Molecular Detection of *Coxiella burnitii* Among Aborted Women in Thi-Qar Province/ Iraq

Hekmat K. Ateya
College of Veterinary Medicine, Thi- Qar University, Iraq.
E-mail: hekmatkadhum@yahoo.com ,
Phone: + 9647807767782

Abstract

Coxiella burnetii bacteria, an obligate intracellular parasite with a worldwide geographical distribution, are the causative agent of Q fever in humans. In this study a total of 47 blood samples collected from aborted women in Bent Al Huda hospital in Thi- Qar province in southern Iraq. By a polymerase chain reactions (PCR) assay targeting IS*1111* and *com1 gene*s of this bacterium.The C. *burnetii* diagnosed in 8(17.02**%**) clinical samples and the frequent association of the pathogen with cases of spontaneous abortion. This first study on this bacterium which remains not diagnosed as causative agents of abortion and its prevalence in Iraq is non-studied.

Keywords: Is 1111 gene, com I gene, Q fever, *Coxiella burnetii*.

Introduction

Q fever, belongs to a group of rickettsial infections, is an important zoonotic disease caused by the obligate intracellular gram-negative bacterium of Coxiella *burnetii* (Marmion *et al*., 2005).The outbreak of Q fever occurs in Netherland in 2007 has affected over 4000 person (Roest *et al.,2012).*

In abortion, up to 10^9 C. burnetii cells per gram of placenta can be excreted.Considering the infective dose (ID) of this pathogen has been reported to be close to one, these products are obviously hazardous to human's health (Aidya et al., 2008). In other study showed with an abortion,

up to 1 billion *C. burnetii* per gram of placenta can be excreted. Also concentrations of C. burnetii in veterinary matrices are found in birth materials, placentas and like amnion fluids(Masala *et al*.**,** 2004).In humans acute Q fever is a flu-like illness, which is self-limiting or easily treated with antibiotics when an appropriate diagnosis is made. While in chronic Q fever is a severe disease that requires prolonged antibiotic therapy because the infection can result in granulomatous hepatitis and endocarditis. In addition, the *C. burnetii* infection can causes abortions, stillbirth and pre-mature deliveries in pregnantwomen (MaurinM. and Raoult D. 1999).

Q fever cases are typically sporadic, as rural populations continue to decrease in most developed nations, cases of Q fever are becoming more common in naive urban populations who have occasional exposure to infeted animals or their products (Maurin and Raoult, 1999; Tozer *et al.,* 2011) .The infections are primarily found in persons occupationally exposed, such as ranchers, veterinarians, and workers in meat packing plants. Domestic ungulates, such as cattle, sheep, and goats, usually acquire and transmit *C burnetii*, domestic pets (cats) can be a primary source of human infection in urban environments.(*Baca et al.,*1983).

In human there are very few reports of Q fever complicating pregnancy.In pregnant women, *Coxiella burnetii* can cause placentitis leading to abortion. The risk of chronic Q fever leading torecurrently spontaneous abortion is very high when the infection\ occurs during pregnancy (Arricau-Bouvery *etal.,* 2005, Raoult and stein 1994) .

French case studies have showed risk of miscarriage, oligohydramnion, intrauterine growth retardation, premature delivery and stillbirth in untreated pregnancies case. (Carcopino *et al.*, 2009, Angelaki *et al.*,2013).

Q fever infections before conception or during pregnancy might result in miscarriage, stillbirth, intrauterine growth retardation, premature birth. Adverse

pregnancy outcomes are caused by vascular thrombosis resulting in placental insufficiency although direct infection of the fetus has been documented(Carcopino *et al.,* 2007, Stein *et al.,* 1998).

The risk on fetus and mother will develop chronic Q fever are highest when an acute infection occurs during the first trimester. Women infected with coxiella burnetiiduring pregnancy, including those who were asymptomatic or experienced no adverse pregnancy outcomes , might be at risk for recrudescent infection during subsequent pregnancies (Stein *et al.,* 1998**,** Carcopino *et al.,* 2009)**.**

Aims of the study

1- Detection prevalence of *C. burnetii in* Thi-Qar.

2- Detection of *C. burnetii* as a causative agent of abortion in Thi-Qar.

Materials and Methods

Samples

A total of 120 bloods with cases abortion were collected from Bent Al huda hospital in Thi- Qar province in southern Iraq. Collected blood samples were brought in ice-pack containers to laboratory, in college of science university of Thi -Qar.

DNA Extraction

A volume of 200 µl blood was extracted by DNAextraction kit (bioneer) as recommended by manufacturer.

PCR assay

A PCR assay targeting IS*1111* genes an element of *C. burnetii* (Maurin and Raoult1999) was used for the detection of *C. burnetii* in clinical samples (blood). The primers that used in study forward (5_-GTA ACG ATG CGC AGG CGA T-3_), and reverse (5_-CCA CCG CTT GGC TCG CTA-3_). The primers were designed to amplify a 243-bp fragment.

The sequence of the primer used in the PCR is Com-1 forward (5'-AGT AGA AGC ATC CCA AGC ATT G-3') and Com-1 reverse (5'-TGC CTG CTA GCT GTA ACG ATT G-3') targeting *Com1* gene corresponding to 27kDa outer membrane protein the primers was designed to amplify a 501-bp fragment.

Table (1) The PCR mixture (25 µl)

DNA templates	5 µl
Mastermix	12.5 µl
Primer forward	1µl
Primer reverse	1 µl
DW	5.5 µl

The PCR conditions for *com-1* gene included 36 cycle of an initial denaturation of DNA at 94°C for 1 min, 54°C and 72 for 1 min.The PCR conditions for *IS1111*gene included an initial denaturation of DNA at 95°C for2 min, followed by five cycles at 94°C for 30 s, 66 to 61°C (the temperature wasdecreased by 1°C between consecutive steps) for 1 min, and 72°C for 1 min. These cycles were followed by 40 cycles consisting of 94°C for 30 s, 61°C for 30 s, and 72°C for 1 min and then a final extension step of 10 min at 72°C.

Results

The objectives of this study were to determine the presence of *C. burnetii* in blood of aborted woman According to this study the abortion had revealed in different period of pregnancy stage in human(table 1).

These results showed that *Coxiella burnetii* is found in blood of aborted women in percent 8(17.02%).out of 8 , 6 samples (12.765%) were positive to COM 1 and 2 samples (4.255%) were posiive for IS1111 genes, amplification revealed a bands at approximately 501 bp and 243bp respectively, which was in agreement with the size for identification as *Coxiella burnetii*. These genes are specific fo diagnosis of *Coxella burnetii* figur1, 2and 3.

Table: 2 Com1and IS1111 genes amplification.

Samples	Samples no.	Com1gene (+ve)	%	Transgene (+ve)	%
Blood	47	**6**	**12.765 %**	2	**4.25 %**

Total genomic DNA extracted from blood using 1% agarose gel electrophoresis.

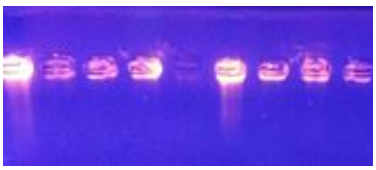

Figure 1: Total genomic

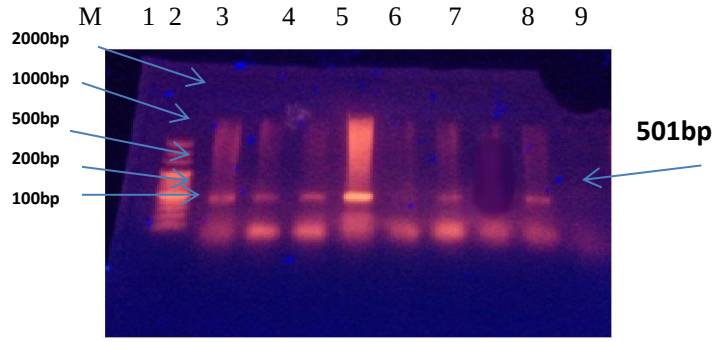

Figure 2: Polymerase chain reaction (PCR) amplification of a 501 bp com1 gene of Coxiella burnetii, line 1 (100 bp ladder). Lines1,2,3,4,6 ,and 8 positive results, line5 negativeresults.;: m line represent ladder : negative control line9.

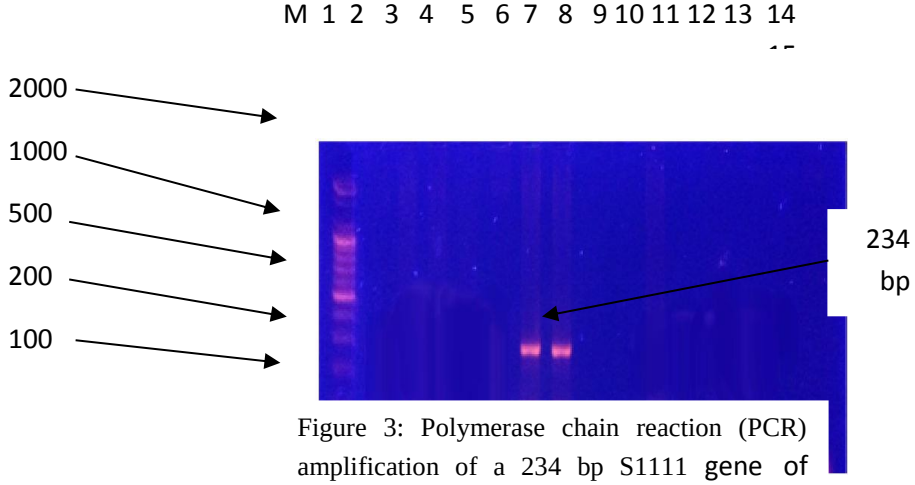

Figure 3: Polymerase chain reaction (PCR) amplification of a 234 bp S1111 gene of Coxiella burnetii, line 1 (100 bp ladder).

Discussion

Many studies conducted in several areas confirmed that is widely geographical distributed in several countries and concluded that animals are important reservoirs of this bacteria.(Weir *et al.*, 1984*;* Rahimi *et al.*,2010, Parisi *et al.*,2006)

In human many disease occurs due to *Coxiella burnetii* infection such as acute flu-like illness, hepatitis, pneumonia, endocarditis

and spontaneous abortion(Vaidya *et al*., 2008,Musso and Raoul1995) .From a few years the large number of abortion case occurs in Iraq, this study showed the high prevalence of Coxiella burnetii in aborted woman and the high number of abortion cases associated with rural women cases*(*Lyytikainen,*et al.*,1998, Javad and Mohammad 2012.)

This result may be due to the direct contact with animal and their products. Animals that carry this organism and shed it into the environment usually do not show any signs of disease. The shedding of *C. burnetii* by animals is an important public health threat. Many research showed the identification of 60% of goats shedding *C. burnetii* into feces, vaginal mucus, and milk. Other studies especially in small ruminant seroprevalence of Q fever occurred in 23.8% and 40.8% in sheep and goats, respectively(Javad and Mohammad 2012,Schimmer *et al.*,2008).In Iraq many of abortion cases occur with unknown causative agent, this study detected of*C. burnetii* as one of these agents.

Conclusion

This study is the first prevalence research of direct identification of *C. burnetii* in aborted woman. The present study demonstrated the high prevalence of *Coxiella burnetii* in aborted woman. Therefore, Q fever could be responsible for considerable numbers of woman abortions in Iraq.

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