Advances in Bioresearch
Adv. Biores., Vol 6 (4) July 2015: 48-52
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

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

Changhiz Ahmadizadeh[1], Farhad Moosakhani*[2], Mahmoud Jamshidian[1]
1-Department of Microbiology, Science and Research Branch, Islamic Azad university,Tehran, Iran
2-Department of Microbiology, Karajbranch, Islamic Azad University, Karaj, Iran
Email:farhadmoosakhani@yahoo.com

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 burnetiiand 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 burnetiiin 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

How to cite this article:
Changhiz A, Farhad M, Mahmoud J .Detection and Identification of *Coxiella burnetii* in Milk Cattles of Tehran Provinc. Adv. Biores., Vol 6 [4] July 2015: 48-52**.** DOI: 10.15515/abr.0976-4585.6.4.4852

INTRODUCTION

Q fever is a zoonotic disease with a worldwide prevalence, which has been reported in different geographic areas with different climate conditions. Q fever (Q stands for Query which means under question) reported for the first time by Dreick in 1973 after the prevalence of a flu-like disease among the workers of a Slaughter house in Brisbane, Australia [1]. Q fever is azoonosis infection and is endemic worldwide except for New Zealand [2]. An obligate intracellular bacterium, *Coxiella burnetii*, is the cause of the disease, which is among the type 3 Rickettsia families [3]. The organism creates Small Cellular Variant (SCV) and large Cellular Variant (LCV) in its life cycle. The small one is crated in environmental conditions and keeps the organism safe from the bad environmental factors. It is considered as the pathogen of infection. It transforms to LCV after it enters host body .Passing from small to large cell variants is associated with genetic changes in protein expression of surface antigens. *Coxiella burnetii*can infect range of animals such as cattle, sheep, goats, dogs, cats, non-human primates, reptiles, amphibians, birds (domestic and wild), fish and lots of ticks [4, 5].Among domestic animals, dairy cattle, sheep and goats are the largest reservoir of bacteria. Uterus and mammary glands of an animal are the first place of infection in the chronic phase with *Coxiella burnetii.* Infected animals dispose the bacteria in large extent through fecal discharge, uterine secretions, and parts of placenta during childbirth, to the environment. One of the most important ways of disposal of *Coxiella burnetii*s infected environment of dairy cattle [6, 7]. The organisms in the environment become spores-like and due to resistance to drought, heat and many disinfectants ,are able to survive for a long time in environment [8].There are no obvious clinical signs in animals. However, miscarriage, premature birth and infertility are of the most important complications of the disease among domestic animals [9, 10]. Symptoms in humans are highly variable and about 60 percent of patients with positive serum have no specific clinical symptoms [11].Acute Q fever is like flu, pneumonia or unclear hepatitis. The disease is primarily an occupational and it has and outbreak which usually can be observed in the breeders of animals, milking workers, slaughterhouse

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 *Coxiell aburnetii* organisms is in their ability to survive in myeloid cells with anticipation on cellular immune mechanisms and abduction of phagosomic changes [15]. In recent years Q fever out 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 2007and 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.

$\acute{5} - TGGTATTCTTGCCGATGAC - \acute{3}$ Primer F

$\acute{5} - GATCGTAACTGCTTAATAAACCG - \acute{3}$ 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 nucluic 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.

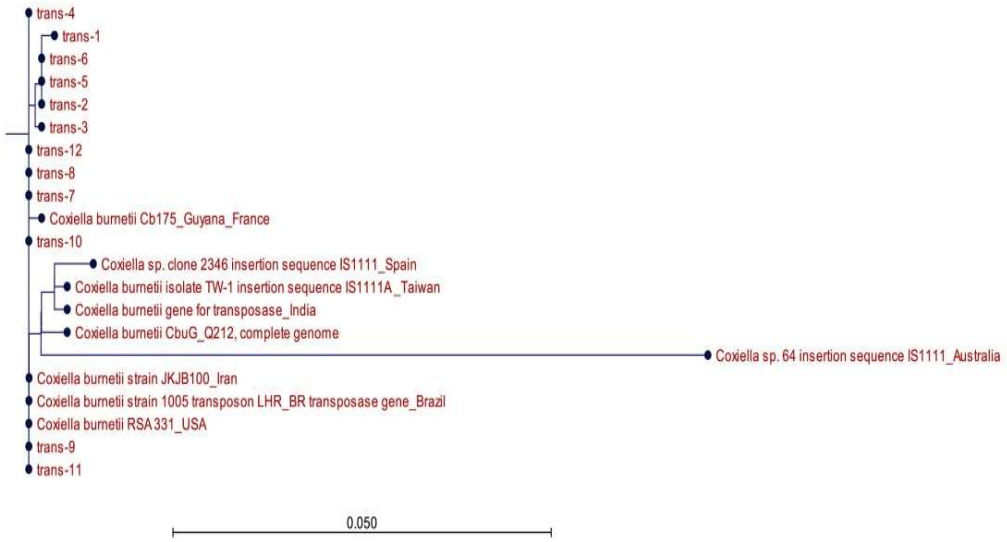

Figure 2: Phylogenetic tree of studied isolates with 8 isolates from around the world

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
a1	1		99.64	99.64	99.64	99.64	99.64	99.64	99.64	99.64	99.64	99.46	99.64	99.64	99.46	99.28	99.28	99.46	99.46	99.46	99.10	99.10	98.75	88.61
a9	2	0.00		100.00	100.00	100.00	100.00	100.00	99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
a10	3	0.00	0.00		100.00	100.00	100.00	100.00	99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
a11	4	0.00	0.00	0.00		100.00	100.00	100.00	99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
Coxiella burnetii strain JKJB100ir insertion sequence transposase IRAN	5	0.00	0.00	0.00	0.00		100.00	100.00	99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
Coxiella burnetii strain 1005 transposon LHR_BR transposase gene BRAZIL	6	0.00	0.00	0.00	0.00	0.00		100.00	99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
Coxiella burnetii RSA 331 USA	7	0.00	0.00	0.00	0.00	0.00	0.00		99.82	100.00	100.00	99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
Coxiella burnetii isolate goat_614 transposase gene BRAZIL	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.82	99.82	99.46	99.64	99.64	99.46	99.46	99.64	99.64	99.82	99.28	99.28	98.92	88.79
Coxiella burnetii gene for transposase_India	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		100.00	99.46	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
Coxiella burnetii Cb175_Guyana FARANCE	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.46	99.64	99.64	99.64	99.64	99.64	99.82	99.82	99.64	99.46	99.46	99.10	88.97
a2	11	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		99.82	99.82	99.64	99.82	99.64	99.64	99.64	99.28	98.93	98.93	98.57	88.45
a5	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		100.00	99.82	99.64	99.64	99.82	99.82	99.46	99.10	99.10	98.75	88.61
a6	13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.82	99.64	99.64	99.82	99.82	99.46	99.10	99.10	98.75	88.61
a3	14	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.64	99.64	99.82	99.82	99.46	99.10	99.10	98.75	88.61
a4	15	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00		99.82	99.82	99.82	99.28	99.11	99.11	98.75	88.63
a7	16	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.82	99.82	99.28	99.11	99.11	98.75	88.63
a8	17	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		100.00	99.46	99.28	99.28	98.92	88.79
a12	18	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		99.46	99.28	99.28	98.92	88.79
Coxiella burnetii isolate goat_618 transposase gene BRAZIL	19	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		99.10	99.10	98.75	88.61
Coxiella burnetii CbuG_Q212 USA	20	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		99.28	98.92	88.79
Coxiella burnetii isolate TW-1 insertion sequence IS1111A transposase gene TAIWAN	21	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		99.28	88.79
Uncultured Coxiella sp. clone 2346 insertion sequence IS1111 transposase gene SPAIN	22	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		88.43
Coxiella sp. 64 insertion sequence IS1111 transposase-like gene ASTURALIA	23	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	

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 Bakhtiariin 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 *Coxiell aburnetii* [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*in 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 to33 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 cow's milk in Chahar Mahal Bakhtiari (n = 210), about 6.2 percent. [32].Karghar*et al* in 2012 with an assessment of 100 samples of milk in the city of Jahrom, reported11%infection of *Coxiella burnetii* [33]. Kim *et al* reported in 2005 that the prevalence of *Coxiella burnetii*in milk collection tanks in America were much higher than 90% [34]. Muskens *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 is29.42 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 positions125 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

1. Derrick EH. (1983). “Q” fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Rev Infect Dis; 5(4): 790-800 .
2. Madariaga MG, Rezai K, (2003). Trenholme GM, Weinstein RA. Q fever: a biological weapon in your backyard. Lancet Infect Dis 3(11): 709–21 .
3. Weiss E, Moulder J. W.(1984). Genus III. Coxiella. In: Krieg NR, Holt J. G. (editors), Bergey’s manual of systematic bacteriology, vol.1. The Williams & Wilkins Co., Baltimore, PP 701-4
4. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999; 12: 518-553.
5. Parker NR, Barralet JH, Bell AM. Q fever. Lancet. 2006; 367: 679-688.
6. Kim SG, Kim EH, Lafferty CJ, Dubovi E.(2005). *Coxiella burnetii*in bulk tank milk samples, United States. Emerg Infect Dis. 11: 619-621.
7. Arricau-Bouvery N, Rodolakis A. (2005). Is Q fever emerging or re-emerging zoonoses? Vet Res. 36: 327-350.
8. Guatteo R, Beaudeau F, Joly A, Seegers H. (2006). Shedding routs of *Coxiella burnetii*in dairy cows: implications for detection and control. Vet Res. 37: 827-833.
9. To H, Htwe KK, Kako N, Kim HJ, Yamaguchi T, Fukushi H, Hieai K. (1998). Prevalence of *Coxiella burnetii*infection in dairy cattle with reproductive disorders. J Vet Med Sci. 60: 859-861.

10. Bildfell RJ, Thomson GW, Haines DM, McEwen BJ, Smart N.(2000). *Coxiella burnetii*infection is associated with placentitis in cases of bovine abortion. J Vet Diagn Invest. 12: 419-425.
11. Raoult D, Marrie T, Mege J. (2005). Natural history and pathophysiology of Q fever. Lancet Infect Dis 5(4): 219–26 .
12. Cutler SJ, Bouzid M, (2007). Cutler RR, Q fever. J Infec. 54: 313-318.
13. Tabatabayi,A.H and Firozi R , (2005). Diseases of animals due to bacteria University of Tehran press. pp499
14. Rad MA. (2005). Zoonoses. 4 th ed., Tehran, University of Tehran Press, PP 116-21 [Persian]
15. Ghigo E, Pretat L, Desnues B, Capo C, Raoult D, Mege JL. (2009). Intracellular life of *Coxiella burnetii*in Macrophages. Ann N Y Acad Sci; 1166 (1): 55-66 .
16. Scheimer B, Dijkstra F, Vellema P, Schneeberger PM, Wijkmans, et al. (2009). Sustained intensive transmission of Q fever in the south of Netherlands, Eurosurveillance; 14(19): 1-3 .
17. Delsing CE, Kullberg BJ. (2008). Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak. Net J Med ; 66 (9): 365-7 .
18. Gozalan A, Esen B, Rolain JM, Akin L, Raoult D. (2005).Is Q fever an emerging infection in Turkey? East Mediterr Health J; 11(3): 384-91 .
19. Faix DJ, Harrison DJ, Riddle MS, Vaughn AF, Yingst SL, Earhart K, Thibault G. (2008). Outbreak of Q fever among US military in Western Iraq, June–July 2005. Clin Infect Dis; 46(7): 65–8 .
20. Gidding HF, Wallace C, Lawrence G, McIntyre PB. (2009).Australia's national Q fever vaccination program. Vaccine; 27(14): 2037–41 .
21. Hirai A, Kaneko S, Nakama A, Ishizaki N, Odagiri M, Kai A, Sadamasu K, Shinkai T, Yano K, Morozumi S. (2005). Investigation of *Coxiella burnetii* contamination in commercial milk and PCR method for the detection of C. burnetii in egg. Shokhin Eiseigaku Zasshi. 46: 86-92.
22. Cerf O, Condron R. (2006). *Coxiella burnetii*and milk pasteurization: an early application of the precautionary principle? Epidemiol Infect. 134: 946-951.
23. Fretz R, Schaeren W, Tanner M, Baumgartner A. (2007). Screening of various foodstuffs for occurrence of *Coxiella burnetii*in Switzerland. Int J Food Microbiol. 116: 414-418.
24. Rahimi E, Doosti A, Ameri M, Kabiri E, Sharifian B. (2009). Detection of *Coxiella burnetii*by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Zoonoses Public Health. 57, 38-41.
25. Kargar M, Rashidi A, Doosti A, Ghorbani-Dalini S, Najafi A. (2012). Prevalence of *Coxiella burnetii*in bovine bulk milk samples in southern Iran. Com Clin Pathol. doi: 10.1007/s00580-012-1406-9.
26. Emerging Infectious Diseases (2011).• www.cdc.gov/eid • Vol. 17, No. 4.24-30.
27. http://www.biomedcentral.com/1746-6148/8/241
28. Hirai K, To H. (1998). Advances in the understanding of *Coxiella burnetii* infection in Japan. J Vet Med Sci ;60(7):781-90.
29. Bashiribod H, Sixl W, Stuenzner D. Q-Fieber in Iran. (1976). Abstracts of II Internationales Arbeitskolloqium Ueber Naturherde von Infektionskrankheiten in Zentraleuropa. Graz-Austria, 25.02-28.02. 1976:323-6.
30. To H, Htwe KK, Kako N, Kim HJ, Yamaguchi T, Fukushi H. (2000). Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. J Vet Med Sci;60(7):859861.
31. Rehacek J, Literak I. (2001). Coxiellosis among domestic animals in the Czech Republic, Vet Med Praha;46(4):54-9.
32. Kim SG, Kim EH, Lafferty CJ, Dubovi E. *Coxiella burnetii*in bulk tank milk samples, United States. Emerg Infect Dis. 2005; 11: 619-621.
33. Rahimi E, Doosti A, Ameri M, Kabiri E, Sharifian B. (2009). Detection of *Coxiella burnetii* by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Zoonoses Public Health. 57, 38-41.
34. Kargar M, Rashidi A, Doosti A, Ghorbani-Dalini S, Najafi A. (2012). Prevalence of *Coxiella burnetii* in bovine bulk milk samples in southern Iran. Com Clin Pathol. doi:10.1007/s00580-012-1406-9.
35. Muskens J, van Engelen E, van Maanen C, Bartels C, Lam TJ. (2011).Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Vet Rec. 168:79.
36. Banazis M.J., Bestall A.S., Reid S.A. and Fenwick S.G. (2010). A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*. Veterinary Microbiology, 143: 337-345.
37. Kennerman E., Rousset E., Golcu E. and Dufour P. (2010). Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey. Comparative Immunology, Microbiology and Infectious Diseases, 33: 37-45.