Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle

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Abstract

Until recently, Q fever was notified in very low numbers annually in Denmark and it was always considered to be acquired abroad. Preliminary reports now describe *Coxiella burnetii* in milk samples from Danish dairy cattle. Serum samples of a large cohort of farmers, veterinarians, inseminators and hoof trimmers, all having occupational contact with dairy cattle, were tested for the presence of IgG to phase I and phase II antigens of *C. burnetii*. In 39 of 359 individuals studied (11%), the presence of antibodies to *C. burnetii* was found. Veterinarians had the highest seropositivity rate (36%). This survey suggests that *C. burnetii* is a recently recognized domestic infection in Denmark and that risk of infection is associated with occupation.

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Introduction

Q fever is a zoonotic infection caused by the obligate intracellular organism *Coxiella burnetii* [1]. *C. burnetii* can be found in high numbers in amniotic fluid, placenta and foetal membranes as well as in milk, urine and faeces of infected animals [2–4]. The primary mode of human infection involves the aerosol route [5], and the domesticated animals most often implicated in human disease are sheep, cattle and goats. Occasional outbreaks have been described in many countries, although the majority of cases are probably not diagnosed as a result of a subclinical or nonspecific self-limiting clinical course of the infection. Patients that have been infected with *C. burnetii* remain seropositive and are considered to be immune to acute Q fever for at least some years. Certain professions have an elevated risk of exposure to *C. burnetii* because of their occupational contact with animals [6–9].

C. burnetii is distributed in wild and domesticated animals worldwide [10]. Earlier literature suggested New Zealand and the Scandinavian countries to be free of Q fever, but human cases linked to sheep farming have been published from Sweden [6,11] and, recently, *C. burnetii* has been reported as one of the causes of abortion in Danish sheep [12]. Until recently, Q fever in Denmark was diagnosed in very low numbers annually and it was always consodered to be acquired abroad. However, we have now encountered veterinarians and farmers with Q fever without travel history, and *C. burnetii* has been identified in placenta material from aborting cows [13]. Preliminary reports suggested a high prevalence of *C. burnetii* in milk samples from Danish dairy cattle. We decided to perform a seroepidemiological survey in a sentinel cohort of farmers, veterinarians, inseminators and hoof trimmers in one of the main agricultural activities in Denmark because all the included individuals have an occupational contact with dairy cattle.

Materials and Methods

A total of 359 adult individuals (age > 18 years) considered to be at potential risk of contracting an infection with *C. burnetii* because of close contact with dairy cattle were studied. The sentinel group consisted of farmers, veterinarians, inseminators and hoof trimmers. The study was approved by

the Regional Scientific Ethics Committee (N-20070060), and all subjects provided their informed consent. Blood samples were obtained between January and October 2008. The farm residents (n = 163) came from 100 randomly selected farms, the veterinarians (n = 87) were recruited from the association of veterinarians working with dairy cattle as their main occupation (membership 120), the inseminators (n = 95) were recruited from the association of Danish inseminators (membership 182) and, lastly, hoof trimmers (n = 14) were also included.

Mailed questionnaires recorded the participants' demographic data, occupation, travel history and clinical illness for the last 2 years. The 359 participants included 101 women and 258 men, aged 18–69 years. There was no difference in age, sex or occupation between the individuals who agreed to participate and those who did not.

Serum samples were tested for the presence of IgG reacting with phase I and phase II antigen of *C. burnetii* strain Nine Mile using a commercially available immunoflourescence assay (Focus Diagnostics, Cypress, CA, USA). The test was performed according to the manufacturer's instructions in a certified laboratory at Statens Serum Institut, Copenhagen, Denmark. On the basis of testing serial dilutions, a semiquantitative titre was obtained. A sample was considered positive for antibodies against *C. burnetii* when a titre of IgG phase I ≥ 512 or IgG phase II ≥ 1024 was determined. These cut-off levels have previously been determined based on a study involving 158 healthy blood donors (assumed not to have Q fever) from three city areas of Denmark [14]. In 27 cases, an indeterminate result was obtained with the first sample (anti-phase I and anti-phase II antigen IgG titres of 128–256 and 256–512, respectively) and a second test was performed after 3 months, although a seroconversion to positivity was not observed in any of these cases.

In all cases of elevated IgG titres, an additional assessment of sedimentation rates, C-reactive protein, liver function and white blood cell counts was performed.

Results

In 39 of 359 study individuals (11%) anti-*C. burnetii* IgG was detected. *C. burnetii* antibodies were found in 31 of 87 veterinarians (36%), in two of 15 inseminators (2%), in five of 163 farmers (3%) and in one of 14 hoof trimmers (7%). Among the seropositive individuals, there were 20 with a positive IgG phase I titre and 26 with a positive IgG phase II titre. The titres of IgG antibodies varied; few very high values were observed (Table 1). There was no correlation between age and the IgG positivity rate (3.7% aged 18–29 years, 16.5% aged 30–39 years, 7.7% aged 40–49 years, 15.9% aged 50–59 years, and 5.6% aged 60–69 years were positive). None of the seropositive subjects had signs or symptoms of acute Q fever infection at the time of examination, and biochemical parameters were within normal range in all cases. In one case, a cardiac murmur was detected and, by echocardiography, a moderate insufficiency of the mitral valves without excrescences was observed. The IgG phase I titre was 4096 and a diagnosis of possible Q fever endocarditis was made. In all of the remaining 38 cases, no abnormalities were found.

TABLE 1. Titre distribution of antibodies against *Coxiella burnetii* phase I and phase II antigens among 359 high-risk subjects in Denmark

	Phase I antigen		Phase II antigen	
Titre	*n*	%	*N*	%
<256	309	86.0	259	72.1
256	30	8.4	40	11.1
512	9	2.5	34	9.5
1024	8	2.2	23	6.4
2048	2	0.6	1	0.3
4096	1	0.3	2	0.6
Totals	359	100.0	359	100.0

Dashed lines denote the separation between seronegative and seropositive subjects based on the cut-off criteria used in the present study.

The percentage of IgG-positive subjects among pooled groups was higher in males (64%) than in females (36%); in addition, the frequency of antibodies among veterinarians was higher in males (58%) than in females (42%), although the difference between positives in the male and in the female group was not statistically significant in the pooled groups (p 0.25) or among the veterinarians (p 0.69). Twenty-three percent of the seropositive individuals and 30% of the seronegative individuals did not have any travel history (traveling out of country) for the last 2 years. There was no statistically significant difference (p 0.32) between these two groups. We found 51% of the seropositive individuals and 40% of the seronegative individuals with at least one episode of influenza-like symptoms during the last 2 years. There was no statistically significant difference (p 0.21) between these two groups.

Discussion

C. burnetii is distributed worldwide, with the exception of New Zealand [9]. The earlier availabale literature reported that the Scandinavian countries were free of Q fever and that the few sporadic cases were acquired abroad. It has been suggested that Denmark and its Scandinavian neighbours are the only exceptions with respect to the

worldwide distribution of Q fever [15]. However, seroepidemiological studies have revealed antibodies against *C. burnetii* in humans from all over Sweden [10,11] and *C. burnetii* has been reported as one of the causes of abortion in Danish sheep [12]. This survey suggests that the Q fever situation in Denmark is similar to that of other countries and that *C. burnetii* infection is a domestic infection in Denmark. There was no difference in seropositivity rates between individuals with and without travel history, which supports our hypothesis that study individuals were infected in Denmark.

The question arises as to whether the increase in the number of diagnosed cases is the result of a recent introduction of Q fever into Denmark or the consequence of increased surveillance and assessment of antibodies in patients with a perceived increased risk. The observation of no correlation between age and seropositivity among adults with risk exposure suggests that *C. burnetii* has not been widespread among Danish dairy cattle for a long time, but may have been introduced recently.

By contrast, reports from other countries with Q fever endemic for many years describe an increased seropositivity at increasing age [16,17].

The survey conducted in the present study demonstrated the highest prevalence in veterinarians, which is in agreement with observations from many other countries [8,11,18,19], whereas surprisingly few farmers and inseminators had antibodies despite their well recognized daily contact with dairy cattle. In Sweden, 13% of veterinarians and 28% of sheep farmers tested had antibodies against *C. burnetii* compared to 7% of hospital employees [11]; 13.5% of Japanese [8] and 9.5% of Australian veterinarians [18] had antibodies. Approximately 25% of Swiss veterinarians were positive in contrast to 3.5% of blood donors [19], and a recent survey found 22.2% seropositivity among US veterinarians [20]. It is difficult to compare the results of our survey with those of serological studies in other countries because different cut-off criteria were used. We used very high cut-off levels and we found an almost three-fold higher percentage of seropositive veterinarians (36%) than that observed in our neighbouring country, Sweden.

It is obvious that the value of the cut-off titre used as a criterion for seropositivity will have a great influence on the results of any study. Differences between assays and between populations make it difficult to directly compare reports from different countries. Consequently, although using a commercial assay from the USA, we decided to use cut-off values that had been determined on the basis of results obtained from a control group of healthy adults from major cities in Denmark. To increase the positive predictive value of the test in a presumed low endemic area, the cut-off values had been selected rather conservatively as one titre above that of any sample obtained from healthy urban controls [14].

We acknowledge that the results obtained in the present study likely represent the low end of the true seroprevalence, and that this definition will potentially make the rate of seropositivity in our study cohort appear to be lower than than that found in other surveys.

Only 3% of Danish farmers were seropositive. This is a low percentage, especially when milk samples of 61% of Danish diary cattle have been found to be positive for *C. burnetii* (J. Agerholm, unpublished data). In other countries, this percentage was much higher: 28.5% of Swedish sheep farmers [6] and 27% of English farmers [9] were seropositive, and 17.8% of Polish farmers had IgG phase I antibodies [21]. In Milwaukee, the seropositivity among residents of dairy farms was much higher (28.5%) than in a local control group (2.2%) [22], which was also observed in Maryland (15.3% vs. 0.15%) [23]. The reason why veterinarians are more exposed than farmers may be that the veterinarians work with sick animals also during parturition and with various types of livestock from many different farms, whereas the farmers usually work with healthy animals and with one livestock only. We have no information on other potential sources of exposure (e.g. dogs or cats), which could explain a difference between risk groups. However, it is unlikely that dog or cat ownership should influence the probability of developing antibodies against *C. burnetii* [24]. However, other unassessed differences in exposure between veterinarians and farmers or controls cannot be excluded.

Among 39 participants with significant, elevated titres of IgG antibodies against *C. burnetii*, only one case of possible chronic Q fever was observed. All other subjects were without symptoms and biochemical measurements were all within normal ranges. We did not find a higher prevalence of clinical illness in the seropositive group within 2 years prior to the study as compared to the seronegative group.

Because the main domestic animals in Denmark, apart from pigs, are cattle, and only very few sheep and goat farms exist, another explanation could be that Q fever acquired from cattle has a milder clinical course. Although clinical illness appears to be very rare, we should consider the possibility of Q fever in cases of unexplained illness in patients with occupational contact to cattle', especially veterinarians.

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Transparency Declaration

The authors have no conflict of interest to report.

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