Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 4, 508–519
ISSN 1311-1477; DOI: 10.15547/bjvm.2322

Original article

FIRST ISOLATION AND MOLECULAR PHYLOGENETIC ANALYSIS OF *COXIELLA BURNETII* IN LACTATING COWS, IRAQ

H. A. J. GHARBAN & A. A. YOUSIF

Department of Internal and Preventive Veterinary Medicine,
College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Summary

Gharban, H. A. J. & A. A. Yousif, 2021. First isolation and molecular phylogenetic analysis of *Coxiella burnetii* in lactating cows, Iraq. *Bulg. J. Vet. Med.,* **24**, No 4, 508–519.

Q fever is an infectious disease of animals and humans, caused by globally distributed *C. burnetii.* In Iraq, there are no previous studies associated with the detection of the organism in cattle. An overall of 130 lactating cows were submitted to direct collection of milk samples. Initially, the samples of milk were tested using the molecular polymerase chain reaction (PCR) assay targeting three genes (*16S rRNA*, *IS1111a transposase*, and *htpB*). However, positive results (18.46%; 24/130) were detected only with the *16s rRNA* gene. Concerning risk factors, the highest prevalence of *C. burnetii* was showed in the district of Badra (42.86%), whereas the lowest – in Al-Numaniyah and Al-Suwaira districts (P=0.025). There was no significant variation in positivity between the months of sampling period (P=0.082) and between age groups (P=0.076). Crossbred cows (20.69%) showed a higher positivity than local and pure breeds (P=0.043). Milk of positive samples (n=24) was used for cultivation of *C. burnetii* into specific pathogen free-embryonated chicken eggs (SPF-ECEs). After three passages into SPF-ECEs, contents of yolk sac were collected, subjected for DNA extraction, and re-tested by PCR assay using the primer of *16s rRNA* gene only. Of 24 cultivated milk samples, 12.5% (3/24) were positive for *C. burnetii*. Finally, the positive local isolates were analysed phylogenetically and reported in NCBI-Genbank under the accession numbers of MN121700.1, MN121701.1, and MN121702.1. In conclusion, this is a unique study as it detected *C. burnetii* in Iraqi lactating cows, and confirmed that organism was shed actively through milk, suggesting that these animals can play a role as a reservoir for organism with potential risk for transmission of infection from these animals to humans as well as to other animal species.

Key words: cow, *Coxiella burnetii*, Iraq, isolation, milk, phylogenetic analysis

INTRODUCTION

Coxiella burnetii is an obligate intracellular bacterial pathogen that infects animals as well as humans resulting in a highly infectious disease named Q fever (″Query fever″). Based on *16S rRNA*-encoding gene sequence, *C. burnetii* was moved from Rickettsiales order to Legionellales order, and reclassified under *Coxiellaceae* family as the only species of *Coxiella* genus (Kim *et al.,* 2005; Honarmand, 2012).

Since the first description of disease in 1935 among slaughterhouse workers of Australia, extensive body of knowledge about *Coxiella* and its infections (Porter *et al.,* 2011) became available. Although domestic ruminants are the main reservoirs, many animals such as ticks, birds, mammals and reptiles have a role in shedding of *C. burnetii* (Bolanos-Rivero *et al.,* 2017). Large amounts of the organism can be excreted into milk, urine, semen, faeces, vaginal fluids, placenta, and aborted foetuses of diseased animals. Cattle may typically acquire *C. burnetii* by inhalation of infectious droplets or direct exposure to contaminated body fluids (Angelakis & Raoult, 2011).

One of the most critical points for controlling the spread of *Coxiella* among animals and from animals to humans is the diagnosis of organism in ruminants (Guatteo *et al.,* 2011). In ruminants, *C. burnetii* infection is strikingly asymptomatic and occurs at any age (Radostits *et al.,* 2006). Recently, several tools are modified to detect the organism using different samples such as blood, milk, faeces, and tissues (Mohammed *et al.,* 2014). Culture is one of the more reliable diagnostic methods used as a reference standard for detection of microorganisms. In *Coxiella*, culturing demands growth and requires biosafety level 3 facilities in addition to the cost and impractical use on large samples. Until recently, investigation of *C. burnetii* infection in domestic animals was hampered by the absence of inexpensive and a high sensitive and specific technique (Chosewood & Wilson, 2009; Porter *et al.,* 2011; Agerholm, 2013). PCR assays have become a more useful tool for detection of *C. burnetii* DNA in acute and chronic infections, and to identify the carrier animals (Fretz *et al.,* 2007; Nicollet & Valognes, 2007; Muskens *et al.,* 2011). In this era of rapid and inexpensive sequencing, genome sequence comparisons often reveal many polymorphisms that can be used to develop new assays for increased discrimination among samples and for better definition of phylogenetic relatedness (Pereira *et al.,* 2008; Li *et al.,* 2009).

In last decade, *Coxiella* was reported to be endemic in most countries in the Middle East, and in different animal species (Jaff & Wilson, 2016; Eldin *et al.,* 2017). In Iraq, no nationwide studies are performed to detect the true prevalence of *Coxiella* among cattle. Hence, the present study aimed to identify *C. burnetii* in milk samples of lactating cows using the molecular PCR assay, to isolate the organism into the SPF-ECE, and to perform phylogenetic analysis of inoculated positive samples. Association of risk factors (region, period, age, and breed) to the PCR-positivity was also targeted.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the College of Veterinary Medicine, University of Baghdad, Iraq. Milk samples were collected and tested without any cost from their owners.

Samples collection

A total of 130 lactating cows were selected randomly from different areas (Al-Suwaira, Al-Aziziyah, Al-Numaniyah, Al-Kut, Badra and Al-Hai) in Wasit province, Iraq, during November 2018 to May 2019. Fifty mL of milk were collected from each animal under aseptic conditions into sterile disposable plastic containers. The samples of milk were transported to the laboratory in a cooled plastic ice-box, then divided into 2 parts (one for DNA

extraction and other for inoculation) using plastic tubes and kept frozen at –20 ºC.

Molecular PCR assay

All tubes of milk samples were thawed firstly in water bath (Poly-Science, USA) at 37 ºC and shaken vigorously by vortex (Scientific Industries, USA) to ensure that the sample was mixed well. According to manufacturer's instructions (Intron, Korea), the protocol (A) was followed. Nanodrop (Thermo-Scientific, USA) was used to evaluate the purity and concentration of extracted DNA. Targeting three genes; the primers of *16S rRNA* [(F: 5′-AGTACGGCCGCAAGGTTAAA-3′) and (*R*: 5′-CTCCAATC CGGACTACGAGC-3′)] (NCBI:NR_104916.1), *IS1111a transposase*, [(F:5′-GATGGGTATCCC AACGCAGT-3′) and (R:5′-ACTGCCGG GAACGATGAAAT-3′)] (NCBI: MG385669.1), and *htpB* [(F:5′-GGCAAT CACCAATAAGG GCCG-3′) and (R:5′-GCGGGTGATGGTACCACAACA-3′)] (To *et al.,* 1996), were used to amplify *C. burnetii* DNA at 425 bp, 674 bp and 501 bp respectively. PCR mastermix of each primer was prepared in a ready to use AccuPower PCR-PreMix kit (Bioneer, South Korea) at a final volume of 20 µL. PCR reaction for each primer was performed in Thermocycler (Bio-Rad, USA) under optimised conditions (Table 1).

Ten µL of each amplimer was analysed in 2% agarose gel targeting three genes, using 100–2000 bp of DNA marker (Qiagen, Germany). Agarose was stained with 1 µg/mL ethidium bromide (Biotech, Canada), and electrophoresis was carried out (100 V, 80 mA, 1 h). Resultant DNA fragments were visualised under a ultraviolet transilluminator (Clinx Science, China).

Isolation of C. burnetii using SPF-ECE

To isolate *C. burnetii*, the PCR-positive milk sampleswere inoculated into SPF-ECEs under strict sanitary conditions following previously described protocols (Ho *et al.,* 1995; Cooper, 2011; Kılıç *et al.,* 2016) with some modification. The steps were as followed: 2 mL of each milk sample were thawed in a water bath at 37 °C and centrifuged at 4000 rpm/15 min. Then, 200 µL supernatant was inoculated without further preparation into the yolk sac, and incubated for 6–7 days (37 °C, 60% humidity) with regular daily ovo-

Table 1. Thermocycler conditions for PCR amplification of targeted genes

Step	Gene								
	16S rRNA			*IS1111a transposase*			*htpB*		
	Temp.	Time	Cycle	Temp.	Time	Cycle	Temp.	Time	Cycle
Initial denaturation	95 °C	5 min	1	95 °C	2 min	1	95 °C	5min	1
Denaturation	95 °C	40 s	30	94 °C	30 s	35	94 °C	1 min	30
Annealing	56 °C	40 s		61 °C	30 s		56 °C	1 min	
Extension	72 °C	1 min		72 °C	1 min		72 °C	1 min	
Final extension	72 °C	7 min	1	72 °C	10 min	1	72 °C	5 min	1
Hold	4 °C	Forever		4 °C	Forever		4 °C	Forever	

scopy to ensure that all embryos were live. On the seventh day, yolk sac of living embryo was harvested, homogenised with three portions of purified buffered saline (PBS), centrifuged, and 0.5 mL of supernatants were re-inoculated. After third passage, all SPF-ECEs were examined macroscopically and microscopically to detect and evaluate any abnormalities. Finally, a volume of 0.5 mL of harvested yolk sac was homogenised in 9.5 mL PBS, mixed well and subjected for DNA extraction to be tested by PCR.

Phylogenetic analysis

To document the strains of *C. burnetii* local isolates, the extracted DNAs from positive SPF-ECEs were sent for sequencing in Bioneer Company (South Korea). Multiple Sequence Alignment Program and phylogenetic tree was constructed by MEGA-6 software; whereas the nucleotide sequence data were compared with several *C. burnetii*-16S rRNA sequences from GenBank to determine the homology.

Statistical analysis

All data were analysed by the computerised programs; Microsoft Office Excel v.16, Paint v. 6.1 and SPSS v. 23. At a level of $P<0.05$, odds ratio and chi-square (χ^2) values were calculated to estimate significant prevalence of *C. burnetii* between the groups of each risk factor (George & Mallery, 2011; Neyeloff *et al.,* 2012).

RESULTS

Targeting three genes (*16S rRNA, IS1111a transposase*, and *htpB*), the PCR assay revealed that 18.46% (24/130) lactating cows were positive for *C. burnetii* infection by the primers of *16S rRNA* gene only; whereas, no positive samples were observed with the primers of *IS1111a transposase*, and *htpB* genes (Table 2, Fig. 1).

Table 2. Total results of milk samples by PCR assay

Gene	Positive	Negative
16S rRNA	24/130 (18.46%)*	106
IS1111a transposase	0	130
htpB	0	130

* Significant variation in positive results of genes ($P<0.05$).

Concerning risk factors, the study revealed significant differences for the fac-

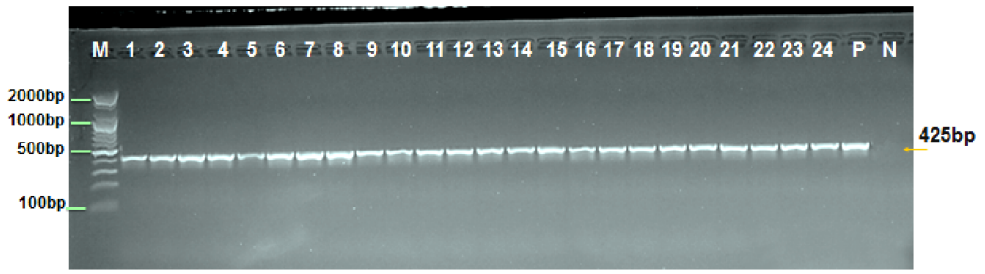

Fig. 1. Agarose gel electrophoresis for the 425 bp PCR product of positive milk samples with *C. burnetii.* M: DNA marker at 100–2000 bp; Lanes 1–24: Positive samples to *16S rRNA*; P: Positive control; N: Negative control.

Table 3. Association of positivity with the risk factors

Group	Positive/ Tested	Prevalence %	Risk	Odds ratio	
Region (district)					
Al-Suwaira	1/27	3.70%	0.037	0.02	P= 0.025
Al-Aziziyah	4/21	19.05%	0.19	0.24	
Al-Numaniyah	0/19	0%	0	0	
Al-Kut	7/22	31.82%	0.318	0.47	
Badra	9/21	42.86%	0.429	0.75	
Al-Hai	3/20	15%	0.15	0.18	
Period (month)					
November	3/7	42.86%	0.429	0.75	P= 0.082
December	2/8	25%	0.25	0.33	
January	5/19	29.41%	0.26	0.36	
February	3/17	17.65%	0.176	0.21	
March	4/32	12.5%	0.125	0.14	
April	4/26	15.38%	0.154	0.18	
May	3/21	14.29%	0.143	0.17	
Age (years)					
≤ 3-4	1/11	9.09%	0.091	0.1	P= 0.076
> 4-5	8/51	15.69%	0.157	0.19	
> 5	15/68	22.06%	0.221	0.28	
Breed					
Local	0/5	0%	0	0	P= 0.043
Crossbred	24/116	20.69%	0.207	0.26	
Pure	0/9	0%	0	0	

tor region: the highest prevalence of *C. burnetii* was showed in the district of Badra (42.86%), whereas the lowest prevalence was recorded in Al-Numaniyah and Al-Suwaira districts (P=0.025). For period factor, a high positivity was seen in November (42.86%) in comparison to other months, however these differences were not significant (P=0.082). For age as a factor, though the prevalence of *C. burnetii* was increased obviously with advanced age, no significant (P=0.076) correlation was showed between study age groups. Crossbred cows (20.69%) showed a statistically significantly higher positivity than local and pure breeds (P=0.043; Table 3).

Among inoculated SPF-ECEs, no alterations or lesions were observed among all positive and negative samples with absence of mortalities during the periods of inoculation. Of 24 yolk sac samples subjected to DNA extraction and PCR examination, the results revealed 12.5% positive samples (Fig. 2).

Genomic DNAs of local isolates were analysed phylogenetically targeting the *16S rRNA* gene. Sequencing results of local isolates were named IQ-No.1, IQ-No.2, and IQ-No.3 and recorded in NCBI under accession numbers MN121700.1, MN121701.1, and MN121702.1 respectively. Comparative analysis of *16S* rRNA nucleotide sequences of study strains with

the *C. burnetii* strains of GenBank database was constructed using the ClustalW Alignment of MEGA-6 software (Fig. 3).

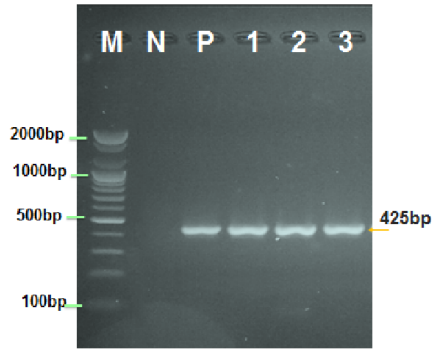

Fig. 2. Agarose gel electrophoresis of the 425 bp PCR product for total positive isolates of *C. burnetii.* M: DNA marker at 100–2000 bp; P: Positive control; N: Negative control; Lanes 1-3: Total positive samples to 16S rRNA which identified by inoculation of previous 24 positive samples into yolk sacs followed by DNA isolation and PCR analysis.

Phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in MEGA-6 software. Comparative identity of study local isolates with the global GenBank isolates reported 99% of similarity and 0.2–1.2% of genetic changes (Fig. 4). NCBI-BLAST Homology Sequence showed that there was a significant identity between the local *C. burnetii* strains and *C. burnetii* CB015LAMP (KU215908.1) of Italian strains (Table 4).

DISCUSSION

Coxiella burnetii is one of the most important pathogens of a worldwide distribution except New Zealand (Reichel *et al.,* 2018). In Iraq, there is a clear lack of information on the role of cattle in shedding of *C. burnetii*, particularly in lactating cows. Globally, dairy cows are implicated to play a great and important role in transmission of infection for humans through their secretions (indirect) or consumption of raw milk and dairy products (direct). Using PCR assay, 18.46% of study cows were positives for 16S rRNA gene of *C. burnetii*. This finding demonstrates that the prevalence of the pathogen in lactating cows was relatively high, meaning that the disease is endemic in cattle throughout study areas. In comparison to other countries, molecular reports detected that the percentage of bovine *C. burnetii* infection was 4.7% in Ethiopia (Yewondwosen *et al.,* 2018), 5.35% in India (Dhaka *et al.,* 2019), 7.43% in Saudi Arabia (Mohammed *et al.,* 2014), 8.7% in Hungary (Gyuranecz *et al.,* 2012), 15.9% in Iran (Esmaeili *et al.,* 2019), 31.54% in Poland (Szymańska-Czerwińska *et al.,* 2019), 48% in Turkey (Parin & Osman,

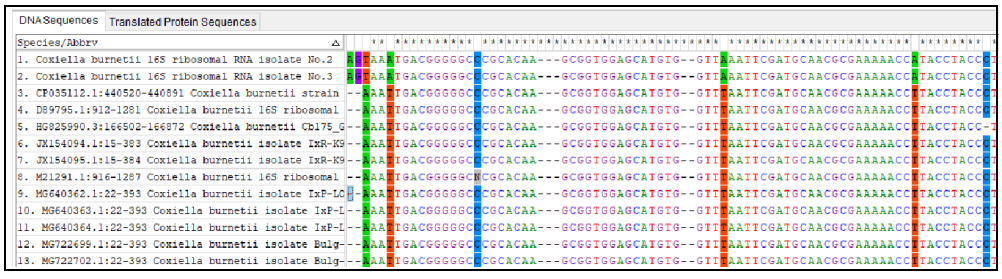

Fig. 3. Multiple sequence alignment analysis in partial *16S rRNA* gene of local *C. burnetii* IQ strains with the strains of NCBI-Blast *C. burnetii 16S rRNA* gene.

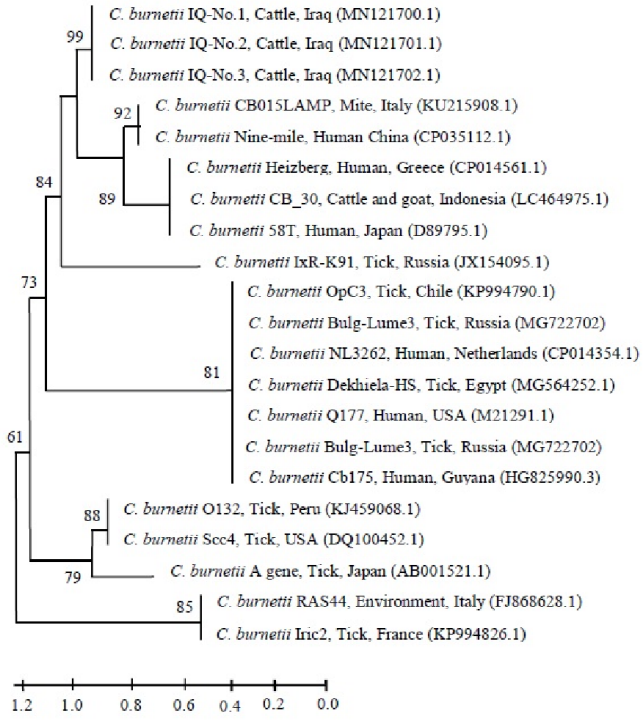

Fig. 4. Phylogenetic tree analysis based on 16S rRNA gene partial sequence in local *C. burnetii* IQ isolates in comparison to global Genbank-NCBI isolates.

Table 4. NCBI-BLAST Homology Sequence identity between local and NCBI-BLAST *C. burnetii* isolates

C. burnetii study strain	GenBank Accession No.	NCBI-BLAST Homology Sequence Identity (%)		
		NCBI BLAST	GenBank Accession No.	Identity (%)
C. burnetii IQ-No.1	MN121700.1	*C. burnetii* isolate CB015LAMP	KU215908.1	97.50%
C. burnetii IQ-No.2	MN121701.1	*C. burnetii* isolate CB015LAMP	KU215908.1	97.58%
C. burnetii IQ-No.3	MN121702.1	*C. burnetii* isolate CB015LAMP	KU215908.1	97.55%

2015), and 94.3% in United States (Kim *et al.,* 2005). However, the rate of infections in farm animals could be varied considerably between locations, countries, and with time (Radostits *et al.,* 2006). Also, diagnostic method and/or type of tested sample might affect the obtained results. Many studies suggested that the most common route for shedding of *Coxiella* is milk (Cerf & Condron, 2006; Guatteo *et al.,* 2007; Rodolakis *et al.,* 2007). Nonetheless, it should be taken into account that the percentage of infected animals may have been underesti-

mated due to occurrence of intermittent shedding of organisms and the possibility of shedding by several routes (Guatteo *et al.,* 2006; Rodolakis *et al.,* 2007).

The association of risk factors (region, period, age, and breed) to positive prevalence of *C. burnetii* reported significant variations ($P<0.05$) in results. Several reports showed that the prevalence rates of *C. burnetii* in animals varied widely depending on the geographical location, climatic conditions, and differences in farming practices or management systems (Psaroulaki *et al.,* 2014; Mobarez *et al.,* 2017). The persistence of animals in a geographically restricted area can reflect on the low prevalence of infection due to the restricted movement of animals which is important in prevention and control of infection (Nusinovici *et al.,* 2013). The recent global warming and climate change has possibly contributed to the widespread distribution of ticks and vector-borne disease as Q fever (Dantas-Torres, 2015). Evidence of age susceptibility to infection showed that the positivity was increased insignificantly with the advanced age. This finding could be interpreted by the facts that animals have more infection in advanced age due to the decrement of immunity, and infected animal remain carrier for all life. The influences of cattle breed on susceptibility to *Coxiella* infection were discussed in present study. However, the obtained findings could be explained with either the low number of local and purebred subjects selected for examination, high resistance of local breed to infection, and superior management conditions for purebred cattle, or to the potential effect of genetic variation in breed infectivity of cattle.

Isolation of *C. burnetii* can be achieved from a wide range of clinical samples; however, it requires specialised laboratories with biosafety level 3, cell culture facilities, and is time consuming. In addition, this organism does not grow easily within most standard tissue cultures, and isolation is not used routinely for diagnosis of Q fever (Dalton *et al.,* 2014; Yewondwosen *et al.,* 2018). Full absence of macroscopic and microscopic lesions and alterations in positive and negative SPF-ECEs seen in this study might be ascribed to failure of *C. burnetii* to produce lesions or presence of minimal lesions. We suggested that the local strain might have no or low pathogenic action on SPF-ECEs. Also, considerable variations in the ability of *Coxiella* to create infection might be due to the effect of challenge dose, in addition to the route and period of infection. In this study, the low positive numbers of inoculated SPF-ECEs could be attributed to many reasons such as that the SPF-ECE is not the suitable method for cultivation, presence of certain materials in milk that reduce or prevent the growth of organism in SPF-ECE, and existence of a low number of viable organisms. Omsland *et al.* (2009) summarised that *C. burnetii* was characterised by inability to propagate under axenic (host cell-free) culture conditions that imposes severe experimental constraints, and suggested a new developed complex medium [Acidified Citrate Cysteine Medium (ACCM)]. Other reasons include failure of the organisms to grow and errors during inoculation, incubation, and harvesting steps. However, successful isolation of *C. burnetii* in SPF-ECEs with application of PCR as a confirmative method provided further evidence that the organism is present in raw milk of study cows. These findings indicate that the people as well as animals were exposed efficiently to pathogenic agent that is deemed as one of the most potential agent for bioterrorism due to its

ability for survival in environment, transmission, and very low infectious dose (Oyston & Davies, 2011; Brooke *et al.,* 2013).

Phylogenetic analysis and the Homology sequence identity reported that the study local isolates had high similarities to global *C. burnetii* isolates existing in NCBI-Genbank. Based on *16S rRNA* gene sequence, phylogenetic analysis has placed *Coxiella* along with *Legionella*, *Francisella*, and *Rickettsiella* in the Gammaproteobacteria class (McLaughlin *et al.,* 2017). *C. burnetii*, as the single species within the *Coxiella* genus, has a particular importance due to its complexity for culturing and restrictions of the agent due to the fact that the collections of this organism are small and rare, making it more important to rely upon existing work in order to facilitate inter-laboratory comparisons among the collections that are available (McQuiston & Childs, 2002; Hornstra *et al.,* 2011). Such comparisons will lead to a better understanding of the distribution of this pathogen historically, at present, and in the future (Hornstra *et al.,* 2011). Consequently, homology of the sequences obtained from three inoculated *C. burnetii* strains in this study, is closely related to several strains isolated from cattle, ticks, and mites. Genotypic characterisation of *C. burnetii* strains is vital to trace the source/s of an outbreak, to determine genotypes circulating in a population, and to establish the potential connection between genotypes and virulence of strains (Szymańska-Czerwińska *et al.,* 2019).

CONCLUSION

For our knowledge, this is the first study in Iraq directed toward detection, isolation and molecular phylogenetic confirmation of local *C. burnetii* isolates. The findings of present study concluded that cattle livestock in Iraq (particularly in study areas) shedding *C. burnetii* actively in their milk. Diagnostic protocol described here can serve as a bridge between culture, PCR and phylogeny. Nonetheless, the percentage of positives reported in this study does not represent cattle herds in Iraq as the sample size was low, and non-lactating cattle (males, calves, and dry cows) were not involved.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad/Iraq, for providing the necessary facilities for this study. The authors did not receive any funds for this study.

REFERENCES

Agerholm, J. S., 2013. *Coxiella burnetii* associated reproductive disorders in domestic animals – a critical review. *Acta Veterinaria Scandinavica*, **55**, 1–11.

Angelakis, E. & D. Raoult, 2011. Emergence of Q fever. *Iranian Journal of Public Health*, **40**, 1–13.

Bolanos-Rivero, M., C. Carranza-Rodríguez, N. F. Rodríguez, C. Gutiérrez & J. L. Pérez-Arellano, 2017. Detection of *Coxiella burnetii* DNA in peridomestic and wild animals and ticks in an endemic region (Canary Islands, Spain). *Vector-Borne and Zoonotic Diseases*, **17**, 630–634.

Brooke, R. J., M. E. Kretzschmar, N. T. Mutters & P. F. Teunis, 2013. Human dose response relation for airborne exposure to *Coxiella burnetii*. *BMC infectious diseases*, **13**, 1–8.

Cerf, O. & R. Condron, 2006. *Coxiella burnetii* and milk pasteurization: An early application of the precautionary princi-

ple? *Epidemiology & Infection*, **134**, 946–951.

Chosewood, L. C. & D. E. Wilson, 2009. Rickettsial agents. *In: Biosafety in Microbiological and Biomedical Laboratories*, 5th edn, Centers for Disease Control and Prevention, National Institutes of Health, Georgia, pp. 195–199.

Cooper, A. E., 2011. Identification of potential reservoirs of Q fever in Queensland, Australia. Doctoral dissertation, James Cook University, Queensland, Australia, pp. 57–58.

Dalton, H. R., J. Dreier, G. Rink, A. Hecker, K. Janetzko, D. Juhl & M. Ziemann, 2014. *Coxiella burnetii*-pathogenic agent of Q (query) fever. *Transfusion Medicine and Hemotherapy*, **41**, 60–72.

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. *International Journal for Parasitology: Parasites and Wildlife*, **4**, 452–461.

Dhaka, P., S. S. Malik, J. P. Yadav, M. Kumar, A. Baranwal, S. B. Barbuddhe & D. B. Rawool, 2019. Seroprevalence and molecular detection of coxiellosis among cattle and their human contacts in an organized dairy farm. *Journal of Infection and Public Health*, **12**, 190–194.

Eldin, C., C. Melenotte, O. Mediannikov, E. Ghigo, M. Million, S. Edouard & D. Raoult, 2017. From Q fever to *Coxiella burnetii* infection: A paradigm change. *Clinical Microbiology Reviews*, **30**, 115–190.

Esmaeili, S., A. M. Mobarez, M. Khalili, E. Mostafavi & P. Moradnejad, 2019. Molecular prevalence of *Coxiella burnetii* in milk in Iran: A systematic review and meta-analysis. *Tropical Animal Health and Production*, **51**, 1345–1355.

Fretz, R., W. Schaeren, M. Tanner & A. Baumgartner, 2007. Screening of various foodstuffs for occurrence of *Coxiella burnetii* in Switzerland. *International Journal of Food Microbiology*, **116**, 414–418.

George, D. & P. Mallery, 2011. SPSS for Windows step by step: A simple guide and reference 18.0 Update. Edited by Allyn & Bacon, pp. 35–79.

Guatteo, R., F. Beaudeau, M. Berri, A. Rodolakis, A. Joly & H. Seegers, 2006. Shedding routes of *Coxiella bur*netii in dairy cows: implications for detection and control. *Veterinary Research*, **37**, 827–833.

Guatteo, R., F. Beaudeau, A. Joly & H. Seegers, 2007. Assessing the within-herd prevalence of *Coxiella burnetii* milk-shedder cows using a real-time PCR applied to bulk tank milk. *Zoonoses and Public Health*, **54**, 191–194.

Guatteo, R., H. Seegers, A. F. Taurel, A. Joly & F. Beaudeau, 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. *Veterinary Microbiology*, **149**, 1–16.

Gyuranecz, M., B. Dénes, S. Hornok, P. Kovács, G. Horváth, V. Jurkovich & N. Vass, 2012. Prevalence of *Coxiella burnetii* in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. *Vector-Borne and Zoonotic Diseases*, **12**, 650–653.

Ho, T., K. K. Htwe, N. Yamasaki, G. Q. Zhang, M. Ogawa, T. Yamaguchi & K. Hirai, 1995. Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. *Microbiology and Immunology*, **39**, 663–671.

Honarmand, H., 2012. Q fever: An old but still a poorly understood disease. *Interdisciplinary Perspectives on Infectious Diseases*, **2012**, 1–8.

Hornstra, H. M., R. A. Priestley, S. M. Georgia, S. Kachur, D. N. Birdsell, R. Hilsabeck & P. Keim, 2011. Rapid typing of *Coxiella burnetii*. *PloS One*, **6**, 1–8.

Jaff, D. & P. Q. Wilson, 2016. Fever: A Neglected disease in the Middle East. *Journal of Health Systems*, **16**, 12–14.

Kılıç, A., H. Kalender, O. Koç, Ü. Kılınç, B. Irehan & M. Berri, 2016. Molecular investigation of *Coxiella burnetii* infections in aborted sheep in eastern Turkey. *Iranian Journal of Veterinary Research*, **17**, 41–44.

Kim, S. G., E. H. Kim, C. J. Lafferty & E. Dubovi, 2005. *Coxiella burnetii* in bulk tank milk samples, United States. *Emerging Infectious Diseases*, **11**, 619–621.

Li, W., D. Raoult & P. E. Fournier, 2009. Bacterial strain typing in the genomic era. *FEMS Microbiology Reviews*, **33**, 892–916.

McLaughlin, H. P., B. Cherney, J. R. Hakovirta, R. A. Priestley, A. Conley, A. Carter & D. Sue, 2017. Phylogenetic inference of *Coxiella burnetii* by 16S rRNA gene sequencing. *PloS One*, **12**, 1–18.

McQuiston, J. H. & J. E. Childs, 2002. Q fever in humans and animals in the United States. *Vector Borne and Zoonotic Diseases*, **2**, 179–191.

Mobarez, A. M., F. B. Amiri & S. Esmaeili, 2017. Seroprevalence of Q fever among human and animal in Iran; A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, **11**, 1–15.

Mohammed, O. B., A. A. Jarelnabi, R. S. Aljumaah, M. A. Alshaikh, A. O. Bakhiet, S. A. Omer & M. F. Hussein, 2014. *Coxiella burnetii*, the causative agent of Q fever in Saudi Arabia: Molecular detection from camel and other domestic livestock. *Asian Pacific Journal of Tropical Medicine*, **7**, 715–719.

Muskens, J., C. Van Maanen & M. H. Mars, 2011. Dairy cows with metritis: *Coxiella burnetii* test results in uterine, blood and bulk milk samples. *Veterinary Microbiology*, **147**, 186–189.

Neyeloff, J. L., S.C. Fuchs & L.B. Moreira, 2012. Meta-analyses and Forest plots using a microsoft excel spreadsheet: Step-by-step guide focusing on descriptive data analysis. *BMC Research Notes*, **5**, 1–6.

Nicollet, P. & A. Valognes, 2007. Current review of Q fever diagnosis in animals. *Bulletin de L'Académie Vétérinaire De France,* **160**, 289–295.

Nusinovici, S., T. Hoch, S. Widgren, A. Joly, A. Lindberg & F. Beaudeau, 2013. Relative contributions of neighbourhood and animal movements to *Coxiella burnetii* infection in dairy cattle herds. *Geospatial Health*, **8**, 471–477.

Omsland, A., D. C. Cockrell, D. Howe, E. R. Fischer, K. Virtaneva, D. E. Sturdevant & R. A. Heinzen, 2009. Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 4430–4434.

Oyston, P. C. F. & C. Davies, 2011. Q fever: The neglected biothreat agent. *Journal of Medical Microbiology*, **60**, 9–21.

Parin, U. & K. A. Osman, 2015. Detection of *Coxiella burnetii* prevalence in bovine, ovine and caprine herds. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, **62**, 177–181.

Pereira, F., J. Carneiro & A. Amorim, 2008. Identification of species with DNA-based technology: Current progress and challenges. *Recent Patents on DNA & Gene Sequences*, **2**, 187–200.

Porter, S. R., G. Czaplicki, J. Mainil, R. Guattéo & C. Saegerman, 2011. Q Fever: Current state of knowledge and perspectives of research of a neglected zoonosis. *International Journal of Microbiology*, **13**, 1–22.

Psaroulaki, A., D. Chochlakis, E. Angelakis, I. Ioannou & Y. Tselentis, 2014. *Coxiella burnetii* in wildlife and ticks in an endemic area. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **108**, 625–631.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff & P. D. Constable, 2006. Q-Fever. *In: Veterinary Medicine E-Book: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. Elsevier Health Sciences, Philadelphia, pp. 1468–1469.

Reichel, M. P., L. C. Wahl & F. I. Hill, 2018. Review of diagnostic procedures and approaches to infectious causes of reproductive failures of cattle in Australia and New Zealand. *Frontiers in Veterinary Science*, **5**, 1–15.

Rodolakis, A., M. Berri, C. Hechard, C. Caudron, A. Souriau, C. C. Bodier & J. P. Vadet, 2007. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. *Journal of Dairy Science*, **90**, 5352–5360.

Szymańska-Czerwińska, M., A. Jodełko, K. Zaręba-Marchewka & K. Niemczuk, 2019. Shedding and genetic diversity of *Coxiella burnetii* in Polish dairy cattle. *PloS One*, **14**, 1–16.

To, H., N. Kako, G. Q. Zhang, H. Otsuka, M. Ogawa, O. Ochiai & M. Akiyama, 1996. Q fever pneumonia in children in Japan. *Journal of Clinical Microbiology*, **34**, 647–651.

Yewondwosen, W., S. Tesfaye, B. Asfaw & V. Afework, 2018. Seroprevalence and molecular epidemiology of *Coxiella burnetii* in Northern Ethiopia. *Clinical Microbiology*, **7**, 52–64.

Paper received 12.12.2019; accepted for publication 20.01.2020

Correspondence:

Hasanain A. J. Gharban
Department of Internal and Preventive Veterinary Medicine,
College of Veterinary Medicine,
University of Baghdad, Baghdad, Iraq,
e-mail: hghirban@ uowasit.edu.iq