008) found 4.5% of positive blood serum in herd, and McQiston et al.(2002) found 3.4% of positive serums. These are herds with small percent of positive serums. In future, we can expect increase percent of positive blood serums because big herds are with tendency of increasing the percent of infected animals (Biberstein et al., 1974).

The lowest presence of *C. burnetii* in blood serum was in the third phase of lactation when the highest concentration of immunoglobulin G was measured (Table 3). Statistical analysis showed that there was negative correlation between IgG concentration and the presence of *C. burnetii* in blood serum, and correlation coefficient was -0.27.

Conclusions

Concentration of immunoglobulin G in blood serum of infected cows was above the reference values during whole production cycle. The presence of *C. burnetii* in blood serum was from 48.6 to 66.6%.The lowest presence of *C. burnetii* in blood serum was when the highest concentration of immunoglobulin G was measured. Negative correlation between these two parameters was calculated.

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