Molecular detection of *Coxiella burnetii* in raw milk of cattle in Karbala province

Nawras Amer Muhye [1*], Kadhim S. Kadhim [1], Mohammed Asad Salih[2]

[1]Veterinary Public Health Department, Veterinary Medicine College, University of Kerbala, Karbala, Iraq.
2 Internal and preventive Department, Veterinary Medicine College, University of Kerbala, Karbala, Iraq
***Corresponding author e-mail: nawras.a@uokerbala.edu.iq .**
https://doi.org/10.59658/jkas.v11i3.2347

Received:
May 13, 2024

Accepted:
July 05, 2024

Published:
Sept. 15, 2024

Abstract

Coxiella burnetii causes Q fever in humans and coxiellosis in animals. It was widely distributed worldwide; the main reservoirs are sheep, goats, and cattle. This study has been conducting to detection *C. burnetii* in raw milk of cattle (50) milk samples were randomly collected from dairy cattle from different areas of major districts of Karbala Province in interval during the period from November 2023 to April 2024 under aseptic conditions, using polymerase chain reaction (PCR) showed that *C. burnetii* IS1111 gene abundance was 14% in seropositive animals. This study presents the initial molecular identification of *C. burnetii* in the province of Karbala, indicating its prevalence among cattle. Furthermore, individuals who consume Unpasteurized milk and/or products may have a heightened susceptibility to infection.

Keywords: *Coxiella burnetii*, cattle, PCR.

Introduction

Coxiella burnetii is a polymorphic gram-negative bacterium that causes Q fever, a globally widespread zoonotic disease first discovered in Australia in 1937 [1], Livestock farming in particular is important for the global economy. When the characteristics of *C.burnetii* infection were examined, its relationship with livestock was determined [2] , Domestic animals (sheep, goats and cattle) are considered the main source of infection and are often associated with the focus of human infection, which is considered the main source of the disease and contaminates the environment through the excretion of *C. burnetii* in milk , feces, urine, saliva, genitals, breasts, and amniotic fluid [3].

Among foods of animal origin, raw milk is considered the most important source of *C. burnetii* with or without clinical signs of infection in animals at different times of lactation [4].

The primary mode of human infection with *C. burnetii* is through the inhalation of aerosols containing the bacteria. Other potential routes of transmission include direct contact, consumption of dairy products that are contaminated with the bacteria, tick bites, and human-to-human transmission through contamination [5].

C. burnetii is stable in the environment and resistant to physical attack such as chemistry, dehydration, radiation or osmosis. Therefore, this bacterium can survive for a long time in dairy and meat products, as well as feces, dirt, wool, pet food, equipment and clothing [4].

Polymerase chain reaction (PCR) tests are often used to detect *C. burnetii* directly in biological materials such as placentas, genital swabs, feces or milk samples and to detect ongoing infections associated with the shedding of bacteria [6]. The objective of this research was to isolate and identify *C. burnetii* from cattle milk samples in the province of Karbala. Additionally, the study aimed to confirm the diagnosis of *C. burnetii* using the PCR technique.

Materials and Methods

Samples

In this cross-sectional study, conducted in various regions of the primary districts in Karbala Province, milk samples were collected from dairy cows between November 2023 and May 2024. A total of 50 milk samples were randomly obtained, with approximately 50 ml collected from each available quarter of the udder. To ensure sample integrity, the milk samples were promptly placed in an icebox and transported to the laboratory under aseptic conditions

Molecular testing

DNA extraction

Molecular methods included the extraction and amplification of C. burnetii DNA, by using commercial kits gSYNCTM DNA Extraction Kit /Geneaid Republic of Korea according to Manufactures Company, was done in laboratory in the University of Karbala, College of Veterinary Medicine.

DNA samples were stored at -20°C until they were used. In this study, we used genomic primers targeting one genes: 16SrRNA.

Table (1): Nucleotide sequences C.burnetii primers

Primer	Nucleotide Sequence	Size	Targeted Gene	Reference
Forward Cocf	AGTACGGCCGCAAGGTTAAA	425bp	16S rRNA	NCBI: NR_104916.1
Reverse Cocr	CTCCAATCCGGACTACGAGC			

Polymerase chain reaction test (PCR)

The PCR reaction was performed by Thermal-Cycler (Bio-Rad, USA) utilizing the following optimized conditions: 1 cycle (95ºC/5 min.) for initial denaturation, 30 cycles comprised (95ºC/40 sec.) denaturation, (56ºC/40 sec.) annealing, and (72ºC/1 min.) extension for each one, and followed by one cycle (72ºC/7 minutes) for the final extension. Electrophoresis in 1.5% agarose gel was examined the PCR products using 100-1500 bp of DNA ladder (Qiagen, Germany). The agarose was stained with ethidium bromide (Biotech, Canada), and electrophoresis was done at 100 V, 80 mA for

one h. The DNA bands were visualized by a UV trans illuminator (Clinx Science, China).

Table (2): Show PCR program for PCR amplification

Step	Repeat cycle	Tempera-ture	Time
Initial Denaturation	1	95C°	5 min
Denaturation	35	95C°	40sec
Annealing		56C°	40sec
Extension		72C°	1min
Final Extension	1	72C°	7min
Hold	4 C°		

The master mix reaction components were added to the standard PCR tube that containing the PCR Premix. PCR products along with 100 bp DNA ladder electro-phoresed in 1% agarose gel containing ethidium bromide (5µl /100 ml) of ethidium bromide dye. Then the agarose was run at 100 V and 80 AM for 1 hour [7].

Results and Discussion

In the semi-nested PCR assay with new primers Coc-F and Coc-R only Four spe-cific band was observed with the ex- pected size (425 bp) of *C. burnetii*.

Table (3): Total results for testing DNA products

No. of samples	Gene	Positive	Negative	Percentage
50	16S rRNA	7	43	14%

In total 50 tested samples 7 samples were positive with primers CocF - CocR and showed the 425 bp PCR products on agarose gel (Figure 1).

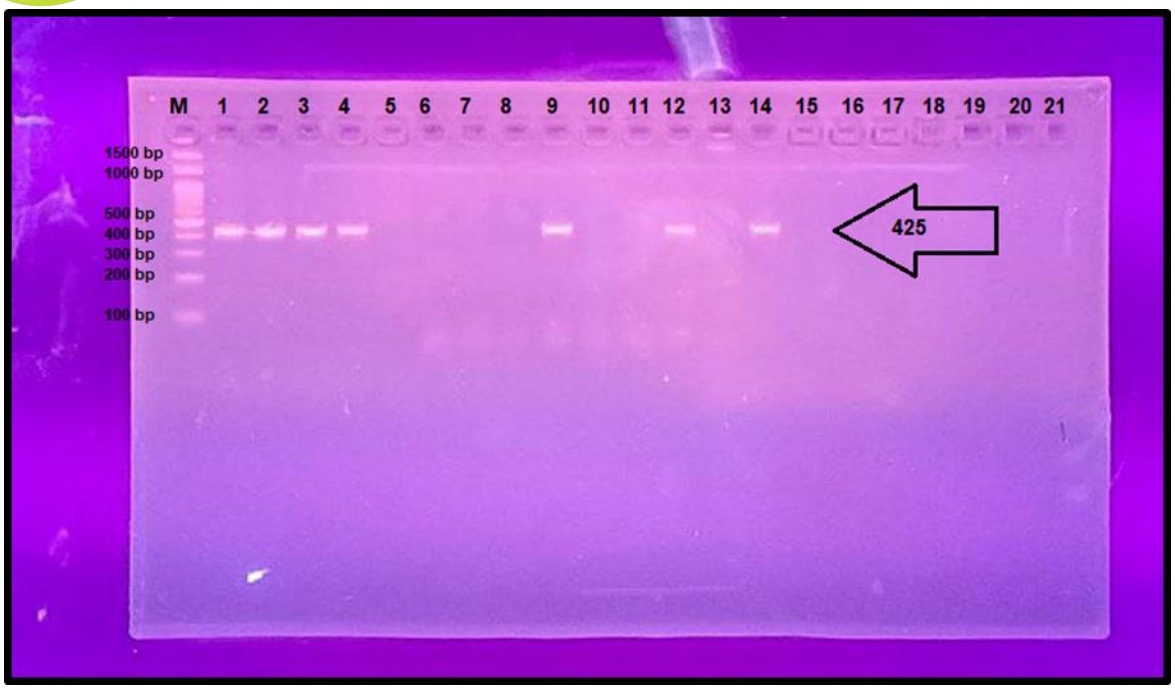

Figure (1): The arrow shows that PCR product as 425 bp represented 16S rRNA on Agarose gel electrophoresis, well 1:DNA ladder marker, well 1,2,3,4,9,12,and 14 represented positive sample.

PCR product analysis of 16S rRNA gene (425bp) of *C. burnetii* on Agarose-gel electrophoresis.

This research took place in Karbala Province, where there was no existing data on *C. burnetii* infection in animals and raw milk. The findings revealed the presence of *C. burnetii* in milk samples obtained from dairy animals across all examined regions. Out of the raw cow milk samples tested, 14% were found to be positive for *C. burnetii* through PCR analysis. It is worth noting that cow's milk is primarily consumed in its unpasteurized form, and there is relatively less interest in consuming raw cow's milk and its unpasteurized products. Nonetheless, in certain rural areas, there remains a preference for consuming raw, unpasteurized milk and its derivatives.

In this study, *C. burnetii* was identified in 14% of the bovine milk sample , which closely align with the ratios reported in other studies (12%) in Al-Diwaniyiah city [8], (18.46%) in waist [9] and (15%) positive cows in Iran[10

Our results suggest that *C. burnetii* infection, particularly in raw milk samples, may a serious risk of Q fever to farmers and consumer.

In our study, *C. burnetii* were detected from clinically healthy animals , this result agreement with previous studies [11] who mentioned to presence of carriers without clinical signs, these latently infected animals contribute to infection persistence at the flock level and pose the highest threat for the silent transmission of bacterium [3,11].

Based on epidemiological studies conducted in animals, it has been observed that *C. burnetii* infection exhibits a high prevalence in certain regions, with variations based on host species and diagnostic techniques used. In our study, we found that the prevalence of Q fever showed no significant difference, indicating that Q fever is

endemic in Iraq [12]. *C. burnetii* displays remarkable resistance to heat, pressure, and chemical stresses, allowing it to survive for extended periods in the environment. This, coupled with its high infectivity and ease of transmission to humans, has classified *C. burnetii* as a potential warfare agent. As an obligate intracellular parasite, it can only replicate within living cells, specifically in phagolysosomes, even under harsh conditions characterized by an acidic pH [13] .

References

1) España, P., Uranga, A., Cillóniz, C., Hirschmann, J., Raoult, D., Marrie, T., Stein, A., Saunders, N., Taylor, A., & Raoult, D. (2020). Q fever (*Coxiella burnetii*). *Seminars in Respiratory and Critical Care Medicine, 41*(4), 509-521. https://doi.org/10.1055/s-0040-1713443

2) Orrego, R. C., Ríos-Osorio, L. A., Keynan, Y., Rueda, Z. V., & Gutiérrez, L. A. (2020). Molecular detection of *Coxiella burnetii* in livestock farmers and cattle from Magdalena Medio in Antioquia, Colombia. *PLoS ONE, 15*(6), 1-16. https://doi.org/10.1371/journal.pone.0234360

3) Abiri, Z., Khalili, M., Kostoulas, P., Sharifi, H., Rad, M., & Babaei, H. (2019). Bayesian estimation of sensitivity and specificity of a PCR method to detect *Coxiella burnetii* in milk and vaginal secretions in sheep and goat samples. *Journal of Dairy Science, 102*(6), 4954-4959. https://doi.org/10.3168/jds.2018-15233

4) Pexara, A., Solomakos, N., & Govaris, A. (2018). Q fever and prevalence of *Coxiella burnetii* in milk. *Trends in Food Science & Technology, 71*, 65-72. https://doi.org/10.1016/j.tifs.2017.11.004

5) Rahravani, M., Moravedji, M., Mostafavi, E., Mohammadi, M., Seyfi, H., Baseri, N., Mozoun, M. M., Latifian, M., & Esmaeili, S. (2022). The epidemiological survey of *Coxiella burnetii* in small ruminants and their ticks in western Iran. *BMC Veterinary Research, 18*(1), 1-7. https://doi.org/10.1186/s12917-022-03396-0

6) Hardi, F., Rauf, H., Mahmood, S., Ahmad, R., Ali, B., & Sheikh, M. (2020). Molecular detection and identification of *Coxiella burnetii* in aborted sheep and goats in Sulaimani Province, Kurdistan-Iraq. *Assiut Veterinary Medical Journal, 66*(164), 133-139. https://doi.org/10.21608/avmj.2020.167268

7) Rashidi, A., Doosti, A., Najafi, A., & Ghorbani-Dalini, S. (2015). The sensitivity of the PCR method for detection of *Coxiella burnetii* in milk samples. *Zahedan Journal of Research in Medical Sciences, 17*(6). https://doi.org/10.17795/zjrms988

8) Ayyez, H. N. (2017). Molecular detection and phylogenetic analysis of *Coxiella burnetii* in goat milk. *Al-Qadisiyah Journal of Veterinary Medicine Sciences, 16*(1), 79-83. https://doi.org/10.29079/vol16iss1art40

9) Al-Graibawi, M. A., Yousif, A. A., Gharban, H. A., & Zinsstag, J. (2021). First serodetection and molecular phylogenetic documentation of *Coxiella burnetii* isolates from female camels in Wasit governorate, Iraq. *Iraqi Journal of Veterinary Sciences, 35*, 47-52. https://doi.org/10.33899/ijvs.2021.130888.1890

10) Mobarez, A. M., Mostafavi, E., Khalili, M., & Esmaeili, S. (2021). Identification of *Coxiella burnetii* in raw milk of livestock animals in Iran. *International Journal of Microbiology, 2021*, 6-10. https://doi.org/10.1155/2021/6632036
11) Khademi, P., Ownagh, A., Ataei, B., Kazemnia, A., Enferadi, A., Khalili, M., & Mardani, K. (2020). Prevalence of *C. burnetii* DNA in sheep and goat milk in the northwest of Iran. *International Journal of Food Microbiology, 331*, 108716. https://doi.org/10.1016/j.ijfoodmicro.2020.108716
12) Gleeson, T. D., Decker, C. F., Johnson, M. D., Hartzell, J. D., & Mascola, J. R. (2007). Q fever in U.S. military returning from Iraq. *The American Journal of Medicine, 120*(9), e11-e12. https://doi.org/10.1016/j.amjmed.2007.05.020
13)Kazar, J. (2005). *Coxiella burnetii* infection. *Annals of the New York Academy of Sciences, 1063*, 105-114. https://doi.org/10.1196/annals.1355.018
14)Hassan, S. A., & Saeed, A. A. (2013). Effect of feeding different levels of dietary [incomplete information]. [Complete details needed for full citation].