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Serological and Molecular Phylogenetic Confirmation of *Coxiella burnetii* in Milk of Awassi Sheep, Iraq

Wasan A. Gharbi
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq
Email: wasan@covm.uobaghdad.edu.iq

Abstract
Background: *Coxiella burnetii* is a worldwide ubiquitous bacterial pathogen which is able to infect several animal species as well as human resulting in asymptomatic dangerous disease, Q fever. **Aim:** To detect *C. burnetii* IgG-antibodies and DNA in milk samples of Awassi sheep and to document of local strains in the National Centre for Biotechnology Information (NCBI) for first time in Iraq. **Methods:** A total of 182 milk samples were collected from Awassi sheep at different areas of Baghdad province (Iraq), and subjected to ELISA to detect of specific IgG-antibodies (Phase I and II); and to PCR amplification targeting 16SrRNA gene. **Results:** Positive findings were 37.91% by ELISA and 3.85% by PCR. Concerning to titers of seropositive sheep, significant increases were seen in low level category. Three local *C. burnetii* strains reported in Genbank-NCBI under an accession numbers of MN900570.1, MN900571.1, and MN900572.1. **Conclusion:** For our knowledge, this s first Iraqi report confirmed that Awassi sheep are shedding of *C. burnetii* in their milk.
Keywords: *Coxiella burnetii*, Awassi sheep, Milk, Sequence, Iraq.

Introduction
Coxiella burnetii is an obligate intracellular Gram-negative bacterium causing of Q fever, a zoonosis that concerns public health throughout the world (Elhaig *et al.*, 2018). Previously, *C. burnetii* is classified as a Rickettsiales pathogen (Philip, 1956), but then, the organism is reclassified under the order of Legionellales based on 16S rRNA gene (Weisburg *et al.*, 1989). Under environmental conditions, the spores of *C. burnetii* are highly infectious, stable, and can spread easily by many routes for several kilometers away from the primary infection source (Tissot-Dupont *et al.*, 2004). Mammals, birds, arthropods mainly ticks can be infected; however, domestic ruminants such as cattle, sheep and goats are the main reservoirs for other animals and humans (Fard *et al.*, 2011). The organism is present in high concentrations in the placental and fetal fluids, and subsequent in vaginal secretions, and excreted with urine and feces (Contreras *et al.*, 2018). Also, *Coxiella* can present in milk of infected animals variably based on period and duration but can persist at least two years post infection (Radostits *et al.*, 2006). However, transmission of infection occurs directly by inhalation or indirectly through the contaminated environments (Plummer *et al.*, 2018).

In domestic ruminants, though *C. burnetii* infection is mainly asymptomatic, reproductive disorders including abortion at late stage of gestation, stillbirth, and delivery of weak and unviable newborns can occur (Longbottom *et al.*, 2013). Worldwide, many human outbreaks are generally associated with proximity to sheep and goats particularly during parturition or abortion during dry and windy weather (Khaled *et al.*, 2016). Since then, important work has been made to prevent the spread of *C. burnetii* from sheep or goats to humans; however, these efforts have been hindered by the limitation of effective veterinary interventions in these small ruminants (Cruz *et al.*, 2018). In fact, the few longitudinal follow-up studies performed with sheep have

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been particularly valuable in providing descriptive data on individual shedding patterns. In order to investigate the prevalence of C. burnetii infection, several serological methods are developed and conducted to identify specific antibodies (Van der Hoek *et al.*, 2011).

Enzyme-linked immunosorbent assay (ELISA) is the most commercially available test for detection of IgG antibodies against *C. burnetii* phase I and phase II, as it high in sensitivity, specificity, safety, and can performed easily when compared to other serological methods (Van den Brom *et al.*, 2015). It is great importance to be able to trace back the source of infection and to characterize the strains of the pathogen present in certain areas both for epidemiological and public health reasons. Recently, the most reliable diagnostic tool is the DNA-based methods such as polymerase chain reaction (PCR) which have more capability, sensitivity and specificity for detection of organism in different samples than culture techniques (Fard *et al.*, 2011; Guidi *et al.*, 2017).

To date, no Iraqi study has documented that sheep can demonstrate undetectable levels for shedding of *C. burnetii* in their milk. Hence, the present study was aimed to provide data on seroprevalence of Coxiella antibodies in milk samples of sheep using ELISA, confirm shedding of organism by PCR, and phylogenetic analysis of local strain to document them in the National Center for Biotechnology Information (NCBI) for the first time in Iraq.

Materials and methods

Samples

A total of 182 adult female Awassi sheep selected randomly from different areas at Baghdad province (Iraq) were subjected for the present study during June to December/2018. From each animal, approximately 50ml of raw milk was collected directly from udder under aseptic conditions into plastic containers. At laboratory, the samples of milk were shacked vigorously, and divided into two equal parts, one subjected for centrifugation at 4000rpm/30 minutes, pipetting of supernatants under the skim to be kept into numbered eppendorf frozen until be used for serology; whereas, the second part frozen directly at -20°C for molecular testing.

Serology

Following the manufacturer's instruction (Applied Biosystems, ThermoFisher Scientific, USA), the PrioCHECK™ Ruminant Q fever Ab Plate Kit (indirect ELISA) was used for detection of specific IgG (phase I and phase II) antibodies against *C. burnetii*. After preparation of samples and reagents, the test was performed and the resultants were read at a wavelength of 450nm using the automated microplate reader (MICROTECH, USA). For each sample, the S/P ratio and titer were calculated as following:

$$S/P = (OD_{Sample} - OD_{NC}) / (OD_{PC} - OD_{NC})$$

$$Titers = S/P \times 100$$

Then, the test was validated if $OD_{PC} > 0.4$ and $OD_{PC}/OD_{NC} > 2$; and the results were interpreted as following:

Result	Interpretation
Titer ≤ 40	Negative
40 < Titer ≤ 100	Positive + (Low)
100 < Titer ≤ 200	Positive ++ (Intermediate)
Titer > 200	Positive +++ (Severe)

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Note: S/P = Sample/Positivity, OD= Optical density, PC= Positive control, NC= Negative control

Molecular assay

According to manufacturer's instruction of G-spin ^{TM} Total DNA Extraction Kit (INTRON Biotechnology, South Korea), DNA was extracted from the milk samples of study animals. The concentration and purity of extracted DNA were checked using the system of Nanodrop (Therm Scientific, UK) at an absorbance of A260/1280nm. Targeting 16SrRNA gene, one set of primers [(F: 5´AGT ACG GCC GCA AGG TTA AA´3) and (R: 5´CTC CAA TCC GGA CTA CGA GC´3)] designed (Gharban and Yousif, 2020) and supplied (Macrogen, South Korea) was utilized for detection of *C. burnetii*. A ready to use AccuPower PCR-Premix Kit (Bioneer, South Korea) was used for preparation of a Mastermix at a final volume of 20µl [5µl DNA template, 1µl Forward primer, 1µl Reverse primer, and 13µl Free-nuclease water). PCR reaction was performed in Thermal Cycler (Bio-Rad, USA) to amplify the primers of target gene under the following optimized conditions: initial denaturation (95°C/5min.)/1 cycle; denaturation (95°C/40sec.), annealing (56°C/40sec.), and extension (72°C/1min.)/1 cycle; final extension (72°C/7min.)/1 cycle; and hold at 4°C forever. In 1.5% agarose gel-electrophoresis stained with ethidium promide, resultants of PCR products were examined at 100volt and 80mA, for 1 hour, to detect of positive samples at 425bp using of an ultraviolet illuminator.

Phylogenetic analysis

Of PCR-positive samples, three samples were sent for sequencing in the Bionner Company (South Korea). Received data by private emails was analyzed using the software of Multiple Sequence Alignment Program; whereas, the program of MEGA-6 was used to to construct of the phylogenetic tree. Nucleotide sequence data of each local *C. burnetii* strain were reported in the National Centers for Biotechnology Information (NCBI) and compared with global Genbank-NCBI *C. burnetii* strains/isolates to detect of identity.

Statistical analysis

The study results were collected and arranged initially in the program of Microsoft Office Excel (version ^{2007}) and analyzed by IBM/SPSS (version ^{17}). Chi-square (x^2) test was used to detect significant differences between the positive findings of ELISA and PCR assay, and the titer levels of positive ELISA results at (P<0.05), (Released, 2008).

Results

Among 182 milk samples, the results showed that there were 37.91% (69/182) and 3.85% (7/182) positive sheep by ELISA and PCR respectively (Table 1, Figure 1). Concerning to seropositive samples by ELISA, the levels of titers were revealed on significant differences (P<0.05) between their values; however, significant increases (P<0.05) were detected in a category of low titers (42.03) in comparison with intermediate (34.78) and high titers (23.19), (Table 2).

Table (1): Total results of both diagnostic assays (ELISA and PCR) for a totally 182 study sheep

Total No.	Diagnostic assay	Positives	Negatives
	ELISA	69 (37.91%)	113

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182	PCR	7 (3.85%)	178

Significance * (*P<0.05*)

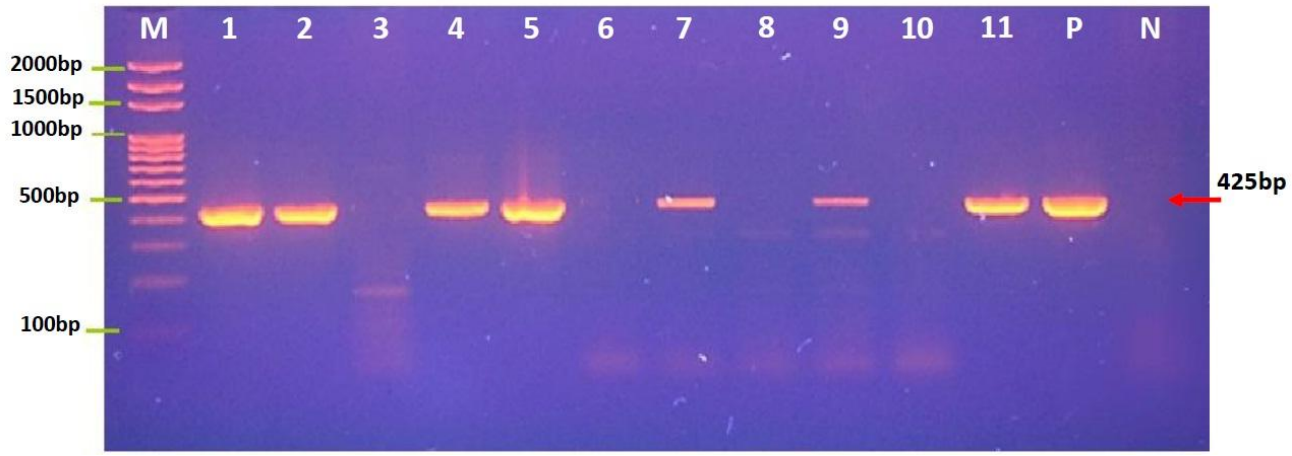


Figure (1): Agarose gel electrophoresis for PCR products of total positive milk samples with *C. burnetii* at 425bp.

M: DNA marker at 100-2000bp; Lanes (1, 2, 4, 5, 7, 9, and 11): Total positive samples to 16SrRNA; Lanes (3, 6, 8, and 10): Negative samples; P: Positive control; N: Negative control

Table (2): Interpretation results for the titers of seropositive milk samples

Interpretation result	No.	%
Low titers	29	42.03 **
Intermediate titers	24	34.78 *
High titers	16	23.19
Total	**69**	**37.91**

Significance * (*P<0.05*)

Genomic DNAs of three PCR positive samples were sequenced and reported in NCBI under the accession numbers of IRAQ/SHEEP-No.1, IRAQ/SHEEP-No.2, and IRAQ/SHEEP-No.3. Multiple sequence alignment for partial analysis of 16SrRNA gene in local *C. burnetii* strains which constructed based on NCBI-BLAST analysis was showed that there were a nucleotide similarity and mutations in targeted gene (Figures 2, 3, 4).

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Score	Expect	Identities	Gaps	Strand
536 bits (290)	9e-157	312/323 (97%)	0/323 (0%)	Plus / Plus

```
Query 1   ATGCAACGCGAAAAACCTTACCTACCCTTGACATCCTCGGAACTTGTCAGAGATGATTTG  60
Sbjct 1   ................A.....................T.A.............  60
Query 61  GTGCCTTCGGGAACCGAGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT  120
Sbjct 61  ....G.....................................................  120
Query 121 GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCGAGTCAAGTCG  180
Sbjct 121 .......C.......................A...A.....................  180
Query 181 GGAACTCTAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGATGTCAAGTCAT  240
Sbjct 181 .....C.............G........T.............................  240
Query 241 CATGGCCCTTACGGGTAGGGCTACACACGTGCTACAATGGGCAGTACAAAGGGTTGCCAA  300
Sbjct 241 ............................T.............................  300
Query 301 GCCGCGAGGTGGAGCTAATCCCA  323
Sbjct 301 .......................  323
```

Figure (2): Partial sequence of 16SrRNA gene of *C. burnetii* local strain (IRAQ/SHEEP-No. 1)

Score	Expect	Identities	Gaps	Strand
542 bits(293)	2e-158	313/323(97%)	0/323(0%)	Plus/Plus

```
Query 1   ATGCAACGCGAAAAACCTTACCTACCCTTGACATCCTCGGAACTTGTCAGAGATGATTTG  60
Sbjct 14  ................A.....................T.A.............  73
Query 61  GTGCCTTCGGGAACCGAGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT  120
Sbjct 74  ....G.....................................................  133
Query 121 GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCGAGTCAAGTCG  180
Sbjct 134 .......C.......................A.....................  193
Query 181 GGAACTCTAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGATGTCAAGTCAT  240
Sbjct 194 .....C.............G........T.............................  253
Query 241 CATGGCCCTTACGGGTAGGGCTACACACGTGCTACAATGGGCAGTACAAAGGGTTGCCAA  300
Sbjct 254 ............................A.............................  313
Query 301 GCCGCGAGGTGGAGCTAATCCCA  323
Sbjct 314 .......................  336
```

Figure (3): Partial sequence of 16SrRNA gene of *C. burnetii* local strain (IRAQ/SHEEP-No. 2)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14172 - 14184
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Score	Expect	Identities	Gaps	Strand
531 bits(287)	4e-155	307/317(97%)	0/317(0%)	Plus/Plus

```
Query 1   ATGCAACGCGAAAAACCTTACCTACCCTTGACATCCTCGGAACTTGTCAGAGATGATTTG  60
Sbjct 7   ................A.....................T.A.............  66
Query 61  GTGCCTTCGGGAACCGAGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT  120
Sbjct 67  ....G.....................................................  126
Query 121 GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCGAGTCAAGTCG  180
Sbjct 127 .......C.........................A.........................  186
Query 181 GGAACTCTAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGATGTCAAGTCAT  240
Sbjct 187 .....C.............G........T.............................  246
Query 241 CATGGCCCTTACGGGTAGGGCTACACACGTGCTACAATGGGCAGTACAAAGGGTTGCCAA  300
Sbjct 247 ...............................A.........................  306
Query 301 GCCGCGAGGTGGAGCTA  317
Sbjct 307 .................  323
```

Figure (4): Partial sequence of 16SrRNA gene of *C. burnetii* local strain (IRAQ/SHEEP-No. 3)

Phylogenetic tree that constructed using of Mega-6 software and comparative identity of study local isolates with the global Genbank-NCBI isolates were showed a significant genetic variation (96.90-95.38) and total genetic changes at 0.005%. Based on NCBI-BLAST Homology Sequences, statistical analysis showed that there were significant identities between the local *C. burnetii strains* and many Genbank-NCBI strains/isolates (Table 3, Figure 5).

Table (3): Comparative identity between the local *C. burnetii* strains and global Genbank-NCBI strain/isolate

Strain/Isolate	Identity (%) of local (IRAQ/SHEEP) strains		
	No.1	No.2	No.3
Coxiella burnetii isolate IRAQ/SHEEP-No.1 16SrRNA gene, partial sequence	-	99.09	99.05
Coxiella burnetii isolate IRAQ/SHEEP-No.2 16SrRNA gene, partial sequence	99.09	-	100.00
Coxiella burnetii isolate IRAQ/SHEEP-No.3 16SrRNA gene, partial sequence	99.05	100.00	-
Coxiella burnetii isolate *Hyalomma asiaticum* 16S ribosomal RNA gene, partial sequence	96.62	96.74	96.90
Uncultured *Coxiella* sp. Clone JGC06 16S ribosomal RNA gene, partial	96.62	96.74	96.90

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14172 - 14184
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sequence			
Coxiella burnetii CB-30 gene for 16S ribosomal RNA, partial sequence	96.62	96.74	96.90
Coxiella burnetii strain RSA439 chromosome, complete genome	96.62	96.74	96.90
Coxiella burnetii strain nine mile phase II chromosome, complete genome	96.62	96.74	96.90
Coxiella burnetii strain 2014-PE-15890 chromosome, complete genome	96.62	96.74	96.90
Coxiella burnetii isolate Bulg-Lume 1 16S ribosomal RNA gene, partial sequence	96.62	96.74	96.90
Coxiella burnetii str. Schperling chromosome	96.62	96.74	96.90
Coxiella burnetii strain Heizberg chromosome	96.62	96.74	96.90
Coxiella burnetii strain 18430 chromosome	96.62	96.74	96.90
Coxiella burnetii strain 701CbB1 chromosome	96.62	96.74	96.90
Coxiella burnetii strain14160-001 chromosome	96.62	96.74	96.90
Coxiella burnetii strain KZQ2 16S ribosomal RNA gene, partial sequence	96.62	96.74	96.90
Uncultured *Coxiella* sp. Partial 16S rRNA gene, isolate S014	96.62	96.74	96.90
Coxiella burnetii ´ MSU Goat Q177´ chromosome, complete genome	96.62	96.74	96.90
Coxiella burnetii str. Namibia genome	96.62	96.74	96.90
Coxiella burnetii strain Ammassalik 16S ribosomal RNA gene, partial sequence	96.62	96.74	96.90
Coxiella burnetii isolate IxR-K92 16S ribosomal RNA gene, partial sequence	96.59	96.72	96.90
Uncultured *Coxiella* sp. Clone Ocap1 16S ribosomal RNA gene, partial sequence	95.69	95.85	95.98
Coxiella endosymbiont of Ornithodoros brasiliensis isolate Obr01 16S ribosomal RNA gene, partial sequence	95.38	95.55	95.67
Coxiella sp. 64e2 16S ribosomal RNA gene, partial sequence	95.38	95.55	95.67

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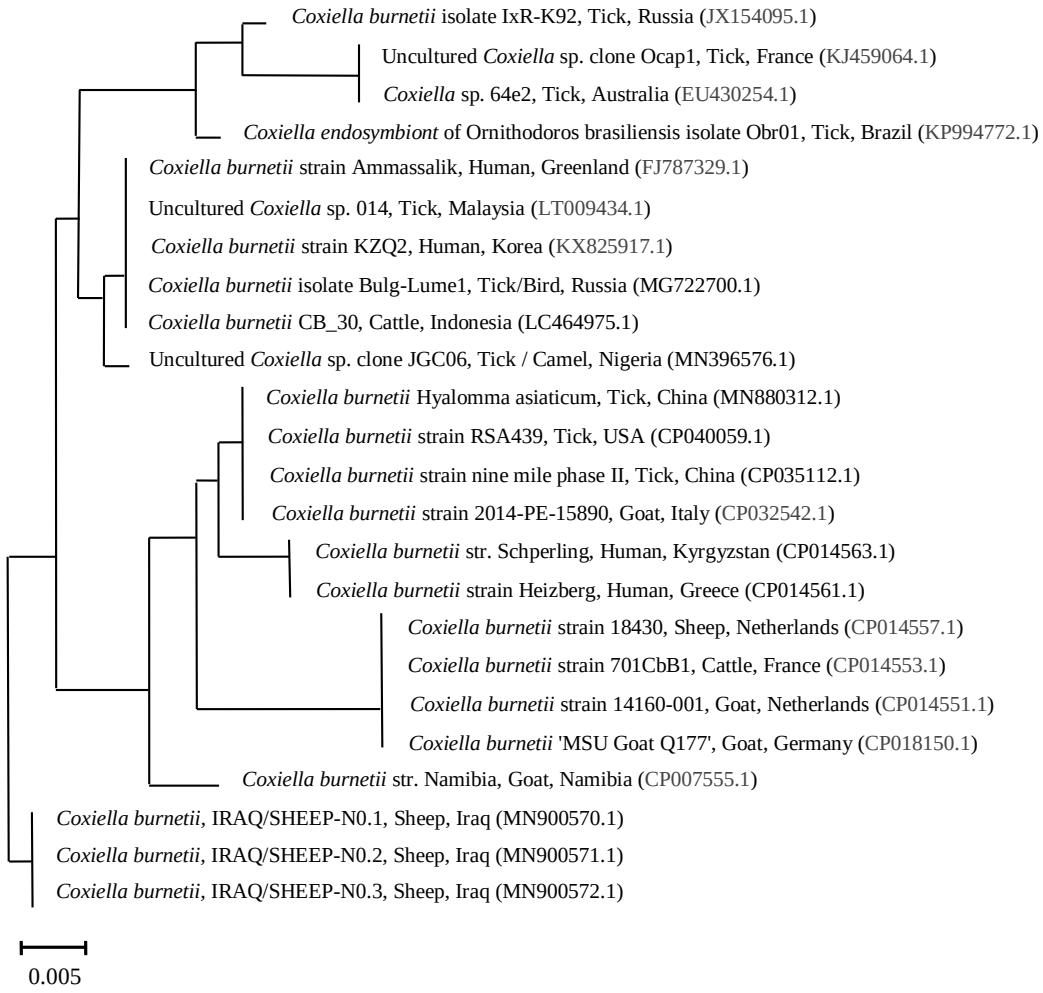


Figure (5): Phylogenetic tree analysis based on 16SrRNA gene partial sequence of local *C. burnetii* isolates as a confirmative genetic detection

Discussion

The shedding of *C. burnetii* in sheep and other animals is a major issue for public health, since diagnosing of infection according to the clinical symptoms is difficult as most cases occur sub-clinically, and studies on frequency of infection in ruminants are uncommon (Radostits *et al.*, 2006; Guatteo *et al.*, 2007). Herein, the knowledge is important to determine the risk of transmission between animals and from animals to humans. Testing of bulk milk tank samples for C. burnetii is relatively new in sheep, and can be used for measuring of serological responses as well as for confirmation of *C. burnetii* shedding. In the present study, individual raw milk was collected to assess the prevalence and shedding of *C. burnetii* in sheep was revealed on positive findings by both serological and molecular applied assays. In addition, the study showed that there were significant increases in serological ELISA positive results (37.91%) in comparison to molecular PCR (3.85%) findings. The latter was in compatible with that detected previously (Saglam and Sahin, 2016; Ullah *et al.*, 2019), and conflicted with others (PARIN and Osman, 2015; Gangoliya *et al.*, 2019). In comparison to other studies, the prevalence of *C. burnetii* in sheep was 5% in USA (Oliveira *et al.*, 2017), 6% in Hungary (Gyuranecz *et al.*, 2012), 8% in Greece (Filioussis *et al.*, 2017), 24.66% in Iran (Mobarez *et al.*, 2017), 28% in Germany (Hilbert

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et al., 2012), 33% in Egypt (Nahed and Khaled, 2012), 36% in Slovenia (Knap *et al.*, 2019), 52.1% in Jordan (Obaidat and Kersh, 2017), and 56.84% in Tunisia (License *et al.*, 2015).

The lower titers of antibodies among seropositive animals detected in this study might be due to that these animals either at an early cute or at late stage of infection or at late stage of infection. Emery *et al.* (2012) showed that the low positive or negative results showed with ELISA may caused by presence of IgM antibodies. Roest *et al.* (2013) refer to that the duration of antibody response and the role of cellular immunity during Coxiella's infection are not exactly determined, and concluded that the immune response can last for several months or even up to years. However, Joulie *et al.* 2017; Najim *et al.* 2020) found that antibody levels in milk of dairy ewes were indicative for assessing within flock-seroprevalence; and suggested analyzing of serum and milk samples to confirm the observed status and to identify potential discordant test results. Moreover, antigen used for coating of ELISA kit used in this study was prepared from *C. burnetii* strain that responsible for abortion in sheep; therefore, the kit exhibits greater sensitivity for detecting of animals shedding the organism than Nine Mile ELISA kits.

To determine which animals represent a current risk for transmission and actively shed of bacterium, Khalifa *et al.* (2016) refer to that some animals may seroconvert without shedding or remain seropositive long after the acute infection, and some others may pose a risk for infection prior to the development of antibodies by shedding the bacteria. The using of ELISA and PCR together can provide a more reliable, reproducible, and discriminatory power as they do not required cultivation of organism which not only is it difficult to achieve but also obliges Biosafety Level 3 facilities (Kowalczewska *et al.*, 2011; Eldin *et al.*, 2017). Many studies were found that PCR assay is a highly specific and sensitive for direct detection of *C. burnetii* in several samples from different animals (Oliveira *et al.*, 2017; Canevari *et al.*, 2018). Rodolakis *et al.* (2007) reported differences between the prevalence of *C. burnetii* in cattle, sheep and goats and linked a part of the variation to the fact that there are different routes of shedding of the organism in these animal species. Mohammed *et al.* (2014) detected that the main routes of shedding by sheep was feces and vaginal discharges but not milk. This might explain why there were low detectable positives by PCR in current study; however; high efficiency of PCR to detect one *C. burnetii* cell in 1 ml of raw milk or unpasteurized milk product can overcome this obstacle. In addition, though shedding of *C. burnetii* could be intermittent, different between species, and vary between the types of samples, concomitant analysis of various and different types of samples from the same animal might increase the probability of finding an infected animal with *C. burnetii* (Contreras *et al.*, 2018; Ali et al., 2019). Worldwide, most human *C. burnetii* outbreaks were linked epidemiologically to domestic animals particularly sheep (Guatteo *et al.*, 2007). Given the large flock size, experimental evidence that *C. burnetii* can be shed for multiple parturitions, extremely high ratio of shedding from different routes to infectious dose, and environmental persistence of organism ongoing intra-flock transmission overtime (Oliveira *et al.*, 2017).

Molecular methods are increasingly used to characterize isolates and to determine relationship between isolates that cause disease in different contexts and geographical areas. In last two decades, the identification and typing of *C. burnetii* is achievable without access to bio-safety level-3 (BLV-3) contaminant. Although, there many genotyping methods are available worldwide, phylogenetic analysis based on 16SrRNA gene were showed a high efficacy in detection and confirmation of the local strains of *C. burnetii* in this study. The finding of present

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study was agreed with that detected previously by McLaughlin *et al.* (2017) who demonstrated that 16SrRNA sequencing does indeed differentiate *C. burnetii* strains and can be used as a simple, unambiguous, and transferrable genotyping approach. Comparative identity between three local and global Genbank-NCBI *C. burnetii* demonstrated high significant identities with many *C. burnetii* strains isolated mainly from ticks, goats, cattle, sheep, human, and birds. According to these results, the study suggested that ticks could be the main source of infection, and the local strains of Coxiella circulated in Iraqi sheep might be widespread globally. Also, bird migration, and importation of many animal species from different countries with lack of national biosecurity, may play a role in those findings.

Conclusion

The present study seen that sheep may act as a reservoir of *C. burnetii* pathogen, and humans as well as animals in Baghdad province are at risk of exposure to infection. Also, raw milk of infected sheep is an important source for transmission of infection and contamination of environment. ELISA and PCR assays, when used together, can provide more reliable data that will be improve the understanding of *C. burnetii* circulation in infected flocks. Furthermore studies should be increased for ruminant husbandry areas in Baghdad or other Iraqi provinces to interpret the results at different levels, understand the dynamics of shedding, and to establish a preliminary step for future epidemiological studies on animals and human. Certainly, much more work needs to be done and many more samples required to be examined in order to record as many different genotypes as possible to cover most of the country territory.

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Competing interests

There no conflict to interest.

References

1. Elhaig, M. M., Selim, A., Mandour, A. S., Schulz, C., & Hoffmann, B. (2018). Prevalence and molecular characterization of peste des petits ruminants virus from Ismailia and Suez, Northeastern Egypt, 2014–2016. *Small ruminant research*, *169*, 94-98.
2. Philip, C. B. (1956). Comments on the classification of the order Rickettsiales. *Canadian journal of microbiology*, *2*(3), 261-270.
3. Weisburg, W. G., Dobson, M. E., Samuel, J. E., Dasch, G. A., Mallavia, L. P., Baca, O., & Woese, C. R. (1989). Phylogenetic diversity of the Rickettsiae. *Journal of bacteriology*, *171*(8), 4202-4206.
4. Ali, A. F., Mohammed, Th. T. & Al-Bandar, L. K. 2019. Effect of adding different levels of Optifeed®, Vêo® Premium and Oleobiotec® to the diets as appetite stimulants in the production and physiological performance of Male broiler under heat stress conditions. Plant Archives, 19(1): 1491-1498.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14172 - 14184
Received 05 March 2021; Accepted 01 April 2021.

5. Tissot-Dupont, H., Amadei, M. A., Nezri, M., & Raoult, D. (2004). Wind in November, Q fever in December. *Emerging infectious diseases*, *10*(7), 1264.
6. Fard, S. N., & Khalili, M. O. H. A. M. M. A. D. (2011). PCR-detection of Coxiella burnetii in ticks collected from sheep and goats in southeast Iran. *Iranian journal of arthropod-borne diseases*, *5*(1), 1.
7. Contreras, V., Gonzalez, M., Alvarez, J., & Mattar, S. (2018). Coxiella burnetii infection in sheep and goats: a public risk health, Colombia. *Infectio*, *22*(4), 173-177.
8. Radostits, O.M., Gay C.C., Hinchcliff K.W., Constable, P.D.: Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Edited by Elsevier Health Sciences, Philadelphia, USA. 2006. Pp: 1468-1469.
9. Plummer, P. J., McClure, J. T., Menzies, P., Morley, P. S., Van den Brom, R., & Van Metre, D. C. (2018). Management of C oxiella burnetii infection in livestock populations and the associated zoonotic risk: A consensus statement. *Journal of veterinary internal medicine*, *32*(5), 1481-1494.
10. Longbottom, D., Entrican, G., Wheelhouse, N., Brough, H., & Milne, C. (2013). Evaluation of the impact and control of enzootic abortion of ewes. *The Veterinary Journal*, *195*(2), 257-259.
11. Khaled, H., Sidi-Boumedine, K., Merdja, S., Dufour, P., Dahmani, A., Thiéry, R., & Bouyoucef, A. (2016). Serological and molecular evidence of Q fever among small ruminant flocks in Algeria. *Comparative immunology, microbiology and infectious diseases*, *47*, 19-25.
12. Cruz, R., Esteves, F., Vasconcelos-Nóbrega, C., Santos, C., Ferreira, A. S., Mega, C., & Mesquita, J. R. (2018). Outbreaks of abortions by Coxiella burnetii in small ruminant flocks and a longitudinal serological approach on archived bulk tank milk suggest Q fever emergence in Central Portugal. *Transboundary and emerging diseases*, *65*(4), 972-975.
13. Van der Hoek, W., Meekelenkamp, J. C., Leenders, A. C., Wijers, N., Notermans, D. W., & Hukkelhoven, C. W. (2011). Antibodies against Coxiella burnetii and pregnancy outcome during the 2007-2008 Q fever outbreaks in the Netherlands. *BMC infectious diseases*, *11*(1), 44.
14. Van den Brom, R., Van Engelen, E., Roest, H. I. J., Van der Hoek, W., & Vellema, P. (2015). Coxiella burnetii infections in sheep or goats: an opinionated review. *Veterinary microbiology*, *181*(1-2), 119-129.
15. Guidi, F., Petruzzelli, A., Ciarrocchi, F., Duranti, A., Valiani, A., Amagliani, G., & Blasi, G. (2017). Prevalence of Coxiella burnetii in cows' and ewes' bulk tank milk samples from selected dairy farms of Central Italy. *Italian Journal of Animal Science*, *16*(4), 673-676.
16. Gharban, H.J. & Yousif, A.A. (2020). First isolation and molecular phylogenetic analysis of *Coxiella burnetii* in lactating cows, Iraq. Bulg. J. Vet. Med, 23 (1).
17. Released, S. I. (2008). SPSS statistics for windows, version 17.0. Chicago: SPSS Inc. PP: 161-179.
18. Guatteo, R., Beaudeau, F., Joly, A., & Seegers, H. (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*(5), 191-194.
19. Saglam, A. G., & Sahin, M. (2016). Coxiella burnetii in samples from cattle herds and sheep flocks in the Kars region of Turkey. *Vet Med*, *1*, 17-22.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14172 - 14184
Received 05 March 2021; Accepted 01 April 2021.

20. Ullah, Q., El-Adawy, H., Jamil, T., Jamil, H., Qureshi, Z. I., Saqib, M., & Khan, I. (2019). Serological and Molecular Investigation of Coxiella burnetii in Small Ruminants and Ticks in Punjab, Pakistan. *International journal of environmental research and public health*, *16*(21), 4271.
21. PARIN, U., & Osman, K. A. Y. A. (2015). Detection of Coxiella burnetii prevalence in bovine, ovine and caprine herds. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *62*(3), 177-181.
22. Gangoliya, S. R., Kumar, S., Alam, S. I., Sharma, H. K., Singh, M., Kotwal, S. K., ... & Kamboj, D. V. (2019). First molecular and serological evidence of Coxiella burnetti infection among sheep and goats of Jammu province of India. *Microbial pathogenesis*, *130*, 100-103.
23. Oliveira, R. D., Mousel, M. R., Pabilonia, K. L., Highland, M. A., Taylor, J. B., Knowles, D. P., & White, S. N. (2017). Domestic sheep show average Coxiella burnetii seropositivity generations after a sheep-associated human Q fever outbreak and lack detectable shedding by placental, vaginal, and fecal routes. *PloS one*, *12*(11).
24. Gyuranecz, M., Dénes, B., Hornok, S., Kovács, P., Horváth, G., Jurkovich, V., & Vass, N. (2012). Prevalence of Coxiella burnetii in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. *Vector-Borne and Zoonotic Diseases*, *12*(8), 650-653.
25. Filioussis, G., Theodoridis, A., Papadopoulos, D., Gelasakis, A. I., Vouraki, S., Bramis, G., & Arsenos, G. (2017). Serological prevalence of Coxiella burnetii in dairy goats and ewes diagnosed with adverse pregnancy outcomes in Greece. *Ann Agric Environ Med*, *24*(4), 702-705.
26. Mobarez, A. M., Amiri, F. B., & Esmaeili, S. (2017). Seroprevalence of Q fever among human and animal in Iran; A systematic review and meta-analysis. *PLoS neglected tropical diseases*, *11*(4), e0005521.
27. Hilbert, A., Schmoock, G., Lenzko, H., Moog, U., Diller, R., Fröhlich, A., & Henning, K. (2012). Prevalence of Coxiella burnetii in clinically healthy German sheep flocks. *BMC research notes*, *5*(1), 152.
28. Nahed, H. G., & Khaled, A. A. M. (2012). Seroprevalence of Coxiella burnetii antibodies among farm animals and human contacts in Egypt. *J Am Sci*, *8*, 619-621.
29. Knap, N., Žele, D., Biškup, U. G., Avšič-Županc, T., & Vengušt, G. (2019). The prevalence of Coxiella burnetii in ticks and animals in Slovenia. *BMC veterinary research*, *15*(1), 368.
30. Obaidat, M. M., & Kersh, G. J. (2017). Prevalence and risk factors of Coxiella burnetii antibodies in bulk milk from cattle, sheep, and goats in Jordan. *Journal of food protection*, *80*(4), 561-566.
31. License, C. C. B. Y., Elandalousi, R. B., Ghram, A., Maaroufi, A., & Mnif, W. (2015). Seroprevalence of zoonotic abortive diseases in ruminants in the north of Tunisia. *Research*, 2, 1-8.
32. Emery, M. P., Ostlund, E. N., & Schmitt, B. J. (2012). Comparison of Q fever serology methods in cattle, goats, and sheep. *Journal of Veterinary Diagnostic Investigation*, *24*(2), 379-382.
33. Roest, H. I., Post, J., van Gelderen, B., van Zijderveld, F. G., & Rebel, J. M. (2013). Q fever in pregnant goats: humoral and cellular immune responses. *Veterinary research*, *44*(1), 67.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14172 - 14184
Received 05 March 2021; Accepted 01 April 2021.

34. Joulie, A., Rousset, E., Gasqui, P., Lepetitcolin, E., Leblond, A., Sidi-Boumedine, K., & Jourdain, E. (2017). Coxiella burnetii circulation in a naturally infected flock of sheep: individual follow-up of antibodies in serum and milk. *Appl. Environ. Microbiol.*, *83*(13), e00222-17.
35. Khalifa, N. O., Elhofy, F. I., Fahmy, H. A., Sobhy, M. M., & Agag, M. A. (2016). Seropervelance and molecular detection of Coxiella burnetii infection in sheep, goats and human in Egypt. *ISOI J Microbiol Biotechnol Food Sci*, *2*, 1-7.
36. Kowalczewska, M., Sekeyová, Z., & Raoult, D. (2011). Proteomics paves the way for Q fever diagnostics. *Genome medicine*, *3*(7), 50.
37. Eldin, C., Melenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., & Raoult, D. (2017). From Q fever to Coxiella burnetii infection: a paradigm change. *Clinical microbiology reviews*, *30*(1), 115-190.
38. Canevari, J. T., Firestone, S. M., Vincent, G., Campbell, A., Tan, T., Muleme, M., & Stevenson, M. A. (2018). The prevalence of Coxiella burnetii shedding in dairy goats at the time of parturition in an endemically infected enterprise and associated milk yield losses. *BMC veterinary research*, *14*(1), 353.
39. Rodolakis, A., Berri, M., Hechard, C., Caudron, C., Souriau, A., Bodier, C. C., & Vadet, J. P. (2007). Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. *Journal of dairy science*, *90*(12), 5352-5360.
40. Mohammed, O. B., Jarelnabi, A. A., Aljumaah, R. S., Alshaikh, M. A., Bakhiet, A. O., Omer, S. A., & Hussein, M. F. (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*(9), 715-719.
41. McLaughlin, H. P., Cherney, B., Hakovirta, J. R., Priestley, R. A., Conley, A., Carter, A., & Sue, D. (2017). Phylogenetic inference of Coxiella burnetii by 16S rRNA gene sequencing. *PloS one*, *12*(12).
42. Najim, Y. S., Mohammed, Th. T. & Al-Khalani, F. M. H. 2020. The effect of the use of different levels of Azolla to male broilers diets in the production and physiological performance and economic feasibility. Biochemical and Cellular Archives, 20(1): 573-580. Doi: 10.35124/bca.2020.20.1.573