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Short Communication

The First Serological Study of *Coxiella burnetii* among Pregnant Women in Iran

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Abstract

Background: Q fever is a zoonotic disease caused by *Coxiella burnetii*. There is no information about this disease in pregnant women in Iran. The aim of this study was to investigate the seroprevalence of *C. burnetii* infection among pregnant women in southwestern (Ahvaz) and northern (Parsabad) Iran and further to comparison its prevalence in normal and abnormal pregnancies.

Methods: A total of 400 samples were collected randomly from pregnant women who referred to diagnostic laboratories of Ahvaz and Parsabad in 2014. An indirect ELISA kit, designed in Veterinary Faculty, was used to detect the specific antibodies against phase II human*C. burnetii* in serum samples.

Results: The overall prevalence of *C. burnetii* in sera from pregnant women was 29.3% (95% confidence interval (CI): 25-34%). The prevalence of *C. burnetii* infection was significantly different in Ahvaz and Parsabad with, respectively, 22 (95% CI: 16-28%) and 36.5% (95% CI: 30-43%). Total prevalence of *C. burnetii* infection in serum was significantly higher in women with abnormal pregnancy history (39.8%) compared with normal pregnancies (23.8%). Furthermore, maternal age had significant association with seropositivity and the prevalence increased with maternal age. This could be due to higher probability of encountering *C. burnetii* in older women.

Conclusion: The present study demonstrated ahigh seroprevalence of *C. burnetii* infection among pregnant women in Iran for the first time. Seropositivity was associated with adverse pregnancy outcomes and maternal age. The pregnant women who experienced abnormal pregnancy had higher seroprevalence of *C. burnetii* compared to women with normal pregnancy.

Keywords: *Coxiella burnetii*, Prevalence, Pregnant women, Iran

Introduction

Coxiella burnetii is a gram negative, immotile, obligate intracellular, and small (0.2-0.4 µm in width and 0.4-1 µm in length) bacterium, which has a membrane similar to those of gram-negative bacteria and completes its life cycle in infected cell's phagolysosomes (1). It propagates in large numbers inside the parasite-like vacuoles of the host eukaryotic cell and has a doubling time of 20-45 h (2). The slow rate of intracellular propagation of *C. burnetii* (20 h doubling times, which is similar to eukaryotic cells) prevents damaging the infected cells.

C. burnetii is capable of developing stable infection (Q fever) in both human and livestock. The infected animals excrete the bacteria in a high concentration in birth products, urine, stool, and milk. The most important route of transmission of infection to human beings is inhalation of contaminated aerosols or dust containing *C. burnetii*

(1). Such stable infection is usually without symptoms; however, in human, the acute form of illness causes a flulike illness, hepatitis, and pneumonia. *C. burnetii* can cause chronic infection in people with heart valve disease history or suppressed immune system and in pregnant women. Endocarditis is the major clinical manifestation of chronic Q fever (1, 3).

This disease has been reported from everywhere but New Zealand. From 1999 to 2004, 18 cases of Q fever outbreak were reported from 12 countries with extents of 2 to 289 people (4). The serologic prevalence of Q fever in sheep, goat and dairy cattle was 29.24%, 65.78%, and 10.75%, respectively in Kerman Province, Iran (5, 6). In addition, the prevalence of anti-*C. burnetii* antibodies in Kerman dairy cattle reservoir milk was reported 45.4% (7). In Bardsir, Iran, 24% and 36% of cases were positive when tested for, respectively, anti-phase I and anti-phase II *C. burnetii* (8). The interesting point of this study was the fact that serologic prevalence of Q fever was significantly higher than that of Malta fever (8). Recently, in four grand cities of Iran in addition to assessment of the disease risk factors, also the serologic prevalence of *C. burnetii* infection in sheep and goat were determined to be 19.5 and 27.2 %, respectively (9, 10).

Potentially, pregnant women are a high risk group of Q fever as well as by far the largest risk group in size (11, 12).During pregnancy, Q fever has serious complications, both for the fetus and the mother, especially when it occurs in early of pregnancy. Q fever may lead to adverse pregnancy outcome, including spontaneous abortion, intrauterine fetal death, intrauterine growth retardation, oligoamnios, and premature delivery. The mother is at the risk of acute and chronic Q fever (13-15). Although it's serious potential consequences during pregnancy, there is no data from pregnant women in Iran.

Therefore, the present study was undertaken to estimate the serologic prevalence of *C. burnetii* infection in pregnant women of two cities, Ahvaz and Parsabad as well as comparison its prevalence between normal and abnormal pregnancies.

Materials and Methods

Sample collection

In this cross-sectional study in order to investigate the serologic prevalence of C. burnetii infection, a total of 400 serum samples were collected from pregnant women of Ahvaz and Parsabad. Ahvaz is located in southwestern Iran (31°20´N, 48°40´E) and Parsabad is Iran's northernmost city in Ardabil. Sampling process started in May 2014 and lasted until December. A well trained nurse randomly collected the blood samples in aseptic conditions from women who referred to diagnostic laboratories in Ahvaz and Parsabad for routine pregnancy examinations. Sera were immediately removed from samples using centrifugation at 3500 g for 10 min in room temperature and stored in -20 °C until the time of experiments.

Before starting the sampling, informed consent from the participants was taken and local Ethics Committee approved the study.

A total of 200 samples were collected from Ahvaz from which 181 samples were from pregnant women with normal conditions and 19 were from women faced abnormalities (such as abortion, intrauterine growth retardation, neonatal death, and preterm birth) during their pregnancies in past. From the other 200 samples from Parsabad these numbers were 59 and 89 respectively, and there were no information about the pregnancy conditions of remaining 52 samples.

In order to assess the possible risk factors, subjects' information including age, occupation, animal contact, and pregnancy history were collected at the time of their referral to laboratory for bloodletting using predesigned questionnaires.

ELISA test

An indirect ELISA kit developed at Faculty of Veterinary Medicine was used to detect specific antibodies against human phase II *C. burnetii* in serum samples (16). In summary, 50µl antigen of phase II *C. burnetii* (fully purified Dolfinin, Slovakia) diluted with the ratio of 1.39 was added to all the wells in the ELISA kit micro-plate stored inside a refrigerator for one night so that the antigen was at-

tached to the bottom of the wells. In the next step, all the wells in the ELISA kit micro-plate were evacuated and every well was rinsed three times with 400 µl PBS. 150 µl from 2.5% casein solution was added to all the micro-plate wells as the blocker agent and the solution was stored inside the incubator at 37 °C for 2 h. Contents of the wells were emptied and every well was rinsed three times. 50 µl from the serum samples diluted with the ratio of 1:500 was added to the micro-plate wells as suspicious, negative control, and positive control serum samples and the kits were stored in the incubator at 37 °C for 1 h. Contents of the wells were evacuated and every well was rinsed three times. 50 µl from the 1/2000 diluted antibody conjugate (Serotech, England) was added to all the wells and the micro-plate was stored in the incubator at 37 °C for 1 h. Contents of the wells were evacuated and every well was rinsed three times. After the rinsing solution was completely removed from the wells, 50 µl from the substrate TMB solution was added to all the wells and the micro-plate was stored in the incubator at 37 °C for 20 min. Finally, the micro-plates were removed from the incubator and 50 µl from 1M sulfuric acid was added to all the wells to stop the reaction and to prepare the micro-plate for the reading. Then, optical density (OD) of the samples was read by ELISA reader (Anthos 2020, Austria) with the wavelength of 450 nm against the reference filter 620 (16). To interpret the results and identify the positive, suspicious, and negative samples, first, doubled values of standard deviation were summed with the mean value of OD for the negative controls, the cut-off value for the designed kit was calculated, and value of OD was obtained as 0.100. 10% higher and lower than the cut-off value determined for the designed kit was considered as, Border Line respectively 0.09 - 0.110 (16).

Statistical analysis

Seroprevalence of *C. burnetii* infection was estimated according to ELISA test results. SPSS statistical software version 16 (SPSS Inc., Chicago, USA) was used to analyze data. Chi-square test and variance analysis were implemented in order to assess the association between different risk factors and *C. burnetii* infection. In all procedures, significance level was adjusted to $P<0.05$.

Results

Serologic prevalence of C. burnetii infection

A total of 400 samples were collected from pregnant women in Ahvaz and Parsabad and tested for anti-*C. burnetii* antibodies. A total of 117 subjects (29.3%, 95% CI: 25-34%) had anti-*C. burnetii* specific antibodies. The serologic prevalence of *C. burnetii* infection was significantly higher in pregnant women of Parsabad compared with those of Ahvaz ($P=0.001$). The prevalence was 36.5% (95% CI: 30-43%) in Parsabad which is comparable to 22% (95% CI: 16-28%) of Ahvaz.

Seroprevalence of C. burnetii infection in normal and abnormal pregnancies

The samples were collected from women with normal and abnormal pregnancies with adverse pregnancy outcome such as abortion, intrauterine growth retardation, embryonic death, neonatal death, and preterm birth from both cities. Total abnormal pregnancies in Ahvaz consisted of 19 cases including 16 abortions, 2 preterm births, and one embryonic death. There were 89 cases of abnormalities in Parsabad including 80 abortions, 4 intrauterine growth retardations, 2 embryonic deaths, 2 neonatal deaths, and one preterm birth. The results demonstrated that overall prevalence of *C. burnetii* was significantly higher in women with history of abortion and other abnormalities compared with normal pregnant women ($P=0.002$). The prevalence in women with abnormal pregnancy history was 39.8 % (95% CI: 30-49%) while it was 23.8 % (95% CI: 18-29%) in normal subjects.

Although the infection prevalence in abnormal pregnancies was higher in each city compared to normal pregnancies, the difference was not significant in each city ($P>0.05$). The result of each city is presented in Table 1. It is worth mentioning that in some cases the information was not completely available.

Analysis of other C. burnetii infection risk factors

In this study based on accessible information of subjects collected via predesigned questionnaires, in addition to city and pregnancy status, the association of some other factors including maternal age (<25 years, 26-29 yr, 30-34 yr, >34 yr), gestational age at the time of sampling, gestational age at the time of abnormal pregnancy occurrence, contact to animals, and type of accommodation area (rural/urban) with *C. burnetii* infection were also examined. Maternal age had significant association with seropositivity (P=0.004).

Table 1: Seroprevalence of *C. burnetii* infection in pregnant women with history of normal and abnormal pregnancy

Region	Positive n (%)	Negative n (%)
Ahvaz		
Normal pregnancy	37 (20.4)	144 (79.6)
Abnormal pregnancy	7 (36.8)	12 (63.2)
Parsabad		
Normal pregnancy	20 (33.9)	39 (66.1)
Abnormal pregnancy	36 (40.4)	53 (59.6)

Numbers represent the prevalence in pregnant women in each city.

In addition, the difference between age groups was significant and the age groups of <25 and>34 yr had the lowest and highest prevalence of *C. burnetii* infection (13.8 vs 40.7%, P=0.008). Results for the age groups of studied pregnant women for *C. burnetii* seroprevalence in Ahvaz and Parsabad are summarized in Table 2. The examined pregnant women's age was in ranges of 18-44 and 14-48 yr in Ahvaz and Parsabad, respectively.

Table 2: Seroprevalence of *C. burnetii* infection in different age groups of pregnant women in Ahvaz and Parsabad, Iran

Age groups (yr)	*C. burnetii* infection	
	Positive n (%)	Negative n (%)
<25	9 (13.8)	56 (86.2)
26-29	17 (29.3)	41 (70.7)
30-34	15 (22.7)	51 (77.3)
>34	22 (40.7)	32 (59.3)
Total	63 (25.9)	180 (74.1)

From all examined pregnant women only 8 cases had animal contact experience from which 3 cases (37.5%) were serologically positive for *C. burnetii* infection. Despite the fact that the prevalence was higher in these cases compared with subjects with no animal contact history (28.4%), this difference was not statistically significant (P>0.05).

From all subjects only 27 cases lived in rural areas and the rest lived in urban regions. The prevalence in rural and urban areas was 29.6 and 42.1 %, respectively. However, this difference was not statistically significant (P>0.05).

Gestational age at the time of sampling varied from one to 25 wk. The mean gestational age at the time of sampling was 12.1 and 13.7 wk in serum-positive and -negative women, respectively. Gestational age at the time of abnormal pregnancy occurrence in under-study women varied from

one to 32 wk. The mean gestational age in these cases was 10.2 wk for both serum-positive and -negative women. There was no significant association between *C. burnetii* infection andgestational age (at the times of sampling and abnormal occurrences) (P>0.05).

Discussion

The present study demonstrated that the overall seroprevalence of *C. burnetii* in pregnant women was 29.3%. The seroprevalence of *C. burnetii* infection in pregnant women has been estimated in several other countries such as France (2.6%), Canada (4%), London (4.6%), Bulgaria (7.7%) and Netherlands (9.1%) (15, 17-20). This is the first study in Iran to address the prevalence of serologic *C. burnetii* infection in pregnant women (to be 29.3%) which was higher than the reported results from other studies in other countries. However, this value is comparable with other risked groups of Q fever in Iran such as febrile patients suspicious of brucellosis, livestock abattoir employees, hunters and their families, health care employees, and laboratory employees (21-23). In one of these studies carried out on febrile patients suspicious of brucellosis in Bardsir, 24 % of cases were positive for anti-phase I *C. burnetii* antibodies and 36 % for anti-phase II *C. burnetii* antibodies (8). Besides, in a recent study by Naderipour et al. on 45 sera samples from Q fever-suspicious patients in the region of Kuhpayeh, Kerman, the serologic prevalence of Q fever was reported to be 20 % (16).

The higher*C. burnetii* infection prevalence in pregnant women of Parsabad (36.5%) in comparison with Ahvaz (22%) could be in part due to the high density of livestock in Parsabad (Moghan region) which is one of the largest husbandry centers in the country. Considering the high proportion of infected livestock in the country (5, 6, 9, 10) and ease of the disease transmission, the denser livestock (major sources of infection for human) in Parsabad would lead to the more probable of human infection especially in pregnant women who are amongst the high risk groups. Another possible reason for the different prevalence between the two cities may be attributable to the composition of the collected samples. Meaning that considering to high occurrence of abortion in Parsabad, most of the samples were collected from women with adverse pregnancy outcome such as abortion while most of those of Ahvaz collected from normal pregnant women without abnormalities (89 vs 19 cases, respectively). Additionally, previous studies have indicated the relationship between abnormal pregnancy consequences and *C. burnetii* infection (15, 24). Therefore, it is more probable that more subjects in Parsabad which were mainly from women with adverse pregnancy outcome to be seropositive. Finally, some of the differences in the results of different studies can be due to the distinction of experiment designation.

The findings of this study showed a significant association between *C. burnetii* infection and the pregnancy status of subjects. The pregnant women with abnormal pregnancy consequences had higher prevalence (39.8%) than normal pregnant women (23.8%).

In a study in an endemic region of Q fever in Canada approximately 4 % of women delivered showed evidence (presence of antibody in blood) of previous contact with *C. burnetii* and there was a significant association between serologic *C. burnetii* infection and abnormal pregnancy consequences. In that study, the risk of abnormal pregnancy consequences was duplicated in women who were positive for the presence of *C. burnetii* in serum (15). This report was warning and emphasizes that *C. burnetii* infection is a potential threat for pregnant women and their embryos. In a recent study in Denmark, the relation between serologic symptoms of Q fever and adverse pregnancy outcomes was also demonstrated (24). In this project carried out between years 2007 and 2011, in 19 pregnancies of 12 women, the positive titer of anti *C. burnetii* antibody was observed and the abnormal pregnancy consequences were detected in 4 women which constituted 9 pregnancies (47%) (24). The result of the present study is in consistence with the findings of above mentioned researches and emphasizes on the undesirable effects of *C. burnetii* infection

on pregnancy. It necessitates the higher attention of specialists to prevent dangerous consequences of the disease for mother and embryo with detecting the infected women on time. Since the majority of *C. burnetii* infected pregnant women remain without symptoms and encounter the consequences during the pregnancy or before, medical symptom-based precautionary strategies are not appropriate. Instead, regular serological screening in endemic Q fever regions can be of high value for preventing its consequences in this high risk group especially when pregnant women are the most populated class in the *C. burnetii* infection high risk group (13, 15).

However, some studies investigated the Q fever during pregnancy, demonstrated that there were no additional risk of abnormal pregnancy consequences in serologically positive pregnant women (25-27). Different levels of tocological consequences of *C. burnetii* in different geographical locations can be due to the dissimilarities of features and acuity of the strain which are potentially because of variations in plasmids (28).

Among other risk factors, only the maternal age had significant association with *C. burnetii* infection. Seropositivity gradually increased with maternal age, and pregnant women older than 34 yr had the highest prevalence (40.7%). So, based on our findings, the probability of seropositivity in pregnant women grows as well as maternal age. The reason is the fact that the chances of contact with pathogen raise as people age. Other studies have also demonstrated the association between age and infection which is consistent with the results of the present study (15, 29). Nilsen et al. showed that seropositivity in women younger than 25 yr old is at its minimum (13.5%) and is higher in women between 25 and 34 (22.7%) and older than 35 yr (18.1%). However, there was no relationship between seropositivity and age in this study (27). Among all subjects, only 8 cases had a history of contacting dog, cat, and sheep. Although seropositivity in these subjects was higher than others (without contact), the difference was not statistically significant (37.5% against 28.4%). It may be attributedto few numbers of cases with animal contact history in this study. However, the findings of other studies regarding relationship between animal contact and seropositivity are also controversial (27, 30). In addition, since the most common mode of transmission is airborne, people do not need to have direct contact with infected animals to be exposed.

As this is the first study in Iran to include pregnant women regarding *C. burnetii* infection, its outcomes are highly valuable and will pave the way for further scrutinizing investigations on Q fever in pregnant women.

Conclusion

The present study, for the first time, demonstrated the high prevalence of *C. burnetii* infection among pregnant women in Iran. The prevalence was different in studied regions which could be due to the difference in geographical location, weather conditions, and livestock density. Seropositivity was associated with adverse pregnancy outcomes and maternal age. The pregnant women who experienced spontaneous abortion, intrauterine growth retardation, embryonic death, neonatal death, and preterm birth had higher seroprevalence of *C. burnetii* compared to women with normal pregnancies.

Ethical consideration

Ethical issues have been completely observed by the authors. This study was approved by Ethical Committee of AJA University of Medical Sciences. Furthermore, informed consent from the participants was taken.

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sample collection. The authors declare that they have no conflict of interests.

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