Prevalence of *Coxiella burnetii* in dairy herds – diagnostic methods and risk to humans - a review

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Abstract

Q fever is a zoonotic disease caused by *Coxiella burnetii*. The main source of infection are ruminants (cattle, sheep, and goats). *C. burnetii* is excreted via birth products, vaginal mucus, milk, and faeces. Raw milk is considered useful for epidemiological examinations of animals and evaluation of infection dynamics at the herd level. This article summarises data on prevalence studies on *C. burnetii* in bulk-tank milk in different European countries with the means of serological tests and PCR. It also summarises the results of studies to evaluate the actual risk of disease transmission to humans through consumption of raw milk. Moreover, the available diagnostic tools for detection *C. burnetii* infection are presented.

**Keywords:** *Coxiella burnetii*, Q fever, ruminants, diagnostic methods.

Introduction

*Coxiella burnetii*, the aetiologic agent of Q fever, is an obligate intracellular bacterium that produces dense, small, and highly resistant spore-like forms. Infection in animals is mainly asymptomatic; however, clinical signs can appear during pregnancy. They can result in abortion or stillbirth. *C. burnetii* is excreted via vaginal mucus, birth products, milk, and very rarely via faeces. The clinical signs, duration, and the shedding routes for cattle are quite different from those of small ruminants (sheep and goats). The disease in cows is mainly asymptomatic and the animals shed *C. burnetii* with milk. Goats with clinical signs (abortion) shed the bacteria in milk, while ewes with clinical signs shed the pathogen in faeces and vaginal mucus (13, 14, 29). Excretion of the pathogen by ruminants (cattle, sheep, and goats) is the main source of environmental contamination and the cause of human infection. Current epidemiological studies indicate that human infections are mostly noted in people who have close contact with domestic animals (mainly with cattle, sheep and goats) and that Q fever should be considered a public health problem in many countries. The largest human epidemic occurred between 2007 and 2010 in the Netherlands (16). During this epidemic transmitted by infected dairy goats, 4108 cases of Q fever were documented in humans (21). Infection with *C. burnetii* by ingestion of contaminated milk or dairy products remains controversial, but cases of Q fever were described in regions where raw milk and raw milk products were commonly consumed (7, 24). Therefore, in the twentieth century, pasteurisation became the international standard means to destroy *C. burnetii* and protect milk consumers' health. Bulk-tank milk (BTM) is considered useful for epidemiological surveys of dairy herds. In the laboratory diagnosis of Q fever, either serological (ELISA) or molecular (PCR) methods are useful but for the effective evaluation of the infection and detection of shedders at the herd level it is necessary to use both techniques (27). The main diagnostic problem is the use of vaccination against *C. burnetii*, because the commonly used serological and molecular methods are unable to differentiate the wild strains from vaccine strains. Recently, this has become possible by using genotyping (19).

Prevalence of *C. burnetii* in ruminants

Many data is available about investigations of BTM in ruminant farms throughout Europe. The
studies were performed mainly on bovine BTM. Easily and inexpensively collected BTM, is the best choice for herd level evaluation of excretion of *C. burnetii* by infected cows (12). The available data shows that seroprevalence in European countries was estimated between 7% and 88% in the years following 2000 (11). The studies from recent years presented similar results. PCR assays in south-west England disclosed that the prevalence of *C. burnetii* in herd was 69.7% (34) and serological analyses of BTM samples in northern Spain revealed that 67% of dairy cattle herds were seropositive and that *C. burnetii* DNA was present in 52% of the herds (1). Muskens *et al.* (25) reported that in the Netherlands 78.6% of Dutch herds tested had antibodies against *C. burnetii* in BTM, out of the tested animals 56.6% presented detectable bacterial DNA. Studies conducted in Belgium revealed positive PCR results to 30% of BTM samples, while the prevalence of *C. burnetii* in BTM in the UK was 69.7% for PCR (8, 33). An epidemiological survey in the USA quantified the prevalence of *C. burnetii* at 94.3% (30). Q fever can occur endemically in south-eastern Poland and in the German region of Baden-Württemberg, where seroprevalence was 78.3% (17, 26, 31).

**Shedding of *C. burnetii***

There are different routes of *C. burnetii* shedding in cattle: mainly via birth products (birth fluids and placenta) but also via vaginal mucus, milk, faeces, urine, and semen. In bovines, *C. burnetii* DNA is detected more often in milk than in vaginal mucus after abortion. The shedding of the pathogen in milk was described for its taking one of two different profiles (5). The first profile is active and associated with the SNP1 or CbNL01-like MLVA genotype of *C. burnetii*, and the second one is discontinuous and is caused by the other genotypes (5). According to the data from the European Food Safety Authority (EFSA), Q fever is present in a herd when abortions or reproductive disorders are observed, about 50% of animals are seropositive, and *C. burnetii* DNA is detected in vaginal mucus or placentas (9). However, according to the available literature data, the examination of environmental samples is an important additional diagnostic step (27). It should be noted that the presence of *C. burnetii* in bovine faecal samples is noted very rarely, so faeces should not be included in sampling protocols described to evaluate *C. burnetii* in bovine herds (25). Dust from surfaces, air, or slurry are appropriate environmental samples for identification of the infection status. The presence of *C. burnetii* DNA in the environment confirms the presence of infection and active shedders in the herd (28). Shedders, mostly among older cows, and seronegative groups of younger animals can together indicate past infection rather than active infection. The presence of *C. burnetii* antibodies in heifers would give valuable information about recent infection in the herd (9, 28). A moderate-to-high seroprevalence in young animals indicates that the pathogen is circulating in the herd. Böttcher *et al.* (6) demonstrated that seroprevalence of *C. burnetii* depends on the animals’ age. The seroprevalence is less than 5% in one to two-year-old animals, increases to 15% in two to three-year-olds, and the highest level of 20-30% is detected in cows older than four years of age. It was observed in young animals with acute infection that cattle seropositive at the age of one to two years were negative upon subsequent testing.

**Coxiella burnetii in dairy products and human risk of infection**

After the 2nd World War there was a high prevalence of Q fever in humans in Europe and North America, mainly in the region where raw milk and raw milk products were consumed (7). In the 1950s, the pasteurisation of milk became an international standard (7). Prevalence of *C. burnetii* in dairy products seems to play a smaller role in transmission of Q fever than shedding the pathogen in vaginal mucus, birth products, or faeces (10). The hypothesis that infection with *C. burnetii* by the alimentary route is possible remains controversial. The previous experiments performed by Benson *et al.* (3) revealed that a person who consumed raw milk had higher rates of Q fever antibodies than a person from the control group. On the other hand, the results obtained by Krumbiegel and Wiśniewski (22) did not indicate seroconversion or clinical illness in a group of humans who consumed infected raw milk. Opposite results were described by Beck and Bell (2) who reported that 32% of 300 Q fever cases in Los Angeles were detected in people who consumed raw milk. Similar results were described by Klimberley *et al.* (20). They reported two cases of infection in humans who consumed raw milk in circumstances excluding the possibility of infection by any other route, because these people had never visited a farm. In recent years, consumption of raw milk has increased, also in Poland: a few Polish cities have machines vending raw milk directly from farms. Taking into account the frequency of *C. burnetii* in BTM described in the other European countries, there is a high risk of a similar epidemiological situation in Poland but it requires further studies. A preliminary evaluation performed in Poland indicates that DNA of *C. burnetii* is present in commercially available dairy products (32). The studies of dairy products in France suggest that DNA of *C. burnetii* is highly prevalent in cheese and yogurts (10, 18). Products made from cow’s milk are more frequently *C. burnetii* positive compared to products made from goat’s or ewe’s milk. This greater prevalence of *C. burnetii* in milk products derived from cattle rather than those derived from small ruminants might be explained by the higher number of cattle farms compared to small ruminant farms. So far,
despite the presence of *C. burnetii* DNA in many dairy products no viable bacteria have been isolated from them. Thus, the transmission of Q fever by consumption of these products probably does not occur, whereas potential sources of human infection are in dairy products manufactured from unpasteurised milk (*e.g.* cheese) (10). Epidemiological studies performed in Greece showed that consumption of cheese from rural areas increased the risk of Q fever. Although the risk of infection by the alimentary route is pervasive, cheese is frequently distributed directly from stockbreeders and farms (24). Products made from unpasteurised milk have a higher number of *C. burnetii* than those made from pasteurised milk. The pasteurisation inactivates the bacteria and reduces the number of viable cells in milk but does not fully destroy them, leaving genetic material of *C. burnetii* enabling its detection in pasteurised milk and milk products (10).

**Diagnostic methods for Q fever detection in bovine herds**

The correct diagnosis of Q fever is possible based on results obtained by serological and molecular methods. Serological testing by ELISA and the use of polymerase chain reaction (PCR) are the preferred methods for the detection of the causative agent. The first step in detecting infected animals is based on serological methods (ELISA or complement fixation test (CFT). These methods are useful but they are not suitable for an early detection. Furthermore, cross-reactivity between *C. burnetii* and other pathogens involved in reproductive disorders including *Chlamydia* sp. has been observed (23). Molecular methods such as PCR have been developed for detection of an individual pathogen or duplex PCR for simultaneous detection of *C. abortus*, *C. pecorum* and *C. burnetii* (4).

**BTM** is the appropriate biological material for detection of *C. burnetii* shedders in cattle or small ruminants (goats and sheep). The quantitative analysis of BTM by real-time PCR allows evaluation of the infection status at the herd level. Increased estimated titres over time could suggest the existence of an active bacterial infection. Moreover, repeated testing of BTM limits false negative results which could be due to non-lactating cows excluded for one iteration (dry cows or treated animal in milk withdrawal time). Serological testing of BTM samples is a useful epidemiological tool at the population level and can be used to differentiate seropositive and seronegative herds. However, additional assays are required to evaluate whether Q fever is a potential problem. The next attempts in investigating exposure of a cattle herd to *C. burnetii* should include a serological test on heifers. Seropositivity in heifers could confirm recent infection in the herd. Testing placentas or vaginal mucus from heifers and cows with reproductive disorders is the further step to be performed. Due to the very short duration of *C. burnetii* shedding in the vaginal mucus after abortion, samples should be collected rapidly. Guatteo et al. (15) observed that *C. burnetii* in vaginal mucus was present only in 8 out of the initial 24 shedder cows, 14 days after abortion. The detection of shedders is crucial in controlling the spread of the *C. burnetii* infection among animals and from animals to humans.

Several studies revealed that ELISA failed to detect *C. burnetii* antibodies in cows which were positive in PCR. Previously, it was considered that this observation was connected with lack of sensitivity of available ELISAs which included the tick-derived Nine Mile strain of *C. burnetii*. Nevertheless, changing the antigen for a ruminant strain caused a higher test sensitivity but some PCR positive animals remained seronegative. It could be assumed that animals may build up an efficient cellular immunity with low or undetectable levels of antibodies. Animals with cellular immune response in absence of detectable antibodies could be how endemic infection with *C. burnetii* persists in dairy herds. These animals would give birth to non-exposed, without immune response calves which could be infected as pregnant primiparous cows and cause endemic infection at the herd level (6).

The literature data showed that in the endemically infected herd, the seropositive cows at calving were equalled to 50%. The infection of calves *in utero* could have been masked by maternally derived antibodies, which decreases almost to zero between six and 12 months of their lives (6).

A vaccine (Coxevac, Ceva Santé Animale, France) against *C. burnetii* is available in a few European countries including Poland but the major problem associated with vaccination is the impossibility of distinguishing vaccinated from naturally infected animals using serological methods such ELISA or CFT. Performing monitoring or epidemiological studies, it is very important to evaluate whether antibodies are produced due to natural infection or as a result of vaccination. The suitable tool for assessment of an outbreak of Q fever and for differentiation of the Nine Mile vaccine strain from strains circulating naturally in the environment is a single-nucleotide-polymorphism (SNP) method described by Huijsmans et. al. (19). The method is rapid, sensitive, easy to perform, and unambiguous in result interpretation, while Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) is useful for epidemiological studies (16).

**References**


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