Seroprevalence of *Coxiella burnetii* and *Brucella abortus* among pregnant women

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Abstract

Coxiella burnetii and *Brucella abortus* are two intracellular bacteria implicated in zoonotic miscarriage. In the present study, *C. burnetii* and *B. abortus* seroprevalence was compared among women from London with and without miscarriage. *Coxiella burnetii* seroprevalence was high (4.6%, 95% CI 2.8–7.1) despite the rare apparent exposure of this urban population. Only two patients exhibited anti-*B. abortus* antibodies. As a result of the risk of chronic Q fever with endocarditis and/or hepatitis, the mode of *Coxiella burnetii* infection in this population merits further investigation.

Keywords: *Chlamydia*-like, *Coxiella burnetii*, cross-reaction, miscarriage, *Parachlamydia acanthamoebae*, Q fever, seroprevalence, *Waddlia chondrophila*

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Coxiella burnetii, the agent of Q fever, is an intracellular bacterium that may cause systemic infections in humans. Acute Q fever is often asymptomatic or manifests only as an influenza-like unrecognized illness [1,2]. It may also present as hepatitis or atypical pneumonia. Blood culture-negative endocarditis, vascular infections, osteoarticular involvement and chronic liver diseases are the main clinical pictures of chronic Q fever, which represents a rare complication of acute Coxiella infection [1]. Domestic animals and pets are the most frequent source of *Coxiella* infections in humans [1]. *Brucella abortus* is another zoonotic intracellular bacterium that may cause systemic infection. This infection frequently presents as a sustained fever (91% of cases), rarely associated with hepatomegaly (17%), splenomegaly (16%) and lymphadenopathy (6%) [3,4].

Both bacterial species have been associated with human miscarriage [1–4]. *Coxiella burnetii*, when contracted during human pregnancy, may result in a fatal outcome [5–9]. However, other studies performed in more than 12 000 pregnant women failed to confirm an association of *Coxiella* seropositivity with pregnancy outcome [10]. The reproductive system is the second most common site of brucellosis, being associated with a substantial risk of miscarriage during pregnancy [11,12].

The main aim of the present study was to evaluate *C. burnetii* and *B. abortus* seroprevalence among a population of pregnant women in a non-endemic area. In addition, the role of *Coxiella* and *Brucella* as agents causing miscarriage was also assessed.

Sera were obtained from women attending the Recurrent Miscarriage Clinic of St Mary's Hospital, London. The 438 sera had previously been investigated for *Waddlia chondrophila* and other *Chlamydiae* [13]. This allowed the assessment of any serological cross-reactivity among *C. burnetii*, *B. abortus* and several other previously studied intracellular bacteria, which are listed in Table 1.

Sera were tested for the presence of antibodies directed against *C. burnetii* using indirect immunofluorescence [14]. Briefly, sera were screened using an indirect immunofluorescence assay (IFA) at a 1/50 dilution with *C. burnetii* phase I and II antigens (strain Nine Miles, kindly provided by W. Burgdorfer, Rocky Mountain Laboratories, Hamilton, MT, USA). We used fluorescein isothiocyanate goat anti-human specific IgG conjugate (bioMérieux, Marcy-l'Etoile, France). Positive sera were then serially two-fold diluted starting at 1/20.

Serological *B. abortus* diagnosis was established using the Wright's tube agglutination test (Brucella Antigen, Sanofi Diagnostics, Marnes-la-Coquette, France). Antibody reactivity against *Toxoplasma gondii* was assessed using a commercial latex agglutination kit, Toxo-Screen DA (bioMérieux). Prevalence and p values were calculated using STATA software (StataCorp, College Station, TX, USA).

TABLE 1. Characteristics of patients according to their *Coxiella burnetii* serostatus

	Coxiella negative (n = 418)		*Coxiella* positive (n = 20)		p value*
Age					
Median (IQR)	33	(28–38)	33	(29–37)	0.843
Number of pregnancy					
1	115	(27.5%)	7	(35%)	0.239
2	77	(18.4%)	1	(5%)	
>2	226	(54.1%)	12	(60%)	
Parity					
0	159	(38%)	3	(15%)	0.067
1	175	(41.9%)	10	(50%)	
2	50	(12%)	4	(20%)	
>2	34	(8.1%)	3	(15%)	
Miscarriages					
Early ≤12 weeks	242	(57.9%)	9	(45%)	0.355
Late >12 weeks	61	(14.6%)	3	(15%)	1.000
Stillbirth >24 weeks	15	(3.6%)	0	(0%)	1.000
Ethnicity					
White	235	(56.2%)	11	(55%)	1.000
Black	63	(15.1%)	4	(20%)	0.526
Asian	78	(18.7%)	5	(25%)	0.557
Other	42	(10.5%)	0	(0%)	0.241
Born in the UK	224	(53.6%)	10	(50%)	0.821
Contact with animals	106	(25.4%)	4	(20%)	0.793
Cat	57	(13.6%)	3	(15%)	0.745
Dog	46	(11%)	3	(15%)	0.480
Additional serologies					
Waddlia chondrophila (IgG≥1/64)	93	(22.3%)	4	(20%)	1.000
Parachlamydia (IgG ≥1/64)	7	(1.7%)	0	(0%)	1.000
Chlamydia trachomatis (IgG ≥1/50)	61	(14.6%)	2	(10%)	0.752
Chlamydia pneumoniae (IgG ≥1/64)	187	(44.7%)	12	(60%)	0.250
Chlamydia psittaci (IgG ≥1/64)	26	(6.2%)	0	(0%)	0.622
Brucella abortus (Ig ≥1/20)	2	(0.48%)	0	(0%)	1.000
Toxoplasma gondii (Ig ≥1/20)	96	(22.3%)	4	(20%)	1.000

IQR, interquartile range.
*Fisher's exact chi-squared test.

Among the 438 women enrolled in the study [13], 20 (4.6%, 95% CI 2.8–7.1) were positive for *C. burnetii* phase II IgG antibodies. None was positive for *C. burnetii* phase I IgG antibodies. Only two (0.5%, 95% CI 0.1–1.7) also exhibited IgM antibodies against *C. burnetii*. No statistical differences were observed between *C. burnetii* IgG seroprevalences in women with sporadic (1/69, 1.4%, 95% CI 0.1–8.1, p 0.188) or recurrent miscarriage (8/200, 4%, 95% CI 1.7–7.9%, p 0.346) compared to controls (11/169, 6.5%, 95% CI 3.2–11.6).

The demographic characteristics and potential risk factors for IgG seropositivity for *C. burnetii* are shown in Table 1. Although outbreaks of Q fever in humans result from inhalation of aerosols from infected parturient animals, the frequency of animal contacts was similar between women who were *C. burnetii* IgG positive and those who were *C. burnetii* IgG negative. *Toxoplasma gondii* seroprevalence was similar in women with and without anti-*Coxiella* antibodies, suggesting that *Coxiella* and the protozoon do not have similar modes of transmission (i.e. contact with cats). One healthy control woman, who was a cat and dog owner, and one woman with recurrent miscarriage were *C. burnetii* IgM positive. Both were UK-born Caucasians.

Serological cross-reactions with zoonotic (*W. chondrophila*, *Parachlamydia acanthamoebae*, *Chlamydophila psittaci*, *B. abortus*) and human (*Chlamydia trachomatis*) potential agents of miscarriage were also studied. None of these organisms showed evidence of cross-reaction with *Coxiella*.

The serum of only two women (0.5%, 95% CI 0.1–1.6) contained antibodies directed against *Brucella*. Both were immigrants from Sudan. One was healthy, with an at-term pregnancy, whereas the other recurrently miscarried, a total of five times, and no other aetiology of miscarriage was identified.

In conclusion, exposure to *C. burnetii* is common and exposure to *B. abortus* is uncommon in women in London, with a seroprevalence of 4.6% and 0.5%, respectively.

Coxiella burnetii serosurveys, mainly conducted in endemic areas, are difficult to compare because of the different methods and cut-off values employed [2]. In the present study, an IgG anti-phase II *C. burnetii* cut-off of 1/50 was chosen, as recommended elsewhere [1]. Surprisingly, the overall *Coxiella* IgG seroprevalence of 4.6% observed in this urban population from an area considered to be non-endemic was similar to the seroprevalence reported from endemic areas [1]. In an endemic area of France, 0.15% of 12 716 pregnant women had serum anti-*Coxiella* IgG titres ≥1/100 [10]. In an

endemic area of Canada, 3.8% (291/7658) of pregnant women exhibited a phase II titre ≥1/32 [7]. Moreover, among 200 healthy Japanese pregnant women, only four (2%) had anti-*Coxiella* IgG antibodies ≥1/16 [9].

Q fever is frequently under-diagnosed because of the intracellular nature of *C. burnetii*, and because as many as 60% of infections remain asymptomatic [1]. Among 2% of patients requiring hospitalization, approximately 10% develop chronic Q fever, including endocarditis and/or chronic hepatitis [2,15]. Considering the 4.5% prevalence observed in the present study, we estimate that, among the 7.5 million inhabitants of London [16], 300 000 have serological evidence of prior *Coxiella* infection, 6000 will require hospitalization, and 600 may develop chronic Q fever if left untreated.

The present study failed to show an association between *Coxiella*-positive serology and contact with domestic animals. Other modes of transmission, including person-to-person contact [2], or via arthropod exposure [1], raw milk or *Coxiella*-infected amoebae in water [17,18], should be investigated in an attempt to understand infection with Coxiella in an urban area such as London. We found no association between *Coxiella*-positive serology and miscarriage. However, the small sample size limits conclusions concerning this issue. The present study also showed the absence of serological cross-reactivity between *Coxiella* and other agents of miscarriage, such as *W. chondrophila*. Only two women exhibited evidence of previous infection with *Brucella* spp., which is consistent with previously obtained data [3,4]. Q fever might be under-reported in London. As a result of the considerable morbidity associated with Q fever, physicians should be aware of the relatively common exposure to this bacterium, even in urban, non-endemic settings.

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Transparency declaration

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