Detection and Identification of *Coxiella burnetii* in Milk Cattles of Tehran Province

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**ABSTRACT**

*Q fever* is a zoonotic disease with worldwide prevalence that is due to an obligatory intracellular Rickettsia called *Coxiella burnetii*. This study aims to detect and analysis phylogenetic *Coxiella burnetii* isolated from milk farms in Tehran. In this study, 150 samples were taken from the milk tank and 150 samples were collected from 50 dairy plants. The polymerase chain reaction tested to find *Coxiella burnetii* and 12 positive samples were sent Macrogen Company of Korea randomly to test sequencing. In the present study, 18 samples were positive among 150 and after sequencing, the positive samples were same as the sequencings available in the Genebank around 98%. Results of the study showed cow milk could be one of the potential sources of *Coxiella burnetii* in Iran. In addition, results for sequencing showed that isolates had the best similarity with isolates of France.

**Keywords:** *Coxiella burnetii*, milk

Received 01/04/2015 Accepted 19/06/2015 ©2015 Society of Education, India
workers, dairy workers, workers in leather, oil, fertilizer factories, or those working in the laboratories [12].

The main way of transmission to humans is by inhalation of droplets. Other modes of transmission include ingestion, tick or accidental items in the laboratory. Epidemiology of the disease and other infections caused by rickettsia is different, so that the mass of the pathogen survives in environmental conditions in the soil for months. Placenta, feces, urine, dust, ticks, and soil contaminates the source of infection by inhaling droplets. The mass is excreted through aborted fetuses, urine, and feces and milk of infected animals in and unpasteurized milk contaminate human being, ticks feeding on infected animals excreted after consumption. Unpasteurized contaminated milk is the source of infection for human. Symptoms include fever, chills, headache, muscle pain and cutaneous granular appearance. In chronic cases liver disorders, endocarditis, are symptoms of the disease. The mortality rate in acute cases of the disease is about one percent and chronic cases is high [13]. Studies in America have shown that a large number of ticks such as Derma centorandersoni and Amblyomma americanum are naturally infected [14]. Virulence of Coxiella burnetii organisms is in their ability to survive in myeloid cells with anticipation on cellular immune mechanisms and abduction of phagosomes changes [15]. In recent years Q fever outbreak breaks in some countries as an emerging disease, for example, in the East of the Netherlands, which has a high concentration on goats breeding, since 2007 three big epidemic have been occurred. To control disease in addition to the removal of infected goats and sheep from late 2009 to lamb season an extensive vaccination was in progress in 2010 [16, 17]. In the neighboring countries of Iran like Turkey, research results suggest that Q fever is an emerging disease in recent years [18]. The recent outbreak of Q fever between American soldiers in Iraq with a high attack rate of 50 percent in 2007 and fever with respiratory and gastrointestinal symptoms, which may originated by the tick bite or inhalation of contaminated dust, showed that Q fever can be an important factor in influencing troops [19]. Vaccination of slaughter house workers and farmers in Australia since 2002 has been very effective in the treatment of Q fever in Australia [20]. Given that this is a zoonotic disease and is important in terms of human health it can be said that it is done for the first time in Iran, we will try to isolate and study samples of milk with suspected agent of Coxiella burnetii, as an important cause of abortion in ruminants, then with a molecular study and phylogenetic analysis we tried to compare isolates to see how much they are similar together and with other isolates in the world.

MATERIALS AND METHODS

To obtain random samples of suspected industrial farms in Tehran, we took samples of milk tank containing the milk of cows which was about 100 cc. They were under sterile conditions with 50 mL of sterile syringes and sent to the laboratory in ice. Samples were centrifuged with refrigerated centrifuge at 1500 rpm for 15 minutes in Falcon pipes and after the separation of fat deposition, 200 microliter were taken for DNA extraction. Samples of abortion collected from vaginal discharge in cattle and aborted fetuses and sent to lab at 4°C at less than 24 hours. To extract DNA samples using the Qiagen kit, it is done according to the kit protocol. Extracted DNA was stored at -20 °C until PCR. Using specific primers of Coxiella burnetii, we proceeded to PCR according to temperature schedule: a 94 °C for 4 min, 30 cycles of 94 °C temperature, 56 °C and 72 °C for 1 min followed by a final stage of 72 °C for 5 min, so a 687 bp long product was amplified. The gene identified was transposon Is1111a.

5′−TGTTATTCCTTGCCGATGAC−3′  Primer F
5′−GATCGTAACTGCTTTAATAAACCG−3′ Primer R

For the PCR concentration of final volume of 25 microliter will be used in the following way: 5 microliter of DNA template, half mili-molar of mgcl2, a micro moles each primer, Taq DNA polymerase enzyme of and 200 micro-molar half NTPs Mix, two to three drops of sterile mineral oil to prevent evaporation and contamination were added to the PCR reaction mixture. Tubes were placed in the thermo cycler. The 12 samples selected from positive samples randomly then we sent them to Macrogen Company in Korea for nuclic Sequencing. The obtained sequences registered in Gene Bank (NCBI) and then obtained sequences tested for BLAST test in gene banks using DNA Star and Bioedit software. The origin of the isolated agents’ pedigrees was drawn and phylogenetic analysis obtained in this study was compared with the findings of other countries and the results were interpreted.

RESULTS AND DISCUSSION

After the PCR test on 150 simples number of milk, 18 positive samples were obtained which confirms to 12% of existence of bacteria in milk samples. This is with respect to fully clear bands in the range of 600BP of marker in the picture below.

Study and Comparison of the sequences of the studied isolates
Sequencing result compared in NCBI Databank with each other and with the Gene Bank isolates. A phylogenetic analysis was performed for obtained sequences using DNA Star and Bioedit software. After alignment of target sequences with sequences listed in the NCBI, tree was drawn as follows. The obtained sequences showed 98% similarity with sequences recorded in the NCBI.

![Phylogenetic tree of studied isolates with 8 isolates from around the world](image1.png)

Table 1: Similarities and differences in the sequences of the studied isolates with isolates from the rest of the world

Although Q fever is initially an occupational disease, but the consumption of contaminated milk products can also play an important role in the epidemiology of the disease in humans [21]. A study in America shows Coxiella burnetii serologic test in people who have used the raw milk is 10.7% positive. But the amount was reported 7.0% in people using pasteurized dairy products, [22]. Similar results also have been reported in the UK, Bulgaria, Slovakia and Spain. Prevalence of Coxiella burnetii in milk samples from the subjects in this study is similar with other studies carried out in the world. Rahimi et al reported 2.6% of the infection prevalence rate of cow’s milk in Chahar Mahal Bakhtiari in year 2009,[23]. Also Karghar et al reported 11% in 2012 with an assessment of 100 samples of milk in the city of Jahrom [24]. On the other hand a similar study in 2007 showed that among 399 collected cheese samples, 17 samples (4.7%) were infected by Coxiella burnetii [25]. In a research Hendrik et al conducted on molecular epidemiology in ruminants in the Netherlands they concluded that the genetic background of the Coxiella burnetii ruminants led to outbreak of Q fever in humans [26]. Astobiza et al in 2012 in a study entitled Coxiellaburnetii genotyping of isolates from domestic ruminants in northern Spain concluded that there
are considerable genetic variations of *Coxiella burnetii* in domestic ruminants in northern Spain (27). Kato *et al* in the scientific study in 1999, refers to 33 percent of people infected cases to the *Coxiella burnetii* in Japan [28]. Bashir Boud *et al* in 1976 showed positive cases of burnetii infection in cattle in Kermanshah province, also there were 7% of infection among cows, 3.2 of sheep and 1.7 percent of the pigs in the Lorestan province [29]. To *et al* in 2000 in a farm located in Gifu, Japan, found that 5.1% of dairy cows that had impaired fertility were infected by burnetii [30]. Rehacek reported in 2001, 12.2% of sheep, 9.7% of goats, and 4.4% of cattle in the southern regions of the Czech Republic are infected [31]. Rahimi *et al* in 2009 reported Prevalence of *Coxiella burnetii* in cows’ milk in Chahar Mahal Bakhtiari (n = 210), about 6.2 percent. [32]. Kargharet *et al* in 2012 with an assessment of 100 samples of milk in the city of Jahrom, reported 11% infection of *Coxiella burnetii* [33]. Kim *et al* reported in 2005 that the prevalence of *Coxiella burnetii* milk collection tanks in America were much higher than 90% [34]. Musakens *et al* in 2011 reported that 78.6% of the cow’s milk samples contain antibodies against *Coxiella burnetii*. These authors showed that the prevalence of these bacteria in the samples examined by polymerase chain reaction method is 56.6% [35]. Sakha’ii and Khalili in 2010 in southeastern Iran reported: in 85 sheep serums, which were collected from 10 herds, seroprevalence using ELISA is 29.42 percent. Banazis *et al* in Australia in 2010, reported that the seroprevalence of Q fever in cattle (329 samples), and sheep (50 samples), which was examined by ELISA showed the rate of 0.61 percent in cattle and zero in sheep. While the study of feces and urine of cattle by PCR showed 4.3% and 6.3%, respectively, and the prevalence in sheep feces has been reported 12% [37].

In isolates 1, 2, 5 and 6 in position 1 there is guanine instead of adenine. In Isolates of Spain in positions 125 and 179 there is adenine instead of guanine. In isolates of Spain, Iran and Taiwan in position 150 there is thymine instead of cytosine. In French isolates in position 183 there is adenine instead of guanine. In Indian isolates in position 201 there is guanine instead of thymine. Instead of cytosine at position 220 in Taiwan Isolates, there is thymine. In isolates of Spain in position 267 there is adenine instead of guanine. In isolates Q212 and French isolates 433 there is guanine instead of thymine. In Isolates 1, in position 479 there is thymine instead of guanine. In isolates Q212 and isolate id India, Taiwan, Spain and India in position 526 there is guanine instead of cytosine. In isolates Q212 in position 551 there is guanine instead of adenine. By examining the differences and similarities, 9 isolates had 100 percent similarity with sequences recorded in NCBI and only isolates 9 and 11 were slightly different. Generally sequences obtained were more than 98% similar with the sequences in the GENE BANK except for an Australian isolates which was (88%) similar.

**CONCLUSION**

According to the results, the isolated bacteria genetically were most similar to the isolates of *Coxiella burnetii* CB175, Guyana_France, the greatest difference was with *Coxiella sp*.64 insertion sequence IS1111 transposase-Like gene ASTURALAIA. The results of latest researches are compatible with the present study.

**REFERENCES**

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