

DYNAMICS OF *COXIELLA BURNETII* DNA IN MILK AND PHASE-SPECIFIC SEROLOGICAL RESPONSE IN DAIRY COWS

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Abstract

In dairy cows shedding of *Coxiella burnetii* in milk can be persistent, sporadic to absent. Persistent heavy shedder cows are mostly highly-seropositive. Serological response due to *C. burnetii* antigenic phase demonstrates acute or chronic infection (serological response to phase II (PhII) or phase I (PhI) antigen, respectively). The aim of this study was to detect dynamics of *C. burnetii* DNA in milk and phase-specific serological response. In this study, the sera samples from 46 animals and milk samples from 34 were collected initially. Milk and sera samples from 36 animals were collected repeatedly. Samples were collected in five herds with previous history of *C. burnetii* infection from different parishes in Latvia – in 2017 and 2018. Milk samples were tested by detection of *C. burnetii* DNA by real-time PCR amplification using ‘ADIAVET™ COX REALTIME’ (ADIAGENE). Sera were tested by ‘VetLine Coxiella Phase1 and Phase2 ELISA’ (NOVATEC). Three cows (9%) in the first sampling and five (14%) in second sampling demonstrated shedding of *C. burnetii* DNA in milk. Six cows (13%) in the first sampling and eight (22%) in second sampling demonstrated positive serological response to PhI. Three cows (7%) in the first sampling and one (3%) in second sampling demonstrated a questionable serological response to PhI. Two cows (6%) in the second sampling demonstrated a questionable serological response to PhII. In herds with previous history of *C. burnetii* infection the number of animals demonstrating PhI positive serological response increases significantly ($p < 0.05$) during six months. In cows demonstrating positive serological response to PhI it continues and mostly remains unchanged during six months.

Key words: Q fever, dairy cows, phase-specific antigen, shedding.

Introduction

Coxiella burnetii is an ethiological agent of zoonosis Q fever. It is Gram negative bacteria and appears as very short, pleomorphic rod (Babudieri, 1959; Fournier, Marrie, & Raoult, 1998). The major mechanism whereby *C. burnetii* is transmitted to humans and animals are contaminated aerosols containing bacteria (Angelakis & Raoult, 2010). *C. burnetii* are shedded from animals by birth products, vaginal mucus, milk, urine and faeces. No predominant shedding route has been identified (Guatteo *et al.*, 2006) although milk is a significant route of shedding (Guatteo *et al.*, 2007). In dairy cows shedding of *C. burnetii* in milk can be persistent or sporadic to absent. Persistent heavy shedder cows are mostly persistently highly-seropositive (Guatteo *et al.*, 2007). Serology allows to differentiate acute and chronic Q fever infection (Maurin *et al.*, 1999). Due to mutational variation of lipopolysaccharide (LPS) in cell membrane, *C. burnetii* displays two phases of antigen – phase I (PhI) and phase II (PhII). This is similar like rough-smooth variation in family *Enterobacteriaceae* although chemical structure of LPS from *C. burnetii* is different from those of Gram negative bacteria (Amano & Williams, 1984; Fournier, Marrie, & Raoult, 1998; Maurin *et al.*, 1999). PhI antigen is very infectious and corresponds to smooth LPS. When infection occurs, PhI is poorly internalized by monocytes and macrophages and can survive within these cells (Maurin *et al.*, 1999). Due to its cell membrane structure, it can block

access of antibodies to its surface (Fournier, Marrie, & Raoult, 1998). After internalization in host cell *C. burnetii* can limit proinflammatory response and protect itself from clearance (Sobotta *et al.*, 2016). This is why serological response to PhI antigen is found only in chronic infections (Angelakis & Raoult, 2010). PhII antigen is not very infectious, it displays truncated LPS and corresponds to rough LPS. When infection occurs, PhII is easily internalized by monocytes and macrophages and rapidly killed via the phagosomal pathway (Maurin *et al.*, 1999). Q fever in animals is mostly asymptomatic, including a lack of fever (Angelakis & Raoult, 2010). Because of difficult clinical diagnosis of Q fever, it can be diagnosed serologically. Antibodies to PhII appear rapidly, reach high titers in 14 days and can persist for 10-12 weeks while antibodies to PhI develop very slowly and presence of them characterises chronic Q fever (Angelakis & Raoult, 2010; Maurin *et al.*, 1999). Animal classification based on a phase patterns is reasonable (Lucchese *et al.*, 2015). Seroprevalence increases by increasing of animal's age (Böttcher *et al.*, 2011). The aim of this study was to detect dynamics of *C. burnetii* DNA in milk and phase-specific serological response. This study is a continuation of the first study ‘Shedding of *Coxiella burnetii* DNA in milk and antigen phase-specific serological response in dairy herds in Latvia’ which has been reported in conference ‘Research and Practice in Veterinary Medicine 2017’ (Jelgava, Latvia).

Materials and Methods

In this study, the sera samples from 46 animals (12 pregnant heifers, 34 milking cows) and milk samples from the same 34 milking cows were collected initially in July and August, 2017. During the time to next sampling 12 pregnant heifers gave birth and became primiparous cows. Also, during this time 10 cows (four primiparous, six multiparous) were culled for different reasons (dystocia with following paresis of hind limbs, lameness, low milk production, ulcer of abomasum *etc.*). No predominant reason of culling was observed. Milk and sera samples from 36 animals were collected repeatedly in February, 2018. Samples were collected in five herds with previous history of *C. burnetii* infection from different parishes in Latvia. As previous history of infection in herd was the evidence of *C. burnetii* DNA in aborted fetuses. Milk samples (approximately 20 mL) were collected from all quarters in sterile plastic containers. Tip of teat was cleaned using wipes impregnated with 70% ethanol. Removing of first milk jet was done before sampling. Blood samples (approximately 5 mL) were collected by caudal venipuncture in sterile vacutainers containing a clot activator. Skin was cleaned using wipes impregnated with 70% ethanol before puncture. Milk and blood samples were chilled until 4 °C immediately. Milk samples were frozen during 2 – 6 hours after sampling. Sera (approximately 1.5 mL) were aspirated from blood samples during 24 h and frozen. Milk and sera samples were stored at -16 °C until analysis. Samples collected in July and August 2017 were tested in August and September, 2017. Samples collected in February 2018 were tested in February, 2018. To achieve the correct diagnosis, a combination of different laboratory methods, preferably PCR for the agent detection and ELISA for serology is suggested (Niemczuk *et al.*, 2014). Milk samples were tested by detection of *C. burnetii* DNA by real-time PCR amplification using 'ADIAVET™ COX REALTIME' (ADIAGENE). The method used allows to quantify count of mobile element IS1111 of *C. burnetii* per 1 mL in milk. Sera were tested by 'VetLine Coxiella Phase1 and Phase2 ELISA' (NOVATEC). The method used allows to detect positive, questionable and negative samples. Data of dynamics of shedding and serological response comparing the first and second sampling were analysed using Z-test (Z Score Calculator for 2 Population Proportions).

Results and Discussion

In our study, 16 cows demonstrated at least one of parameters detected at least once (Table1). In our study, three cows (9%) demonstrated shedding of *C. burnetii* DNA in milk initially. One of them demonstrated shedding repeatedly, one was culled

before repeated sampling. Three cows were shedders only in the second sampling. In total, five cows (14%) demonstrated shedding of *C. burnetii* DNA in milk when tested the second time. There was no significant ($p>0.05$) difference in the number of shedders compared the first and second sampling. Shedding in our study was low comparing with other studies where it has been detected from 32% in 12 herds (Angen *et al.*, 2011) to 50% in five dairy herds (Guatteo *et al.*, 2007). In our study, six cows (13%) demonstrated positive serological response to PhI (PhI⁺) initially. Four cows out of PhI⁺ initially demonstrated the same serological response when tested repeatedly (one of them became PhI⁺/PhII^{+/-}). One PhI⁺ cow was culled before repeated sampling, other one demonstrated no serological response when tested repeatedly. Four cows without serological response initially demonstrated PhI⁺ when tested repeatedly. In total, eight cows (22%) were PhI⁺ when tested repeatedly. There were significant ($p<0.05$) increase of PhI⁺ cows comparing the first and second sampling. The PhI⁺/PhII⁻ pattern as a rare finding was described in seven multiparous cows out of 1932 animals in 105 herds. Subsequently, five of them became negative. In the same study PhI⁺/PhII⁻ pattern developed from pattern PhI⁺/PhII⁺ in six animals (Böttcher *et al.*, 2011). It is in contradiction with our finding where pattern PhI⁺/PhII^{+/-} developed from PhI⁺. However, no shedding of *C. burnetii* DNA in milk in this study was detected. In our study, three cows (9%) demonstrated a questionable serological response to PhI (PhI^{+/-}) initially. One of PhI^{+/-} cows initially had no serological response when tested repeatedly. Other two of them were culled before repeated sampling. One cow (3%) demonstrated PhI^{+/-} serological response when tested repeatedly. No significant ($p>0.05$) increase of PhI^{+/-} cows was observed comparing the first and second sampling. Only two cows (6%) in repeated sampling demonstrated a questionable serological response to PhII (PhII^{+/-}). Both of them demonstrated PhI⁺/PhII^{+/-} serological pattern. To the best of our knowledge, no reports related by questionable phase-specific serological response to *C. burnetii* antigen are available. None of cows demonstrated serological response to PhII initially. This finding is in contradiction with Böttcher *et al.*, 2011 study where serological response to PhII was a frequent finding. In our study, it was one cow shedding *C. burnetii* DNA in milk without any serological response initially. It matches with Barberio *et al.*, 2014 finding where out of 36 cows shedding *C. burnetii* DNA in milk, 11 had no antibodies in sera detectable by ELISA. Shedding may not be the correct tool, especially if a cow is suffering from new infection (Garcia-Ispuerto *et al.*, 2013).

Table 1

Dynamics of *Coxiella burnetii* DNA shedding in milk and phase-specific serological response

First sampling		Second sampling	
Serological response	Shedding	Serological response	Shedding
PhI ^{+/-}	DNA ⁻	Ph ⁻	DNA ⁻
Ph ⁻	DNA ⁻	PhI ⁺	DNA ⁺
Ph ⁻	DNA ⁻	PhI ⁺ /PhII ^{+/-}	DNA ⁻
Ph ⁻	DNA ⁻	Ph ⁻	DNA ⁺
Ph ⁻	DNA ⁻	Ph ⁻	DNA ⁺
PhI ^{+/-}	DNA ⁺	animal culled	
Ph ⁻	DNA ⁻	PhI ⁺	DNA ⁻
PhI ⁺	DNA ⁺	PhI ⁺ /PhII ^{+/-}	DNA ⁻
Ph ⁻	DNA ⁺	PhI ^{+/-}	DNA ⁺
PhI ⁺	DNA ⁻	PhI ⁺	DNA ⁻
PhI ⁺	DNA ⁻	PhI ⁺	DNA ⁻
Ph ⁻	DNA ⁻	PhI ⁺	DNA ⁺
PhI ⁺	DNA ⁻	animal culled	
PhI ⁺	DNA ⁻	PhI ⁺	DNA ⁻
PhI ⁺	DNA ⁻	Ph ⁻	DNA ⁻
PhI ^{+/-}	DNA ⁻	animal culled	

DNA⁺ – shedding of *C. burnetii* in milk, DNA⁻ – non shedding of *C. burnetii* in milk, PhI⁺ – positive serological response to *C. burnetii* Phase I antigen, PhI^{+/-} – questionable serological response to *C. burnetii* Phase I antigen, PhII^{+/-} – questionable serological response to *C. burnetii* PhII antigen, Ph⁻ – no serological response to any of *C. burnetii* phase antigen.

Conclusions

The study demonstrated that PhI^{+/-} serological response without shedding of *C. burnetii* in milk can vanish completely. In cow shedding of *C. burnetii* DNA in milk, serological response to PhI appears during six months. Cows without phase-specific serological response or shedding become shedders with or without serological response to Ph during six months. In herds with previous history of *C. burnetii* infection, the number of shedders or animals with serological response increases over time. The number of animals with PhI⁺ serological response increases significantly (p<0.05). It indicates that infection is active in the herd level. Pattern of serological response PhI⁺ which is characteristic to chronic infection mostly stays unchanged during six months or can change to PhI⁺/PhII^{+/-}. There are difficulties to

permit definitive conclusions due to 21% of animals that were culled before repeated sampling. The study will be continued.

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