



Original article

Molecular detection and phylogenetic analysis of *Coxiella burnetii* in goats milk

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Abstract

Over the last years, the number *Coxiella burnetii* infections increased throughout the world. A molecular detection and phylogenetic analysis were performed in Al-Diwaniyah city in which the first phylogenetic documentation of *C. burnetii* reported in this area with accession numbers KY576802.1, KY576803.1, KY576804.1 and KY576805.1. Molecular detection and phylogenetic analysis of *C. burnetii* targeting the transposase gene partial sequence was done on the milk samples from 50 apparently healthy goats. DNA sequence was amplified in 6 out of 50 (12%) milk samples collected from different regions of Al-Diwaniyah city. Four of these amplicons were submitted to sequences analysis and gave 99% nucleotides sequence similarity. Also the amplicon sequences of local strain were compared with global *C. burnetii* sequences in NCBI which revealed close related to NCBI-Blast *C. burnetii* transposase gene of Indian (AB84899.1) and Brazilian (JF970261.1) strains, whereas there was genetic variation to NCBI-Blast *C. burnetii* transposase gene for USA (DQ379976.1), Portugal (EU009657.1) and Taiwan (EU000273.1). This work confirms goats infection and shedding the *C. burnetii* in Al-Diwaniyah, Iraq and determined the phylogenetic tree of local strain of this bacterium.

Keywords: *Coxiella burnetii*, Query fever, goat, molecular detection, phylogenetic analysis

Introduction:

Coxiella burnetii, gram negative obligate intracellular bacteria, is the causative microbe of query (Q) fever which is a serious zoonotic disease spread everywhere, except New Zealand^(1, 2). This bacterium is present fundamentally in goats, sheep and cattle which considered the main reservoir for human infection⁽³⁾. In animal the infection is often subclinical but has been combined with abortion and stillbirth. Clinical form in cattle may be sporadic while in goats and sheep the same symptoms may be appeared in epidemic form⁽⁴⁾. Q fever in human is typically an acute febrile disease with nonspecific signs and about 60% of infection are asymptomatic. Clinically acute infection in human appears as flu-like symptom often followed by pneumonia, while chronic type of infection showed endocarditis and cause death⁽⁵⁾. High risk

human groups to infection involve persons working with material of infected animals like slaughterhouse worker, veterinarians, people living in or near to farm and laboratory personnel dealing with infected samples⁽⁶⁾. *C. burnetii* may persist in the environment for years and it is transmitted via inhalation of contaminated dust particles and through contact with carrier animal specially their reproductive fluids or other product such as wool⁽⁷⁾. The serological methods may be not useful to discovery of acute infection because of the retard in antibodies development in addition it is difficult to differentiate between current and previous infection due to the antibodies usually persist after the bacteria disappear from the body⁽⁸⁾. Molecular investigations are very useful for detection, specially to clarify and determine sources of infection and for evaluating



control methods ⁽⁹⁾. There are little information about *C. burnetti* infection in Iraq, so the aim of the this study was to investigate the presence of *C. burnetti* DNA in goat raw milk that may contribute to be source of transmission of this bacterium and identify genotypes occurring in Iraq to compare them with strains in other countries.

Material and methods:

The study was conducted from Augat 2016 to March 2017. Milk samples were collected from four different regions and put in ice bag until be taken to the laboratory for extracted DNA immediately or store at -20°C for other time. Specimens were evaluated by PCR assay using primers targeting the gene for transposase of *C. burnetii* F 5'-GCAGCACGTCAAACCGTATG-3' and R 5'-TTCCCCCTCGAATGTTGTCG-3' which producing an amplification product of 549 bp. The primer was desgined depend on NCBI data base to obtain gene sequece and using primer 3 plus software to desgin specific primer for diagnosis *C. burnetii* DNA was exttracted from milk samples by genomic DNA purification kit supplemented by QIAamp DNA mini kit (Qiagen, Hilden, Germany) depending on the manufacturer's prescript. quantification of extracted DNA

concentration and purity was done using a nanodrop to assessment the concentration and purity of the extracted DNA according to the manufacturer's informations. The PCR reaction was done on 5 µl of tamplet DNA from each prepared specimen in atotal volume of 50 µl. The mixture of final reaction contained 10 pmol of each primer (Bioneer R& D center, Korea). The amplification procedure consisted of an initial denaturatnat 95°C for 120 sec, followed by 30 cycles of DNA denaturation at 94 for 30s, primer annealing at 59 for 30s and strand extension at 72 for 60s. Finally one step at 72 for 5 min to complete DNA extension. PCR product were visualized by electrophoresis, using 8 µm of its mixture and DNA ladder in 1.4% agarose gel for 45 min at 80 V, after stained with ethidium bromide and examind using ultraviolat transilluminator. Four amplicons obtained from PCR were used to nucleotides sequencing by AB DNA sequencing system in Bioneer company Korea. The results were analyzed with the multiple sequence alignment program. Phylogenetic analysis were performed by MEGA-6 and aligned sequences of Coxiella tranposase partial sequences were compared among them to determined homology.

Results:

The product of PCR was showed in (Figure. 1) in which the anticipated size (549bp) for the transposase gene parcial sequence of *C. burnetii* were obtained in 12% (6 of 50) of goat milk samples collected from different region of Al-Diwaniyah city Figure (1).

Phylogenetic analysis via DNA sequencing

Only four among the six milk specimens selected according regions that give an

amplicon of the anticipated base pair for *C. burnnetii* transposase gene from different region of Al-Diwaniyah city were studied. The results of four specimens sequeces had similarityin 99%. The sequences of targeting gene (transposase partial gene) obtained from four bacteria were recorded in gene bank (accession numbers KY576802.1, KY576803.1, KY576804.1 and KY576805.1).

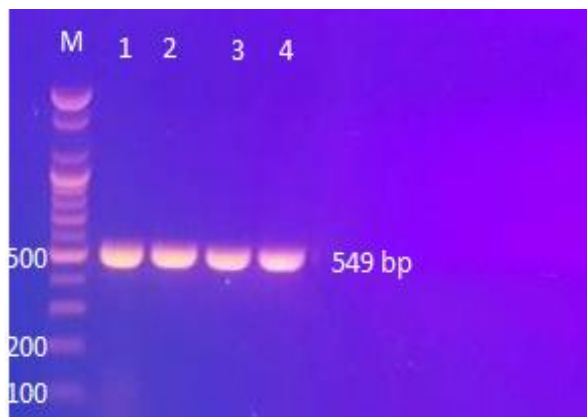


Figure (1): The PCR products of transposase partial sequences gene to identification of *C. burnetii*. M: DNA ladder (1500-100bp), well (1-4) positive samples, at 549 bp PCR product size.

Comparative analysis of transposase nucleotides sequences from Iraq samples with the number of *C. burnetii* strains present in the gene bank database shown in figure 2.

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Iraq-Seq1	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCATCGACAACATTGAGGGGGG-A--A
Iraq-Seq2	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCATCGACAACATTGAGGGGGG-A--A
Iraq-Seq3	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGTGGG-AATA
Iraq-Seq4	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGTGGG-A--T
India	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCATCGACAACATTGAGGGGGG-A--A
China	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGGGGGG-A--A
Taiwan	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGGGGGG-A--A
Portugal	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGGGGGG-A--A
Brazil	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGTGGG-A--A
USA	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTT-----A--T
France	ATTTCATCGTTCCCGGCAGTTGTCGGCGTTTATTGGGTTGGTCCCTCGACAACATTGAGTGGG-A--A

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Figure (2): Multiple sequence alignment analysis of the partial transposase gene of local Iraqi *Coxiella burnetii* isolates (Seq1- Seq4 isolate) with NCBI-Blast *Coxiella burnetii* transposase gene by using (MEGA 6.0, multiple alignment analysis tools). The multiple alignment analysis similarity (*) in transposase gene nucleotide sequences.

Comparative study with other strains of *C. burnetii* transposase gene of several studies showed closed related of Seq-1 and Seq-2 to NCBI-Blast *C. burnetii* transposase gene of Indian strain AB848993.1 in percentage 100% , while Seq-3 and Seq-4 were close related to NCBI-Blast *C. burnetii* transposase gene of Brazilian strain JF970261.1 (Figure 3) and (Table 1).

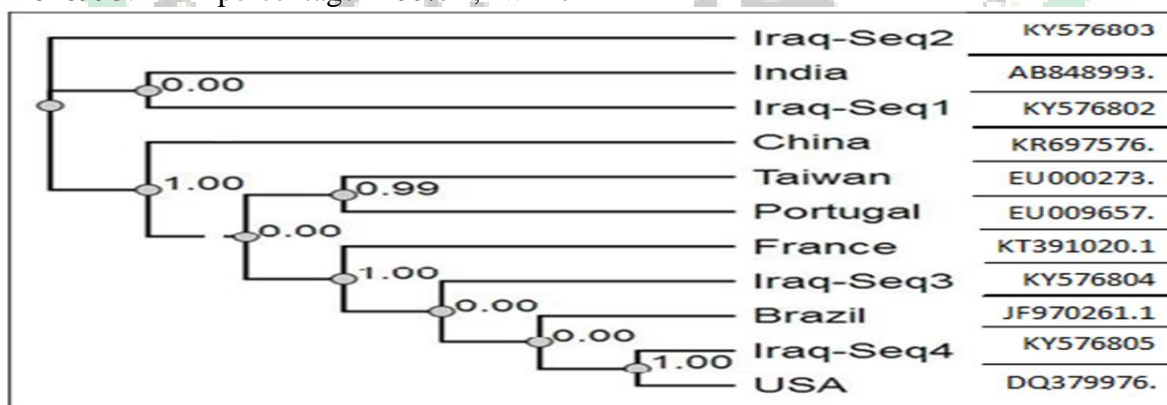


Figure (3): Genetic tree interpretation based on the transposase gene partial sequence that used for *Coxiella burnetii* genetic changes according regions. The genetic tree was constructed according Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Coxiella burnetii* isolates (Seq.1 and Seq.4) were showed closed related to NCBI-Blast *Coxiella burnetii* transposase gene of Indian strains, whereas Seq.3 and Seq.4 revealed identity with Brazilian strains.



Table (1): Comparative study to identify the similarity between local and Global strains

Isolates	Iraq-Seq1	Iraq-Seq2	Iraq-Seq3	Iraq-Seq4
Iraq-Seq1		100.00	99.27	99.27
Iraq-Seq2	100.00		99.27	99.27
Iraq-Seq3	99.27	99.27		100.00
Iraq-Seq4	99.27	99.27	100.00	
Brazil	99.27	99.27	100.00	100.00
India	100.00	100.00	99.27	99.27
China	99.64	99.64	99.64	99.64
USA	99.81	99.81	99.81	99.81
Taiwan	99.45	99.45	99.45	99.45
Portugal	99.45	99.45	99.45	99.45

Discussion:

This study used molecular technique for detection of *C. burnetii* infection compared with the use of serological diagnosis that often has low sensitivity. Cross-reactions have been described with *Legionella* spp. and with *Bartonella* spp. lead to interpretation of serological results difficult (10). Conventional PCR technique targeting the transposase partial sequence gene in specimens collected from apparently healthy goats of different separate farms of Al- Diwaniyah showed the presence of this bacterium DNA in 12% (6 of 50) of the goat milk samples which tested in this study. This result revealed relatively high positive yield of Coxiella compared to study on native Korean goats in which infection was 9.5% (11) and 2% of healthy goat milk samples were positive in Iran (12), while low positive yield compared to other study on Egyptian goats that confirmed the found of *C. burnetii* DNA in 85.2% of goats raw milk (9). The variation in detection rates greatly depends on sampling time after parturition due to shedding of this bacterium by infected goats occurs mainly during parturition (13). Molecular study of *C. burnetii* is an important method to show the genetic diversity in a region and to explore relationships among variants of this

organism. Genotyping methods revealed two genotype prevalence among samples under study. The first was identical to the Brazilian strain, while the other genotype showed a 100% match to the Indian strain. This indicates that the source of these bacteria in Iraq is Brazil and India which entered the country by importing meat and animal products from those countries. The study results refer to the goats is the important reservoir of *C. burnetii* and it was the source of infection in the region and because of ability of this bacterium to survive in the environment and transmission by inhalation, these gave indicated the potential of an increase infections in humans specially persons whose work in contact with animals and to other animals in Al-Diwaniyah city and its surroundings. The sequence results analysis showed the presence of 99% identity among sequenced strains and there are two genotype circulating in the different region and this result highly suggests a clonal spread of Coxiella with this predominant genotype over the goat farms in the Al-Diwaniyah part of the Iraq. **In conclusion:** This is the first work gives information about the genotypic similarity and diversity of *C. burnetii* that found in Iraq with other regions.



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