ORIGINAL ARTICLE

Coxiella burnetii infection with women's febrile spontaneous abortion reported in Algiers

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Abstract

We investigated Q fever infection in Febrile Spontaneous Abortions in women by using a serologic method (Immuno-Fluorescence Assay, IFA) and a molecular method (real-time quantitative PCR, qPCR) in Obstetric-Gynaecology (OB-GYN) services in two hospitals in Algiers. We included in the case group 380 women who experienced Febrile Spontaneous Abortion; the control group comprised 345 women who gave birth without any other infections or complications. Among the 725 women included, antibodies against *Coxiella burnetii* were detected by IFA in three (03) cases patients; all control group samples were IFA negative. In other hand, only four (04) placental samples among the case group came back with q PCR positive for IS1111 and IS30a too. A relationship between *C. burnetii* infection and febrile spontaneous abortion exists in OB-GYN services in Algiers.

Keywords: Immunofluorescence assay, Q fever, qPCR, real-time quantitative, PCR
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Introduction

Coxiella burnetii is the pathogenic agent of Q fever, which remains a worldwide zoonotic disease. The natural cycle of this bacterium is not reported to include humans, which are considered incidental hosts [1,2]. The true reservoir is wide and includes mammals, birds and arthropods, mainly ticks [1]. Q fever is usually an occupational disease, although isolated cases and outbreaks have been reported in people who have had indirect contact with infected animals [3]. Acute or chronic *C. burnetii* infection exhibits a wide spectrum of clinical manifestations; roughly 50% of all infections with *C. burnetii* are asymptomatic [4]. Acute Q fever typically arises from inhalation of aerosolized bacteria; rare but potentially severe chronic disease most commonly manifests as endocarditis [5].

In general, spontaneous abortion affects 10% to 20% of pregnancies, but its cause remains unknown in more than 50% of cases [3]. In animals, Q fever is associated with epizootic abortion in ungulates [6]. In humans, up to 90% of pregnant women have antibodies suggesting recent infection with *C. burnetii* but remain asymptomatic [7]. However, symptomatic or asymptomatic infection during pregnancy has been associated with obstetric complications, including miscarriage, preterm delivery and foetal death [8]. Q fever during pregnancy has been linked to poor obstetric outcomes in southern France, Canada, Scotland and Spain, primarily when the disease is acquired during the first trimester [9]. In pregnant women and other mammals, the bacteria will colonize and multiply in the uterus and placenta, then be reactivated during subsequent pregnancies [3]. When pregnant women have an asymptomatic infection with Q fever, its reactivation increases the risk of chronic Q fever, and the woman may also experience an adverse pregnancy outcome [10].

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The precise mechanisms by which the infection compromises pregnancy are largely unknown, but adverse pregnancy outcome has been reproduced in BALB/c mice in which infection followed by repeated pregnancies resulted in spontaneous abortion and perinatal death [10]. In humans, the role of Q fever during pregnancy has been recently questioned because of the discrepancy between the high risk of obstetric complications among women infected with Q fever in published case series and the absence of an increased risk of adverse pregnancy outcomes in population-based serologic studies [6].

Immunofluorescence assay (IFA) is the reference standard for the diagnosis of Q fever; it is based on detection of antibodies against two antigenic variations of *C. burnetii* lipopolysaccharide, phase I and phase II antigens [11].

In Algeria, little is known about Q fever because diagnostic tools are not readily available. As a result, few studies have been performed studying Q fever in Algeria, and we thus have no precise picture of this disease or its prevalence in this region. After the first cases reported in Algiers in 1948 by Portier et al. [12], outbreaks have been reported in Batna (in the French army in 1955 and 1957) and in Tlemcen (also in the army) [13]. In 1996 Lacheheb and Raoult [14] studied the seroprevalence of Q fever in a population of 729 patients from northeast Algeria; they found 113 positive sera by IFA, with a 15.5% seroprevalence. Furthermore, in order to study infective endocarditis caused by *C. burnetii,* Benslimani et al. [15] in 2005 studied cardiac valves and sera from patients with infective endocarditis and negative blood culture; only two of 61 serum samples were positive for *C. burnetii* antibodies by microimmunofluorescence, and all the cardiac valves came back negative by PCR.

To our knowledge, no study has been done on the abortion aspect of *C. burnetii* in Algeria in women. Thus, the aim of the present study was to investigate Q fever infection in febrile spontaneous abortions in women by using a serologic method (IFA) and a molecular method (real-time quantitative PCR, qPCR) in obstetric-gynaecology (OB-GYN) services in two hospitals in Algiers.

Materials and methods

Study design

In order to evaluate the abortive aspect of *C. burnetii* infection among women in Algiers, we considered it wise to focus our study on OB-GYN services which admit patients from rural areas where livestock of different animal species is widespread and where therefore a high level of contact with animals and their parturition products is reported. We recruited women in OB-GYN services from Hassen Badi Hospital (east of Algiers) and Zéralda Hospital (west of Algiers). The two hospitals receive pregnant women from neighbourhoods with cattle and sheep. Annually, each hospital admits approximately 8865 pregnant women, with 88% (7883) of them giving birth and 12% (982) experiencing miscarriage.

From April 2014 to November 2015, at the two hospitals, we admitted 18 640 pregnant women for delivery, including 2127 women experiencing spontaneous abortion (11.41% prevalence); of these, only women experiencing febrile spontaneous abortion were eligible to be included in the case group, where we sampled 380 women. A total of 345 women who gave birth without any other infections or complications were enrolled onto the study as the control group.

Inclusion criteria and case definitions

In order to select the patients for the case group, we found, by consulting with the obstetric emergency services unit, women who were likely to experience a febrile spontaneous abortion. All miscarriages were confirmed by ultrasound or pathologic examination. Functional signs included pelvic aches and fever (temperature >38.5°C). Physical signs included increased uterine volume and metrorrhagia found by speculum examination; open neck/trophoblastic neck found by manual vaginal examination; and trophoblastic detachment found by ultrasound. We thus selected case patients whose samples were likely to be positive for *C. burnetii* by serology and qPCR.

For the control group, we took into account all women who were admitted for physiologic vaginal delivery. In addition, they had contact with animals and/or came from rural areas. Women with a previous complicated obstetrics history or a complicated partum or other infection history were not included in the control group. Table 1 lists the characteristics of the case and control groups.

Ethics statement

All the women gave us permission to be included the study, including use of interview information and blood and placenta

TABLE 1. Patient information

Characteristic	Group	Variable			
No. of patients		**Age 20–30 years**		**Age 30–40 years**	
	Control	233		112	
	Case	244		136	
Gestational age		**4–8 weeks**	**9–13 weeks**	**4–8 weeks**	**9–13 weeks**
	Control	185	48	79	33
	Case	196	48	114	22
Animal contact		**Yes**	**No**	**Yes**	**No**
	Control	201	32	98	104
	Case	197	47	104	32
Living area		**Rural**	**Nonrural**	**Rural**	**Nonrural**
	Control	174	59	89	23
	Case	181	63	97	39
Abortion history		**Yes**	**No**	**Yes**	**No**
	Control	75	158	43	84
	Case	137	107	69	52

By using chi-square homogeneity test, we confirmed that the two patient groups included in this study were comparable and homogeneous.

samples. Clinical data were obtained by a standardized questionnaire that asked about clinical information, contact with animals and health history. These data were analysed retrospectively when the serologic analysis or molecular tests were positive.

Sample collection

We collected a total of 380 samples comprising placenta or sera samples from women experiencing febrile spontaneous abortion and 345 placenta or sera samples from women giving birth. The samples were collected aseptically in dry tubes and conserved at −20°C so they could be transported to the Emerging Tropical Infectious Diseases Research Unit at the Faculty of Medicine–Marseille for serology (IFA) and qPCR for *C. burnetii.*

Serologic assays

Serologic tests were performed using an indirect IFA, which is the reference method for the serodiagnosis of Q fever. We used reference strains *C. burnetii* Nine Mile I and Nine Mile II as antigens, and antigen preparation and purification were performed as described elsewhere [16].

Polymerase chain reaction

DNA was extracted by using a QIAamp Tissue Kit (Qiagen, Hilden, Germany), and qPCR was performed with a CFX96 thermocycler (Bio-Rad, Marnes-la-Coquette, France) using primers and probes specific for intergenic sequences *IS1111* and *IS30a* as previously described [17]. DNA from the *C. burnetii* Nine Mile II strain was used as positive control and sterile water was used as negative control.

Statistical analyses

In order to calculate the confidence interval and the significance level (p values) of the various results obtained, we used the Web Mediametrie application (http://www.mediametrie.fr/calculettes-mediametrie.php?id=intervalle) as well as the application of the Yates correction for the chi-square test, respectively. $p < 0.05$ was considered statistically significant; p values between 0.05 and 0.1 were considered nonsignificant.

Results

IFA serology

Among the 725 women included, antibodies against *C. burnetii* were detected by IFA in three case patients; all control group samples were IFA negative. Among the positive sera, the titres of antibodies to phase I and II *C. burnetii* antigens varied among immunoglobulin (Ig) G, IgM and IgA (Table 2).

Detection of *C. burnetii* by qPCR

qPCR was used for the detection of *C. burnetii* in placental samples by using *C. burnetii*–specific primers and a probe designed to amplify the *IS1111* gene, and confirmed by the second gene, *IS30a,* which remains highly *C. burnetii* species specific.

Four placental samples from 380 case subjects came back positive for both *IS1111* and *IS30a* genes. All samples from control subjects came back negative for these genes. The C_t values of the positive samples ranged from 29.13 to 32.97 (corresponding to 6.1 and 4.9 $\log_{10}$ DNA copies/mL). The *IS1111* and *IS30a* qPCR results are summarized in Table 3. Table 4 summarizes the case group's serologic and molecular results for *C. burnetii.*

In total, in this study we obtained six of 380 positive results for *C. burnetii* (IFA and/or qPCR) among the case group and no positive results among the control group; these results are statistically significant (p 0.0299). Using the application of the Yates correction for the chi-square test, significance was considered at $p \leq 0.05$.

Description of positive cases

Patient A, a 25-year-old housewife, was admitted to the OB-GYN service of Zéralda Hospital. She was from a south-western suburbs of Algiers, the Rahmania commune (Zéralda

TABLE 2. Acute and chronic titres of antibodies to *Coxiella burnetii* antigens

Group (*n* positive sera)	Total Ig screening[a]	Phase II antigen			Phase I antigen		
		IgG	IgM	IgA	IgG	IgM	IgA
Control (0)							
Case (3)							
A	Positive ≥100	1:200	1:25	1:200	0	0	1:50
B	Positive ≥100	1:200	1:200	1:800	0	1:50	0
C	Positive ≥100	1:200	1:100	1:200	0	0	0

[a]All sera were screened as first-line with total immunoglobulin (Ig). If serum is positive at 1/100 dilution, then antibodies present in this sample are differentially quantified (IgG, IgM, IgA).

TABLE 3. Placental sample results by real-time quantitative PCR

	IS1111 gene					*IS30a* gene				
Group	CB⁻	CB⁺	C_t values	Log_{10} DNA copies/mL	Positive control C_t	CB⁻	CB+	C_t values	Positive control C_t	Log_{10} DNA copies/mL
Control	345	0	—	—	—	345	0			
Case	376	4	31.3	5.4	25.72 = 7.1 log_{10} DNA copies/mL	376	4	31.9	26.37 = 6.9 log_{10} DNA copies/mL	5.2
			29.1	6.1				30.0		5.8
			32.7	5.0				32.9		4.9
			31.0	5.5				31.1		5.5

CB, *Coxiella burnetii*.

TABLE 4. Characteristics of pregnant women according *Coxiella burnetii* results for placental samples

	IFA, *n* (%), 95% CI		qPCR, *n* (%), 95% CI	
Characteristic	Positive (*n* = 3)	Negative (*n* = 377)	Positive (*n* = 4)	Negative (*n* = 376)
Age				
20–30 years	2 (0.82), −0.3 to 2	242 (99.18), 98 to 100.3	2 (0.82), −0.3 to 2	242 (99.18), 98 to 100.3
30–40 years	1 (0.74), −0.7 to 2.2	1135 (99.26), 97.8 to 100.7 p 0.60 NS*	2 (1.47), −0.6 to 3.5	134 (98.27), 96.1 to 100.5 p 0.94 NS*
Gestational age				
4–8 weeks	01 (0.32), −0.3 to 0.9,	309 (99.68), 99.1 to 100.3	03 (0.97), −0.1 to 2.1	307 (99.03), 97.9 to 100.1
9–13 weeks	02 (2.86), −1 to 6.8	68 (97.14), 93.2 to 101 p 0.16 NS*	02 (2.86), −1 to 6.8	68 (97.14), 93.2 to 101 p 0.50 NS*
Animal contact				
Yes	3 (1.00), −0.1 to 2.1	298 (99.00), 97.9 to 100.1	4 (1.33), 0 to 2.6	297 (98.67), 97.4 to 100
No	0 (0.00)	79 (100.00), 100 to 100 p 0.86 NS*	0 (0.00)	79 (100.00), 100 to 100 p 0.68 NS*
Residence				
Rural	2 (0.72), −0.3 to 1.7	276 (99.28), 98.3 to 100.3	3 (1.08), −0.1 to 2.3	275 (98.92),97.7 to 100.1
Nonrural	1 (0.98), −0.9 to 2.9	101 (99.02), 97.1 to 100.9 p 0.69 NS*	1 (0.98), −0.9 to 2.9	101 (99.02), 97.1 to 100.9 p 0.63 NS*
History of abortion				
Yes	2 (0.91), −0.3 to 2.2	219 (99.01), 97.7 to 100.3	3 (1.36), −0.2 to 2.9	218 (98.64), 97.1 to 100.2
No	1 (0.63), −0.6 to 1.9	158 (99.37), 98.1 to 100.6 p 0.87 NS*	1 (0.63), −0.6 to 1.9	158 (99.37),98.1 to 100.6 p 0.86 NS*

CI, confidence interval; IFA, immunofluorescence assay; NS, not significant; qPCR, real-time quantitative PCR.
*Level of significance when $p \leq 0.05$.

division). This commune is situated in a rural zone with livestock. The people who live in this environment are in permanent contact with animals, which is why they remain subject to different zoonotic diseases. This patient was admitted to the obstetrics emergency service while undergoing a spontaneous abortion without pathologic antecedents. It was her third pregnancy (G3). She had previously experienced one live birth (P1) and one abortion (A1). She was in the eighth week of pregnancy. Physical examination revealed a pale patient with high fever (temperature 39°C); she presented with pelvic aches, increasing uterus volume and metrorrhagia. According to the patient's history, she had drunk unpasteurized cow's milk, and she may have inhaled aerosols that came from stored cow's milk. This patient had IFA serology positive against *C. burnetii* phase II (IgG 1:200, IgM 1:25, IgA 1:200). Placental qPCR was negative for both *IS1111* and *IS30a*. The elevated level of antibodies against *C. burnetii* phase II indicated acute Q fever infection, which explains the high fever and infectious condition noted during her admission. It cannot be ruled out that this infection was the cause of abortion.

Patient B (G2 P1 A0) was a 27-year-old teacher admitted to the OB-GYN service of Zéralda Hospital. She came from the western suburbs of Algiers, from the Staouali commune (Zéralda division), which has cattle farms. She was in the 12th week of pregnancy and was admitted with a high fever (temperature 39.5°C) with skin rash and metrorrhagia. She ejected the abortion product 2 hours after admission. Patient history indicated that she had permanent contact with breeding cows, and she had felt feverish and tired during the pregnancy. This patient was the only case that was positive for *C. burnetii* by both IFA serology (IgG 1:200, IgM 1:200, IgA 1:800) and placental qPCR for *IS1111* and *IS30a* (C_t values: *IS1111,* 31.3 = 5.4 log_{10} DNA copies/mL, *IS30a,* 31.9 = 5.2 log_{10} DNA copies/mL) in which the correlation between IFA and qPCR matched well. Serology revealed an acute infection, which would explain the patient's rash, based on the physiopathogenesis of acute *C. burnetii* infection.

Cases C and D, aged 32 and 29 years, were a maid and a housewife, respectively, admitted to the OB-GYN service of Hassen Badi Hospital. The two came from the east region of Algiers, from the Chrarba commune (Eucalyptus division), where there are a large number of animal farms, especially poultry farms, so although these patients were living in nonrural housing, they had occasional animal contact. Patient C (G2 P0 A1) and patient D (G2 P1 A1) were in their 12th week of pregnancy. Physical examination revealed pale patients with a high fever (temperature 38.5°C). Both experienced a typical febrile abortion that took place 24 hours after admission.

Patient C had IFA serology positive for acute infection (IgG 1200, IgM 1100, IgA 1200) with placental qPCR negative for both *IS1111* and *IS30a*. Patient D's placental qPCR also came back positive for *IS1111* (C_t 29.13 = 6.1 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 30.0 = 5.8 $\log_{10}$ DNA copies/mL), but her serologic results were negative. Two different positive responses for the same clinical picture were evident, the interpretation of which is based on the variation of *C. burnetii* physiopathogenesis.

Patient E was a 34-year-old housewife (G3 P2 A0) admitted to the high-risk pregnancy service in Hassen Badi Hospital with a high fever (temperature 40°C) of a week's duration that did not respond to cefalexin 1 g at a rate of 3 g per day for 7 days. All haemocultures were negative for the usually tested germs. Hyperleukocytosis was estimated at 22 000/mm^3. The patient was ill during her admission until the foetus was expelled at 72 hours after admission. Subsequent testing including placental qPCR revealed *IS1111* (C_t 32.7 = 5 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 32.9 = 4.9 $\log_{10}$ DNA copies/mL); IFA serology was negative. This patient came from the El Harrach commune, 800 m away from the hospital, where she lived in nonrural housing. Her history indicated that she was in daily contact with a dog and domestic cats, which remained the only source of animal contamination. She experienced a peak in her fever on the day of spontaneous abortion.

Patient F was a 37-year-old housewife (G4 P2 A1) admitted to the OB-GYN service of Zéralda Hospital for a pneumopathy that occurred 3 weeks ago, which had remained undiagnosed and untreated. At admission, the patient was conscious and had a temperature of 38.5°C. She had a cough, sputum, dyspnoea, localized chest pain and tachycardia in addition to significant bleeding that ended by foetal expulsion. She lived in a rural area located in the commune of Douira, west of Algiers. The people of this region are known to consume raw cow's milk and homemade dairy products. Consumption of such products could be the source of infections caused by excretion of bacteria through animal milk, including *C. burnetii*. This patient's findings included positive placental qPCR for *IS1111* (C_t 31.0 = 5.5 $\log_{10}$ DNA copies/mL) and *IS30a* (C_t 31.1 = 5.5 $\log_{10}$ DNA copies/mL).

Fig. 1 shows the location of the two OB-GYN services studied and the distribution of positive cases on a map of Algiers.

Discussion

No Q fever epidemics have been recorded or verified clinically or biologically in Algeria, likely resulting in the sources of infection of *C. burnetii* being misunderstood. Our study is the first of its kind in Algeria to investigate the outcome of Q fever infection in pregnant women. We sought to learn the relationship between febrile spontaneous abortion and *C. burnetii* infection, hypothesizing an association between febrile spontaneous abortion and Q fever infection.

Our serologic findings indicated a seroprevalence of 0.79% (3/380) for IgG titres $\geq$1:200 phase II (Q fever acute infection) among the case group of women who experienced abortion, whereas women giving birth (control group) had negative serology (0/345). These results accord with those of Nielsen et al. [18] in Denmark, who found a 1.2% seroprevalence of acute Q fever infection among women who experienced

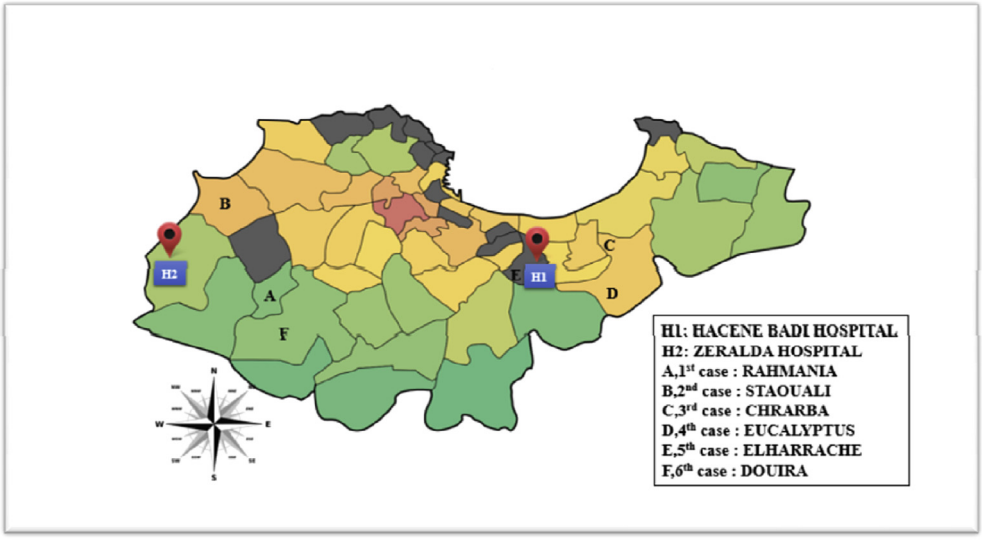

FIG. 1. Map illustrating two obstetric-gynaecology services and locations of positive cases in Algiers.

spontaneous abortion in the first semester of pregnancy, and who thus reported that no increased risk of adverse pregnancy outcome was found in women with verified exposure to *C. burnetii.* Moreover, another study reported a seroprevalence of 0.27% (2/738) in women who experienced spontaneous abortion in southeastern France [13].

Many previously reported findings could not be reproduced in the present study. Langley et al. [19] in 2003 reported that 3.8% (IFA positive) of parturient women in an endemic area had evidence of exposure to *C. burnetii* and that this exposure was associated with adverse pregnancy outcomes. However, Raoult et al. [20] and Quijada et al. [3] confirmed that Q fever acquired during pregnancy is a serious disease. Infection with *C. burnetii* in the first trimester frequently resulted in abortion. Raoult et al. identified 11 women in their first trimester, of whom seven (63.63%) experienced abortion. However, Quijada et al. reported an IFA-positive seroprevalence of 32.2% for women with spontaneous abortion (case group) and 23.3% in women who gave birth (control group); their study also reported that abortion history, rural housing, contact with cattle or sheep and cohabitation with pets were also associated with abortion. Concerning abortion history, McCaughey et al. [21] identified that women with a history of miscarriage or prematurity were more often seropositive than those without such a history (19.5% vs. 9.8%).

We found 1.05% qPCR-positive results for both *C. burnetii IS1111* and *IS30a* for the case group. However, previous results have shown that *C. burnetii* was not identified by qPCR or culture in the placentas investigated, with qPCR results negative for all placental samples [3,19,22]. These results are discordant with our findings; in our study, 1.05% placental samples were qPCR positive in the case group, which leads us to say that the association between febrile spontaneous abortion and the existence of *C. burnetii* in placentas are correlated. The study of Vaidya et al. [23] concluded that qPCR for placental samples among women with spontaneous abortion were 21.62% positive in 74 samples tested; this qPCR positivity explains the presence of *C. burnetii* in the placentas of women with abortion. We emphasize that there is a notable difference between our results (1.05%) and those of Vaidya et al. (21.62%), but in both studies results of qPCR of placental samples from spontaneous abortions were positive for *C. burnetii.*

Statistical analysis of the collected patient data and comparison with serologic and molecular results indicated that for duration of pregnancy, abortion history, rural housing and contact with animals, no significant differences were evident for the cases of febrile spontaneous abortion. In our study, most of the positive cases occurred in women who had had at least one abortion during their previous pregnancies, and they had had contact with animals or their parturition products where they lived; they were surrounded by animal farms, especially in the Algiers suburbs. Such an environment makes people vulnerable to infectious zoonosis.

Conclusions

Our study, which aimed to evaluate the relationship between spontaneous febrile abortion and infection with *C. burnetii* at two OB-GYN services in Algiers, is the first in this region to assess this aspect of *C. burnetii* infection. Our results and their comparison with literature allow us to say that a relationship between *C. burnetii* infection and febrile spontaneous abortion exists in OB-GYN services in Algiers. Looking ahead, we plan to carry out another study with a larger sample size and in other regions in Algeria in order to further assess the relationship between *C. burnetii* infection and febrile spontaneous abortions. Q fever causes a low-noise infection in OB-GYN services, for which an alarm bell must be sounded for every suspected *C. burnetii* infection.

Conflict of interest

None declared.

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