Uttar Pradesh Journal of Zoology

Volume 45, Issue 14, Page 19-25, 2024; Article no.UPJOZ.3655
ISSN: 0256-971X (P)

Molecular Identification of *Coxiella burnetii* and Study Physical Features in Raw Milk of Goat in Karbala Province

Nawras Amer Muhye [a*], Kadhim S. Kadhim [a] and Mohammed Asad Salih [a]

[a] *Department of Public Health, Collage of Veterinary Medicine, University of Kerbala, Iraq.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i144175

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3655

Original Research Article

Received: 09/04/2024
Accepted: 13/06/2024
Published: 20/06/2024

ABSTRACT

This study aims to detect *Coxiella burnetii* in Karbala Province using Chemical Examination of milk and confirmation of infection by polymerase chain reaction test (PCR). The prevalence of *Coxiella burnetii* was found to be 10% targeting 16S rRNA genes from five positive samples, according to the findings of the fifty raw milk samples that were obtained from fifty goats from various regions within the main districts of Karbala city between September 2023 and March 2024. It has been demonstrated by the findings of this study that there is a large amount of *C. burnetii* DNA present in goat raw milk supplies. The finding of the PH was 8, while the results of the catalase were recorded as positive results. On the other hand, the results of the chloride exhibited a milky-gray color. In conclusion, our findings confirmed the prevalence of *C. burnetii* among Iraqi goat, suggesting that these animals might be a source of the pathogen for humans and other animal species.

**Corresponding author: Email: Namer.i@uokerbala.edu.iq;*

Cite as: *Muhye, Nawras Amer, Kadhim S. Kadhim, and Mohammed Asad Salih. 2024. "Molecular Identification of Coxiella Burnetii and Study Physical Features in Raw Milk of Goat in Karbala Province". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (14):19-25. https://doi.org/10.56557/upjoz/2024/v45i144175.*

Keywords: Coxiella burnetiid; polymerase chain reaction test; antibiotic efficacy.

1. INTRODUCTION

Coxiella burnetii is a bacterium that is obligatory and fastidious, and it is found inside of cells. It is responsible for Q fever in both humans and animals. *C. burnetii* is an organism that is exceedingly resistant to harsh environmental circumstances such as physical and chemical stress, and it is also highly contagious [1].

Q-fever is a global public health issue that has been identified in over 59 countries the infection occurs in humans when they inhale contaminated dust generated from infected manure and placenta, leading to the formation of aerosols. *C. burnetii* can be carried by the wind, spreading to urban areas where people may not have direct contact with infected animals [2].

This particular microbe possesses a cell wall that is comparable to that of Gram-negative bacteria; nevertheless, it is not often stainable using the Gram method [3].

It is capable of causing infections in a wide variety of domestic and wild species, such as mammals, birds, reptiles, and arthropods; however, the primary reservoirs are cattle, goats, and sheep, which are also sources of infection in humans. In light of the fact that these bacteria found in a large percentage of cattle, it is essential to determine whether or not it is present in foods that are derived from animals. A significant amount of *C. burnetii* can be found in milk, which is a meal that comes from animals [4].

Milk can be contaminated with *C. burnetii* by the presence of feces, vaginal mucous, or urine. The infected livestock harbour *C. burnetii* in their milk for varying periods of time. Hence, the ingestion of milk or milk products that have been tainted can serve as a potential means of infection for humans [1].

The most common way for humans to contract *C. burnetii* is through inhaling aerosols that are infected with the bacteria [5].

Q fever can infect humans in both acute and chronic forms. Acute Q fever typically manifests as a temporary flu-like condition characterized by headache, rash, and joint pain. Nevertheless, more severe symptoms like pneumonia, hepatitis, and myocarditis can arise, potentially leading to hospitalization [4]. The chronic manifestation of Q fever may develop subsequent to the acute phase and commonly manifests as endocarditis, chronic fatigue syndrome, or complications during pregnancy [4]

The diagnosis of Q fever in animals relies on the identification of particular antibodies by the use of ELISA, as well as the detection of *C. burnetii* DNA in various biological materials such as placenta, vaginal mucus, milk, colostrum, feces, and tissues of aborted fetuses, using PCR [6].

The aim of this study was to isolate and identify *C. burnetii* from goat milk samples collected in the province of Karbala. In addition, the study sought to validate the diagnosis of *C. burnetii* using the PCR approach. Moreover, the study aimed to examine the physical attributes of milk contaminated with *C. burnetii* obtained from goats.

2. MATERIALS AND METHODS

2.1 Samples

This study aimed to analyses milk samples collected under sterile conditions. Approximately 50 samples of goat milk were collected between September 2023 and March 2024. These samples were randomly selected from goats of various ages and breeds in different areas of the main districts of Karbala Province.

The samples were preserved in containers with ice and brought to the laboratory, where they were held at a temperature of -20°C until DNA extraction and subsequent analysis. A centrifugation process was conducted on one milliliter of raw milk in order to separate bacterial cells from the sediment in milk samples. Subsequently, eliminate the layers of cream and milk [7].

2.2 Chemical Examination of Milk

The laboratory examination of milk has been carried out according to (Najim *et al*., 2012) who used these procedures to checking the for chemical composition to endeavor to assess cleanliness; and detect disease or abnormalities which affect the quality of the milk Which included

Milk PH: The measurement of pH in milk is crucial for the detection of contamination, deterioration, and indications of mastitis infection. *C. burnetii* exhibits metabolic activity

within the pH range of 4 to 5,which is normally present in the phagolysosome [8].

Catalase test: In order to ensure their survival, organisms must depend on Defence mechanisms that enable them to repair or evade oxidative damage induced by hydrogen peroxide (H2O2). Certain bacteria synthesized the enzyme catalase, which aids in cellular detoxification [9].

Milk chloride test: The most straightforward technique for measuring chlorides is to directly titrate the milk, employing potassium chromate as an indicator [10].

2.3 DNA Extraction

The molecular techniques employed encompassed the extraction and amplification of C. burnetii DNA. The techniques employed for DNA extraction involve the utilisation of commercially available kits. The gSYNCTM DNA Extraction Kit from Geneaid Republic of Korea was used to extract DNA in the laboratory of the Faculty of Veterinary Medicine, University of Karbala, following the manufacturer's instructions.

The milk samples underwent laboratory processing to exclude cream and precipitation cells, including bacteria, following established techniques that had been previously reported [11].

The final precipitate was dissolved in 1 mL phosphate-buffered saline (PBS) solution and stored at −20°C until use.

In this study, we used genomic primers targeting one gene: 16SrRNA.

The PCR reaction was performed via a thermal cycler (Bio-Rad, USA) with the following optimized parameters: One cycle of initial denaturation at 95 °C for 5 minutes, followed by 30 cycles at 95 °C for 40 seconds each. The process consists of denaturation at 56°C for 5 minutes, followed by annealing for 40 seconds and extension at 72°C for 1 minute. This cycle is repeated once, and then a final renewal step is performed at 72°C for 7 minutes. The PCR results were analyzed using 1.5% Agarose gel electrophoresis, with a 100–1500 bp DNA ladder (Qiagen, Germany) as a reference. The Agarose was dyed with Ethidium bromide (Biotech, Canada) and subjected to electrophoresis at a voltage of 100 V and a current of 80 mA for a duration of 1 hour. The DNA bands were observed using a UV transilluminator (Clinx Science, China).

Table 1. Nucleotide sequences *C.burnetii* primers

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

Table 2. Show PCR program for PCR amplification

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

Table 3. Show result of Chemical examination of milk

No.	Chemical Examination	positive	negative
1	PH	PH 8	PH7
2	Catalase test	rapid formation of bubbles	NO bubbles
3	Chloride test	gray-green milk color	reddish brown color
4	Total 50 sample	5=10%	45

3. THE RESULTS

3.1 Chemical Examination

The results of chemical study have been showed changes in PH, catalase and chloride. The results of PH were showed (8) Alkaline pH of the milk may also indicate neutralization of milk by NaOH, Na_2CO_3, and $NaHCO_3$ etc. (Fig. 1), while results of catalase have been reveled rapid formation of bubbles, Catalase expedites the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen ($2H_2O_2$ + Catalase → $2H_2O$ + O_2). This reaction is evident by the rapid formation of bubbles (Fig. 2), in another hands the results of chloride were showed gray-green milk color (Fig. 3) while a reddish brown color showed that the milk sample was negative (Fig. 4).

3.2 Molecular Examination

Results of DNA Extraction: The results of molecular study have been showed 10% of raw goat milk samples were positive for *C. burnetii* by real-time PCR. Table (4).

Fig. 1. PH Paper indicator for milk analysis

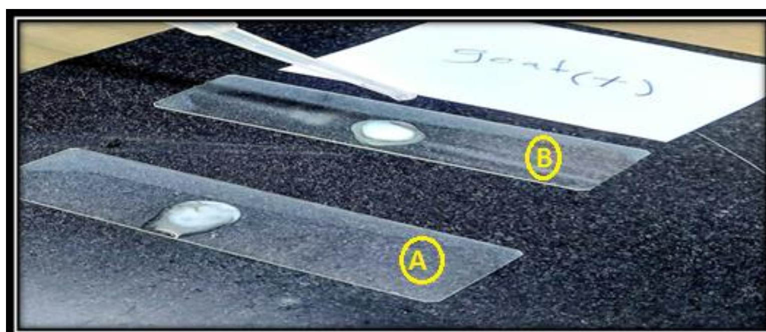

Fig. 2. Slide catalase test results. (A) the negative reaction was produced by no infected milk ; B-The positive reaction was produced by Coxiella burnetii

Fig. 3. Chloride test(positive) for milk analysis.

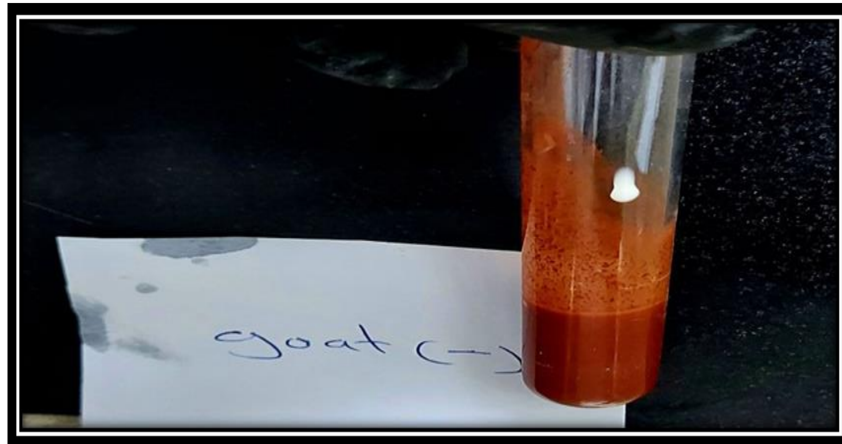

Fig. 4. A g(chloride test(negative) for milk analysis n

Table 4. Total results for testing DNA products

No.of samples	Gene	Positive	Negative	Percentage
50	16S rRNA	**5**	45	10%

3.3 B-Results of PCR Amplification

According to the findings of our investigation, the presence of the DNA of the bacteria *C. burnetii* was found in ten percent (five out of fifty) of the goat milk samples that were examined in our laboratory. Based on these findings, it was determined that the presence of *C. burnetii* infection, particularly in raw milk samples, potentially posed a significant danger of Q fever to farmers and consumers in the city of Karbala (Fig. 5).

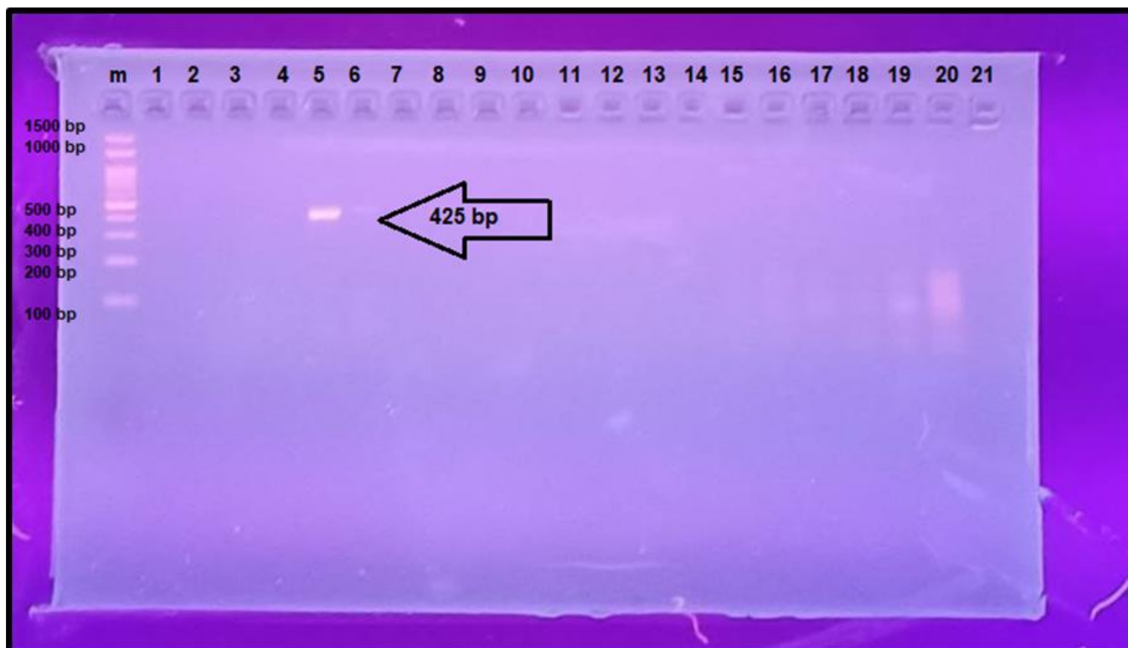

Fig. (5) the arrow shows that PCR product as 425 bp represented 16S rRNA on Agarose gel electrophoresis, well 1:DNA ladder marker, well 5 reped poresentsitive sample

4. DISCUSSION

In contrast to serological diagnosis, which is not always very sensitive, this study used a molecular approach to identify *C. burnetii* infections. Using a convolutional PCR approach that targeted the transposes partial sequence gene, researchers in this study were able to detect the presence of this bacterium DNA in 10% (5 out of 50) of the goat milk samples obtained from seemingly healthy goats from various farms in Karbala.

Comparing this study to one in Iran, the results showed a higher detection rate of *Coxiella.* Use of real-time PCR allowed for the identification of C. burnetii in 10.17 percent of the goat milk samples tested [1].

In comparison to the study conducted in Wasit Province, Iraq, in which (26.34%) of the animals tested positive for *Coxiella* (Shati et al., 2022), this result demonstrated a comparatively low positive yield of *Coxiella*. Additionally, in comparison to another study conducted on Al-Diwaniyia goats, only (12 %) of the animals tested positive for the *C. burnetii*. (Ayyez, 2017).

In contrast, when compared to a study conducted in other countries, the prevalence of disease in Turkey, the positive rate of *Coxiella* detection in this study was relatively low (20%) (T & Dufour, 2010) and (85.2%)in Egyptian goats [12] Q fever cases have been reported from some countries neighboring Iraq, such as Turkey and Iran Serologic evidences indicate people and animals in Iran are exposed to *C. burnetii* Navaei, [13].

The source of these bacteria in Iraq is which entered the country by importing meat and animal products from other countries [14].

The study results refer to the goats are a major source of *C. burnetii* infections in the region. The bacterium can live in the environment and can be transmitted through breathing, so there's a chance that humans, especially those whose jobs put them in touch with animals, could contract the disease more frequently in Karbala and the surrounding areas [4].

Understanding the frequency of shedding is essential for calculating the potential risk of infection transmission among ruminants or herds, as well as between ruminants and humans. Researchers, agricultural organizations, veterinarians, and farmers can all benefit from published, well-designed prevalence studies that outline the sampling and testing procedure [14,15].

5. CONCLUSION

- *Coxiella burnetii* is highly pathogenic bacteria which have zoonotic importance to public health.
- *Coxiella. burnetii* causes multiple changes in Chemical features in raw milk.
- Polymers chain reaction test (PCR) is the best diagnostic procedure to detection of *Coxiella*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mobarez AM, Mostafavi E, Khalili M, Esmaeili S. Identification of Coxiella burnetii in Raw Milk of Livestock Animal in Iran. International Journal of Microbiology. 2021;6–10. Available:https://doi.org/10.1155/2021/6632036
2. Robi DT, Demissie W, Temteme S. Coxiellosis in livestock: Epidemiology, public health significance, and prevalence of Coxiella burnetii Infection in Ethiopia. Veterinary Medicine: Research and Reports. 2023;14:145–158. Available:https://doi.org/10.2147/vmrr.s418346
3. Calahorrano-Moreno MB, Ordoñez-Bailon JJ, Baquerizo-Crespo RJ, Dueñas-Rivadeneira AA, Montenegro MCBSM, Rodríguez-Díaz JM. Contaminants in the cow’s milk we consume? Pasteurization and other technologies in the elimination of contaminants. F1000Research. 2022;11.
4. Pexara A, Solomakos N, Govaris A. Q fever and prevalence of Coxiella burnetii in

milk. Trends in Food Science and Technology. 2018;71:65–72. Available:https://doi.org/10.1016/j.tifs.2017.11.004

5. Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, EdouardS., Mege J-L, Maurin M, Raoult D. From Q fever to Coxiella burnetii infection: A paradigm change. Clinical Microbiology Reviews. 2017;30(1):115–190.
6. Galiero A, Fratini F, Cammà C, Di Domenico M, Curini V, Baronti I, Turchi B, Cerri D. Occurrence of Coxiella burnetii in goat and ewe unpasteurized cheeses: Screening and genotyping. International Journal of Food Microbiology. 2016;237:47–54. Available:https://doi.org/10.1016/J.IJFOODMICRO.2016.08.008
7. Achaw OW, Danso-Boateng E. Milk and dairy products manufacture. In Chemical and process industries: With examples of industries in Ghana. Cham: Springer International Publishing. 2021; 293-374.
8. Smith CB, Evavold C, Kersh GJ. The Effect of pH on Antibiotic Efficacy against Coxiella burnetii in Axenic Media. Scientific Reports. 2019;9(1):1–9. Available:https://doi.org/10.1038/s41598-019-54556-6
9. Taye Y, Degu T, Fesseha H, Mathewos M. Isolation and identification of lactic acid bacteria from cow milk and milk products. The Scientific World Journal. 2021;2021(1):4697445.
10. Upadhyay N, Goyal A, Kumar A, Ghai DL, Singh R. Preservation of milk and milk products for analytical purposes. Food Reviews International. 2014;30(3):203–224.
11. Esmaeili S, Mobarez AM, Khalili M, Mostafavi E. High prevalence and risk factors of Coxiella burnetii in milk of dairy animals with a history of abortion in Iran. Comparative Immunology, Microbiology and Infectious Diseases. 2019;63:127–130.
12. Khalifa NO, Elhofy FI, Fahmy AH, Sobhy MM, Agag M. Seroprevalence and molecular detection of Coxiella burnetii infection in sheep, goats and human in Egypt. ISOI J. Microbiol. Biotechnol. Food Sci. 2016;2(1):1–7.
13. Navaei H. A review of the epidemiology of Q fever disease in Iran. Journal of Zoonotic Diseases. 2021;5(3):1-7.
14. Hardi F, Rauf H, Mahmood S, Ahmad R, Ali B, Sheikh M. Molecular Detection and Identification of Coxiella Burnetii in Aborted Sheep and Goats in Sulaimani Province, Kurdistan-Iraq. Assiut Veterinary Medical Journal. 2020;66(164):133–139. Available:https://doi.org/10.21608/avmj.2020.167268
15. Esmaeili S, Mohabati Mobarez A, Khalili M, Mostafavi E, Moradnejad P. Molecular prevalence of Coxiella burnetii in milk in Iran: A systematic review and meta-analysis. Tropical animal health and production. 2019;51: 1345-55.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3655