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MOLECULAR DETECTION OF *COXIELLA BURNETII* INFECTION IN MILK SAMPLES FROM DAIRY CATTLE IN ASSIUT GOVERNORATE, EGYPT

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ABSTRACT

Coxiella burnetii (*C. burnetii*) is an intracellular bacterium and the cause of query fever (Q fever), which is a serious zoonotic disease that influences numerous animal species globally. Thus, the current investigation's aims were to ascertain the molecular diagnosis of *C. burnetii* and the epidemiological findings' correlation with *C. burnetii* infection. The present investigation was carried out on 100 dairy cows from the Faculty of Agriculture farm, individual farmer houses from El-Fateh and Abnoub cities, and individual cases investigated in the Veterinary Teaching Hospital in Assiut Governorate, Egypt. Milk samples were collected and examined by California Mastitis Test (CMT), and polymerase chain reaction (PCR) to diagnose *C. burnetii*. Results showed that 5%, 9%, 11%, and 75% of 100 milk samples were CMT (++), CMT (+), suspicious, and negative, respectively. *C. burnetii* DNA was reported in the milk of six dairy cows. The percentages of *C. burnetii* infection had no discernible differences ($P<0.05$) with locality, age, breed (native and mixed breed), tick infestation, housing system, and health status of molecularly tested dairy cows. Therefore, the frequency of *C. burnetii* infection in dairy herds emphasizes the critical need for surveillance and adequate biosecurity measures in place to prevent and restrict the spread of Q fever in the Assiut Governorate.

***Keywords**: C. burnetii*, milk, CMT, IS*1111*gene, Epidemiology

INTRODUCTION

Coxiellosis (also referred to as Q fever) is a comprehensive zoonotic disease that is present all over the world, excluding New Zealand (Amin and Ahmed, 2009). In 1935, it was initially noted by Derrick in Queensland, Australia, following a feverish disease outbreak among workers in slaughterhouses (Derrick, 1937). The causative agent of this disease is *C. burnetii*, an obligatory intracellular Gram-negative bacterium, a member of the genus *Coxiella* of the *Coxiellaceae* family, and

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Proteobacteria phylum, in addition to the genera *Legionella* (Selim *et al.*, 2023). Mammalian and non-mammalian animals are impacted by *C. burnetii* (Parker *et al.*, 2006). Domesticated ruminants like cattle, sheep, and goats act as the main carriers of *C. burnetii* for human infection (Kazemeini *et al.*, 2021). These ruminants can primarily excrete the bacteria through their milk, urine, and feces, as well as vaginal discharges (Sadiki *et al.*, 2023). The most common ways that *C. burnetii* spreads are through the inhalation of spore-like phases carried in aerosols, contact with animal excretions or contaminated materials, and, infrequently, tick bites. On the other hand, unpasteurized milk or dairy products containing live *C. burnetii* can potentially infect humans (Szymańska-Czerwińska *et al.*, 2022 and Neare *et al.*, 2023). Q fever in humans typically has no symptoms; nevertheless, in people who are continuously infected, it can lead to acute or chronic illnesses such as influenza-like symptoms, pneumonia, hepatitis, meningoencephalitis, myocarditis, endocarditis, and chronic fatigue syndrome. Furthermore, it raises a pregnant woman's chance of stillbirth and abortion (Rahman *et al.*, 2016). In cattle, the infection typically shows no symptoms for weeks to several months (Grantiòa-Ieviòa *et al.*, 2022). Nonetheless, the most prevalent clinical manifestations are endometritis, abortion, stillbirth, infertility, and mastitis (Saleh *et al.*, 2021). Because of a localized infection of the mammary gland with *C. burnetii*, it can be shed in cow's milk either frequently or continuously (Gwida *et al.*, 2014). The presence of *C. burnetii* in milk poses inquiries about the potential entry routes for this common zoonotic bacterium into humans through unpasteurized raw milk or unpasteurized raw milk products. Therefore, the prevention, management, control, and treatment of Q fever in animals depend on an accurate and precise identification of *C. burnetii* (Kazemeini *et al.*, 2021). Q fever in animals can be identified by looking for bacteria, bacterial DNA, or antibodies (Rahman *et al.*, 2016). These bacteria may grow in axenic (host cell-free) media, although isolation poses a risk to laboratory workers, takes time, and can produce false-negative results (Saleh *et al.*, 2021). Additionally, a Biosafety Level III laboratory is needed for Q fever isolation procedures (Dhaka *et al.*, 2020). Nevertheless, one major disadvantage of using serological tests to diagnose acute infection is the time needed to produce antibodies against this pathogen, which may take several weeks. Also, the antibodies frequently persist for years after an illness, making it difficult to distinguish between infections from the past and present (Amin and Ahmed, 2009). Consequently, molecular techniques such as PCR have been suggested as a rapid and sensitive method to identify *C. burnetii* infection in most ruminants (Kazemeini *et al.*, 2021). The preferred method for detecting pathogens is trans-PCR, a putative sequence (IS*1111*) of *C. burnetii*-targeting PCR. This is because the pathogen's transposon-like repetitive element, which has multiple genomic copies, increases the test's sensitivity (Dhaka *et al.*, 2018). Some studies have been published recently about the epidemiology of *C. burnetii* infection in Egypt (Abdullah *et al.*, 2019; Abbass *et al.*, 2020; Saleh *et al.*, 2021; Selim *et al.*, 2023). Recent reviews declare that scanty research has been done on the *C. burnetii*'s existence in cow milk samples throughout Assiut, Egypt (Amin and Ahmed, 2009). Therefore, the goals of the current investigation were to check for subclinical mastitis in milk samples from dairy cattle, molecular detection of IS*1111* gene of *C. burnetii* and to look into the connection between a few epidemiological factors such as location, age, breed of dairy cattle, tick infestation, housing arrangement, and health and the infection incidence of *C. burnetii* in the Assiut Governorate.

MATERIALS AND METHODS

1. Animals and ethical approval

The study was conducted between August and November 2023. One hundred dairy cattle of different ages and breeds were

investigated clinically and molecularly for the presence of C. burnetii. The examined herd had 45 cows that belonged to the Faculty of Agriculture farm at Assiut University, a total of 28 (62.2%) suspected affected dairy cattle were chosen for the collection of milk samples. Besides, individual dairy cows were from farmer houses in Assiut (El-Fateh and Abnoub cities), and individual dairy cows from different Assiut Governorate villages were examined at the Veterinary Teaching Hospital, Assiut University Faculty of Veterinary Medicine, Assiut University. The dairy cattle under investigation underwent a clinical examination in accordance with Jackson and Cockcroft (2002). Ethical standards were followed in dealing with each dairy cow involved in this study. The research obtained approval with approval number 06/2023/0146 by the Research Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

2. Sampling

2.1. Milk sample

From each individual dairy cow, 15 ml of raw milk was obtained from the udder, placed into a sterile tube, and preserved at -20°C for subsequent DNA extraction. Every milk sample was tested with a California mastitis reagent (Lactotest, Cromasa-Crotales Marcados S.A.) according to the manufacturer's guidelines for the diagnosis of subclinical mastitis.

2.2. Control positive sample

Positive reference *C. burnetii* DNA sample was kindly supplied by Veterinary Research Institute, National Research Center, Cairo, Egypt.

3. Molecular diagnosis

3.1. Extraction of DNA

To separate the layers of cream and whey (supernatant), 1 milliliter of each milk sample was centrifuged at 3000 rpm for 10 minutes. DNA was extracted using the ABT genomic DNA mini extraction kit (Applied Biotechnology, Egypt) from the pellet (sediment), following the manufacturer's instructions.

3.2. Primers

The particularities of the selected primers (Metabion International AG, Germany) for the IS*1111* gene of *C. burnetii* that were utilized in this investigation had already been assessed (Willems *et al.*, 1994). Table 1 shows the primer sequences and their positions in the bacterial genome.

Table 1: The IS*1111* gene of *C. burnetii*'s nucleotide sequence of the primers used and the size of the products developed following PCR.

Primer	Sequence of nucleotides	The size of the DNA product bp
Forward: Trans-1	5'-TAT GTA TCC ACC GTA GCC AGT C-3'	687
Reverse: Trans-2	5'-CCC AAC AAC ACC TCC TTA TTC-3'	

3.3. Molecular detection of *C. burnetii* IS*1111*gene

Possibility of a specific PCR to amplify the IS*1111* gene, which originated from a repetitive area of the *C. burnetii* genome resembling a transposon. To amplify DNA fragments with a length of 687 bp, primer sets Trans-1 forward and Trans-2 reverse were utilized. The ABT red master mix (2X) (Applied Biotechnology, Egypt) was used for this study as a tool for polymerase enzyme and DNTPs. The following reagents were used for PCR, which was carried out in a PCR thermocycler (Peqlab,Germany): 20 µl of the final volume included 10 µl of the ABT red master mix (2X), 0.5 µl from each primer Trans-1 and Trans-2 (5 pmol), 3 µl DNA sample, and 6 µl nuclease-free water. The thermal cycling settings included initial denaturation for 5 minutes at 95°C, followed by successive 40 cycles of denaturation at 95°C for 30 seconds, an annealing step of 1 minute at 54°C, and an extension step of 1 minute at 72°C. This was followed by a final extension for 10 minutes at 72°C.

3.4. PCR products analysis and detection

To visualize the process, 5 µl of amplified PCR products were loaded. Gel electrophoresis was used to analyze the amplicons in a 1.5% agarose gel dyed with ethidium bromide (10 mg/ml) for 60 minutes at 90 V and 155 mA. A gel UV trans-illuminator (Syngene, UK) was used to view the amplicons after their size was determined using size marker DNA of 100 bp.

4. Analytical statistics

Using the statistical program for the social sciences (SPSS) version 16 software, the Chi-square of independence (2007) was employed to enroll and assess the obtained data.

RESULTS

1. Detection of subclinical mastitis by CMT with correlation with *C. burnetii* infection

According to Table (2) from the 100 milk samples examined, 5 (5%), 9 (9%), 11 (11%), and 75 (75%) were CMT (++), CMT (+), suspicious, and negative, respectively. The results of CMT scores of the six molecularly positive milk samples from the examined cows indicate that 1 (20%), 0 (0%), 1 (9.1%), and 4 (5.3%) were classified as CMT (++), CMT (+), suspicious, and negative, respectively. CMT (++) had the mathematically highest rate of *C. burnetii* infection.

Table 2: Subclinical mastitis occurrence in the examined milk samples of examined cows based on the result of CMT with correlation with *C. burnetii* infection

Degrees of reaction on samples (CMT scores)	No. of positive milk samples (%)	No. of *C. burnetii* positive Samples (%)	P-value
Strong positive (++ve)	5 (5)	1 (20)	0.5
Light positive (+ve)	9 (9)	0 (0)	
Suspicious (±ve)	11 (11)	1 (9.1)	
Negative(-ve)	75 (75)	4 (5.3)	
Total	100 (100)	6 (6)	

No significant variation at P<0.05.

2. *C. burnetii* DNA identification using PCR

PCR was performed on the DNA specimens to produce the required bands at 687 bp due to IS*1111* gene of the *C. burnetii* genome (Figure 1). Six (6%) of the 100 milk samples had molecularly positive results.

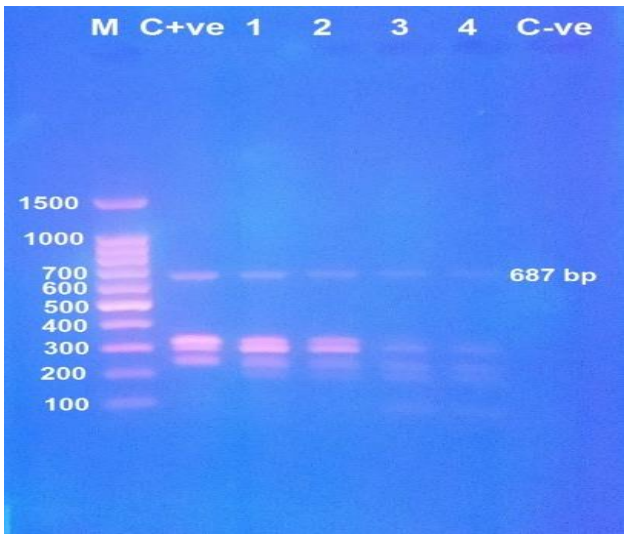

Figure 1: PCR agarose gel electrophoresis following *C. burnetii* IS*1111* gene amplification in milk samples. Line M: 100 bp DNA marker, line C+ve: positive control sample; lines 1, 2, 3, and 4 positive milk samples with the amplification product at 687 bp; and line C-ve: negative control distilled water.

3. Epidemiological findings

3.1. Percentage of *C. burnetii* infection

According to Table 3, the present study revealed that 6% (6/100) of dairy cattle were infected with *C. burnetii*. Regarding the locality, the prevalence was 7.1% (2 of 28) of tested milk samples from dairy cattle at the Faculty of Agriculture farm, while the infection of *C. burnetii* was found in 4% (2 of 50) of milk samples collected from dairy cattle at farmer's houses. In addition, the prevalence was 9.1% (2 of 22) of milk samples taken from dairy cattle that were admitted to the Veterinary Teaching Hospital in Assiut Governorate. The percentage of *C. burnetii* infection did not differ significantly between dairy cattle in farm and individual cow cases (Table 3).

3.2. Age-related vulnerability

The percentage of *C. burnetii* infection in dairy cattle was assessed across two age groups: 3-5 years and >5-7 years. Of the 100 animals investigated, the results showed an infection rate of 5% in the 3-5 year group and 10% in the >5-7 year group. The proportion of *C. burnetii* infection did not significantly differ among dairy cattle in different age groups (Table 3).

3.3. Breed vulnerability

In the current investigation, native and mixed breeds of examined dairy cattle did not exhibit a statistically significant difference in the proportion of infection with *C. burnetii* (Table 3).

3.4. Tick infestation

Based on the presence of tick infestation in the dairy cattle under examination, the analytical results revealed no discernible variation existed in the rate of *C. burnetii* infection (Table 3).

3.5. Housing system

According to the housing system, there was no noticeable distinction in the percentage of *C. burnetii* infection between cattle in the farm and the household (Table 3).

3.6. Health status

Depending on the health status, *C. burnetii* infection was in 5.8% (3/52) of clinically healthy and 6.3% (3/48) of clinically diseased dairy cattle, with no significant variation (Table 3).

Table 3: Association between *C. burnetii* infection and some epidemiological determinants based on the PCR findings.

Variable			No. of studied animals	Positive No. (%)	Negative No. (%)	P-value
Locality	Faculty of Agriculture farm		28	2 (7.1)	26 (92.9)	0.8
	Farmer house	El-Fateh city	25	0 (0)	25 (100)	
		Abnoub city	25	2 (8)	23 (92)	
	Dairy cows admitted to the Veterinary Teaching Hospital		22	2 (9.1)	20 (90.9)	
	Total		100	6 (6)	94 (94)	
Age	3 – 5 years		80	4 (5)	76 (95)	0.4
	>5 - 7 years		20	2 (10)	18 (90)	
	Total		100	6 (6)	94 (94)	
Breed	Native		63	4 (6.3)	59 (93.7)	0.8
	Mixed		37	2 (5.4)	35 (94.6)	
	Total		100	6 (6)	94 (94)	
Tick infestation	Present		35	2 (5.7)	33 (94.3)	0.9
	Absent		65	4 (6.2)	61 (93.8)	
	Total		100	6 (6)	94 (94)	
Housing system	Farms		28	2 (7.1)	26 (92.9)	0.8
	Household		72	4 (5.6)	68 (94.4)	
	Total		100	6 (6)	94 (94)	
Health status	Clinically healthy		52	3 (5.8)	49 (94.2)	0.9
	Clinically diseased		48	3 (6.3)	45 (93.7)	
	Total		100	6 (6)	94 (94)	

No significant variation at P<0.05.

DISCUSSION

Q fever is an emerging zoonotic disease affecting a range of animals, including ruminants (Dhaka *et al.*, 2020). The

frequency of reported outbreaks has increased recently, and the disease's economic impact because of the decreased animal productivity and herd death has raised awareness of Q fever (Abdullah *et al.*, 2019). Consequently, estimating the prevalence of *C. burnetii* is crucial to comprehending the disease's epidemiological situation. In Egypt, Q fever was first detected serologically in a risk group of cattle keepers in 1995 (Botros *et al.*, 1995). Eventually, numerous studies confirmed the occurrence of the disease in cattle (Amin and Ahmed, 2009; Gwida *et al.*, 2014; Horton *et al.*, 2014; Abdel-Moein and Hamza, 2017; Klemmer *et al.*, 2018; Saleh *et al.*, 2021; AlSaadawy *et al.*, 2023; Selim *et al.*, 2023). Nonetheless, there are few reports in the Assiut governorate regarding the incidence and prevalence of *C. burnetii* in dairy animals, especially cattle. Therefore, the current study aimed to detect the percentage of *C. burnetii* infection in milk samples of dairy cows in the Assiut governorate with PCR validation.

The results of the CMT scores of the six molecularly positive milk samples from the examined cows indicate that 1 (20%), 0 (0%), 1 (9.1%), and 4 (5.3%) were classified as CMT (++), CMT (+), suspicious, and negative, respectively. CMT (++) had the mathematically highest rate of *C. burnetii* infection. This finding could be explained by the fact that coxiellosis in cattle has been linked to subclinical mastitis, and that the increased SCC in milk is correlated with the *C. burnetii* PCR status (Barlow *et al.*, 2008 and Dhaka *et al.*, 2020).

The IS*1111* gene was found in 6 (6%) of the 100 milk samples of the studied dairy cows after PCR molecular analysis, in order to identify *C. burnetii* infection. The amplification of the IS*1111* gene enabled the assay's specificity and sensitivity to be increased, as this is a multi-copy gene (7 to 110 copies) (Khalifa *et al.*, 2016 and Hardi *et al.*, 2020). Our molecular outcome (6%) was nearly the same as the conclusions of the past investigation (Menadi *et al.*, 2022), who recorded that 9% (18/200) of the dairy animals under evaluation were positive for *C. burnetii* molecularly. Our finding exceeded the prior report by Cornejo *et al.* (2019), who revealed that 2.1% (2/105) of dairy cattle had a molecular positive rate for *C. burnetii*., while it was less than those of Amin and Ahmed (2009), Dhaka *et al.* (2018), Kalaitzakis *et al.* (2021), and Kazemeini *et al.* (2021), who found that the molecular positive frequencies for *C. burnetii* in dairy cattle were 22% (22/100), 26.7% (58/217), 33.8% (156/462) and 27% (27/100), respectively. This variation in positivity rate could be attributed to differences in sample sizes and geographic distribution.

Epidemiologically, the prevalence of *C. burnetii* infection in dairy cattle under examination was 6% in Assiut Governorate, Egypt. Increased infection rates have been observed in several localities throughout Egypt. Specifically, Saleh *et al.* (2023), who recorded a 36% infection rate in Sharkia, while it was about 15.7% in Assiut (Alsaadawy *et al*., 2023). Additionally, Selim *et al*. (2023) reported an infection rate of about 17.5%, 20.8%, 16.7%, and 24.2% in Gharbia, Kafr El-Shiekh, Menoufia, and Qalyubia, respectively. This may be due to differences in the collection times and numbers of samples, ambient and hygienic conditions, and various diagnostic techniques application. Based on the study location, the percentage of *C. burnetii* infection in the dairy cattle examined from the agriculture farm, farmers' houses, and individual cases admitted to the Veterinary Teaching Hospital of Assiut Governorate did not significantly differ. It is possible that this is because the dairy cattle under investigation were raised using the same method, in the same geography, during the same seasons, and with the same level of hygiene. Concerning age susceptibility, the percentage of *C. burnetii* infection in dairy cattle age groups did not differ significantly. This result corroborated the findings of AlSaadawy *et al.* (2023), who reported that the differences between age groups of cows

were statistically non-significant. Our results could be clarified by the risk of contracting *C. burnetii* infection is the same for cattle of all ages. In the present study, there was no appreciable difference in the rate of *C. burnetii* infection between native and mixed breeds of dairy cattle. This result matched that of Dhaka *et al.* (2020) and AlSaadawy *et al.* (2023), who found statistically no difference between the breeds of cows. Our result would imply that the risk of acquiring a *C. burnetii* infection is similar for all cattle breeds. Although ticks are thought to be crucial for the survival of bacteria in nature, they are neither necessary for animal infection nor crucial for the natural cycle of *C. burnetii* in cattle (Dhaka *et al.*, 2018). In our study, the analytical results showed no appreciable variation in the percentage of *C. burnetii* infection based on the presence of tick infestation in the examined dairy cattle. This outcome differs from those of AlSaadawy *et al.* (2023), who revealed that cows free from ticks exhibited a significantly greater prevalence rate than those infested with ticks. This finding supports previously conducted research showing ticks don't significantly contribute to the spread of *C. burnetii* and may be the result of animals regularly spraying with acaricides to kill ticks (Dhaka *et al.*, 2018 and AlSaadawy *et al.*, 2023). This study demonstrated that no statistically significant difference was found in the *C. burnetii* infection rate between farm rearing and household rearing. This finding may be attributed to animals' rearing with the same methods. In our investigation, no variance was statistically significant in the *C. burnetii* infection rate between dairy cows with clinical signs and those that were clinically healthy. This finding was consistent with that of AlSaadawy *et al.* (2023), who concluded that there was statistically no difference between the apparently healthy and diseased cows. This result may be due to the infrequent appearance of *C. burnetii* infection symptoms in animals (AlSaadawy *et al.*, 2023) and various factors like the resistance of the animal and stress.

CONCLUSION

The rate of *C. burnetii* infection in the Assiut governorate, Egypt, was determined in the present investigation, by detecting the existence of *C. burnetii* DNA in milk samples from dairy cattle using the PCR technique. We discussed some epidemiological aspects observed for *C. burnetii* infection in dairy cattle. Regarding the risks to the public's health posed by consuming infected milk with *C. burnetii*, all milk should be pasteurized, because the organisms are destroyed by high temperatures during the pasteurization process. The identification of risk factors that could otherwise negatively affect livestock wealth highlights the need for effective preventative and surveillance strategies to be implemented all over Egypt, in order to lower the frequency of *C. burnetii* infection.

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REFERENCES

Abbass, H.; Selim, S.A.K.; Sobhy, M.M.; El-Mokhtar, M.A.; Elhariri, M. and Abd-Elhafeez, H.H. (2020): High prevalence of *Coxiella burnetii* infection in humans and livestock in Assiut, Egypt: A serological and molecular survey. Veterinary World, 13(12), 2578-2586. https://doi.org/10.14202/vetworld.2020.2578-2586.

Abdel-Moein, K.A. and Hamza, D.A. (2017): Coxiella burnetii among aborted dairy animals in Egypt and its public health implications. Acta Tropica, 166: 92-

95. https://doi.org/10.1016/j.actatropica.2016.11.011.

Abdullah, H.H.A.M.; Hussein, H.A.; Abd El-Razik, K.A.; Barakat, A.M.A. and Soliman, Y.A. (2019): Q fever: A neglected disease of camels in Giza and Cairo Provinces, Egypt. Veterinary World, 12(12), 1945-1950. https://doi.org/10.14202/vetworld.2019.1945-1950.

AlSaadawy, R.M.; Ahmed, S.O. and Hetta, H.F. (2023): Seroprevalence rate of *Coxiella burnetii* in cows' serum in Assiut Governorate, Egypt. Assiut Veterinary Medical Journal, 69(177), 105-111. https://doi.org/10.21608/AVMJ.2023.189492.1119.

Amin, W.F. and Ahmed, S.O. (2009): Detection of *Coxiella burnetii* in bovine milk samples using Polymerase Chain Reaction. Assiut Veterinary Medical Journal, 55(123), 1-9. https://doi.org/10.21608/AVMJ.2009.174925.

Barlow, J.; Rauch, B.; Welcome, F.; Kim, S.G.; Dubovi, E. and Schukken, Y. (2008): Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle. Veterinary Research, 39(3), 23. https://doi.org/10.1051/vetres:2007060.

Botros, B.A.; Soliman, A.K.; Salib, A.W.; Olsen, J.; Hibbs, R.G.; Williams, J.C.; Darwish, M.; El-Tegani, A. and Watts, D. (1995): Coxiella burnetii antibody prevalence in North-East Africa determined by enzyme immunoassay. American Journal of Tropical Medicine and Hygiene, 98(3), 173-178.

Cornejo, J.; Araya, P.; Ibáñez, D.; Hormazabal, J.C.; Retamal, P.; Fresno, M.; Herve, L.P. and Lapierre, L. (2019): Identification of *Coxiella burnetii* in Tank Raw Cow Milk: First Findings from Chile. Vector Borne and Zoonotic Diseases, 20(3), 228-230. https://doi.org/10.1089/vbz.2019.2535.

Derrick, E.H. (1937): Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Reviews of Infectious Diseases, 5(4), 790-800. https://doi.org/10.1093/clinids/5.4.790.

Dhaka, P.; Malik, S.S.; Yadav, J.P.; Kumar, M.; Baranwal, A.; Barbuddhe, S.B. and Rawool, D.B. (2018): Seroprevalence and molecular detection of coxiellosis among cattle and their human contacts in an organized dairy farm. Journal of Infection and Public Health, 12(2), 190-194. https://doi.org/10.1016/j.jiph.2018.10.001.

Dhaka, P.; Malik, S.V.S.; Yadav, J.P.; Kumar, M.; Barbuddhe, S.B. and Rawool, D.B. (2020): Apparent prevalence and risk factors of coxiellosis (Q fever) among dairy herds in India. PloS one, 15(9), e0239260. https://doi.org/10.1371/journal.pone.0239260.

Grantiòa-Ieviòa, L.; Steingolde, Z.; Boikmanis, G.; Laizâne, L.; Ringa-Oðleja, G.; Bubula, I.; Sergejeva, M.; Maliðevs, A.; Íibilds, J.; Cvetkova, S.; Bergðpica, I.; Eglîte, L,; Cîrule, D.; Kovaïenko, K.; Antâne, V. and Bçrziòð, A. (2022): Shedding of *Coxiella burnetii* in Milk of Dairy Cattle and Evidence of Q Fever in Domestic Ruminants with Emphasis on Abortion Cases in Latvia. Proceedings of the Latvian Academy of Sciences, Section B, 76(2), 295-306. https://doi.org/10.2478/prolas-2022-0043.

Gwida, M.; El-Ashker, M.; El-Diasty, M.; Engelhardt, C.; Khan, I. and Neubauer, H. (2014): Q fever in cattle in some Egyptian governorates: A preliminary study. BMC Research Notes, 7, 881. https://doi.org/10.1186/1756-0500-7-881.

Hardi, F.M.; Rauf, H.S.; Mahmood, S.L.; Ahmad, R.B.; Ali, B.A. and Sheikh, M.O.B. (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.

Horton, K.C.; Wasfy, M.; Samaha, H.; Abdel-Rahman, B.; Safwat, S.; Fadeel, M.A.; Mohareb, E. and Dueger, E. (2014): Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. Vector Borne and Zoonotic Diseases, 14(9), 633-9. https://doi.org/10.1089/vbz.2013.1525.

Jackson, P.G.G. and Cockcroft, P.D. (2002): Clinical examination of farm animals, First Edition, USA.

Kalaitzakis, E.; Fancello, T.; Simons, X.; Chaligiannis, I.; Tomaiuolo, S.; Andreopoulou, M.; Petrone, D.; Papapostolou, A.; Giadinis, N.D.; Panousis, N. and Mori, M. (2021): Coxiella burnetii Shedding in Milk and Molecular Typing of Strains Infecting Dairy Cows in Greece. Pathogens, 10(3), 287. https://doi.org/10.3390/pathogens10030287.

Kazemeini, H.; Partovi, R.; Nazaktabar, A. and Shokri, H. (2021): Detection of *Coxiella burnetii* in raw milk samples collected from dairy farms in Mazandaran province, north of Iran. Journal of Food Safety and Hygiene, 7(3), 171-179.

Khalifa, O.N.; Elhofy, I.F.; Fahmy, A.H.; Mona, M.; Sobhy, M.M. and Agag, M.A. (2016): Seroprevalence and molecular detection of *Coxiella burnetii* infection in sheep, goats and human in Egypt. ISOI Journal of Microbiology, Biotechnology and Food Science, 2(1), 1-7.

Klemmer, J.; Njeru, J.; Emam, A.; El-Sayed, A.; Moawad, A.A.; Henning, K.; Elbeskawy, A.M.; Sauter-Louis, C.; Straubinger, K.R.; Neubauer, H. and El-Diasty, M.M. (2018): Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* specific antibodies in cattle, buffaloes, sheep, goats and camels. PloS One, 13(2), e0192188. https://doi.org/10.1371/journal.pone.0192188.

Menadi, S.E.; Chisu, V.; Santucciu, C.; Di Domenico, M.; Curini, V. and Masala, G. (2022): Serological, Molecular Prevalence and Genotyping of *Coxiella burnetii* in Dairy Cattle Herds in Northeastern Algeria. Veterinary Sciences, 9(2):40. https://doi.org/10.3390/vetsci9020040.

Neare, K.; Tummeleht, L.; Lassen, B. and Viltrop, A. (2023): Coxiella burnetii Seroprevalence and Associated Risk Factors in Cattle, Sheep, and Goats in Estonia. Microorganisms, 11(4), 819. https://doi.org/10.3390/microorganisms11040819.

Parker, N.R.; Barralet, J.H. and Bell, A.M. (2006): Q fever. Lancet, 367(9511), 679-88. https://doi.org/10.1016/S0140-6736(06)68266-4.

Rahman, M.A.; Alam, M.M.; Islam, M.A.; Bhuiyan, A.K. and Rahman, A.K. (2016): Serological and Molecular Evidence of Q Fever in Domestic Ruminants in Bangladesh. Veterinary Medicine International, 2016, 9098416. https://doi.org/10.1155/2016/9098416.

Sadiki, V.; Gcebe, N.; Mangena, M.L.; Ngoshe, Y.B. and Adesiyun, A.A. (2023): Prevalence and risk factors of Q fever (*Coxiella burnetii*) in cattle on farms of Limpopo province, South Africa. Frontiers in Veterinary Science, 10, 1101988. https://doi.org/10.3389/fvets.2023.1101988.

Saleh, M.; El-Hady, A.M.M.; Abdelkader, S.A.; Salem, H.S.S.; Mohammed, M.M.; El Shafei, A.A. and El-Shafei, M. (2021): Sero-Prevalence and Molecular Identification of *Coxiella burnetii* (Q Fever) Among Human and Animals in Egypt. Egyptian Journal of Veterinary Sciences, 52(Issue the 9th International Conference of Veterinary Research Division National Research Centre, Giza, Egypt, 27th -29th

September, 2021), 51-59. https://doi.org/10.21608/ejvs.2021.95033.1291

Selim, A.; Marawan, M.A.; Abdelhady, A.; Alshammari, F.A.; Alqhtani, A.H.; Ba-Awadh, H.A.; Olarinre, I.O. and Swelum, A.A. (2023): Coxiella burnetii and its risk factors in cattle in Egypt: a seroepidemiological survey. BMC Veterinary Research, 19, 29. https://doi.org/10.1186/s12917-023-03577-5.

Szymańska-Czerwińska, M.; Jodełko, A.; Osiński, Z. and Niemczuk, K. (2022): Screening for *Coxiella Burnetii* in Dairy Cattle Herds in Poland. Journal of Veterinary Research, 66(4), 549-557. https://doi.org/10.2478/jvetres-2022-0070.

Willems, H.; Thiele, D.; Frölich-Ritter, R. and Krauss, H. (1994): Detection of *Coxiella burnetii* in Cow's Milk using the Polymerase Chain Reaction (PCR). Journal of Veterinary Medicine, Series B, 41(9), 580-7. https://doi.org/10.1111/j.1439-0450.1994.tb00267.x.

الكشف الجزيئي عن عدوى الكوكسيلا بورينتي في عينات الحليب من الأبقار الحلابة فى محافظة أسيوط ، مصر

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الحمى المجهولة (حمى كيو) هي مرض مشترك خطير تسببه بكتيريا الكوكسيلا بورينتي داخل الخلايا التي تؤثر على العديد من الحيوانات في جميع أنحاء العالم. وبالتالي فإن أهداف الدراسة الحالية هي التشخيص الجزيئي لعدوى الكوكسيلا بورينتي ودراسة مدى ارتباط هذه العدوى ببعض المؤشرات الوبائية. أجريت الدراسة الحالية على ١٠٠ بقرة حلابة تابعة لمزرعة كلية الزراعية، وحالات فردية لبيوت بعض المزارعين (مدن الفتح وأبنوب)، وحالات فردية تم إدخالها إلى المستشفى البيطري التعليمي بمحافظة أسيوط بمصر. تم جمع عينات الحليب للتحليل المختبري. تم إجراء اختبار كاليفورنيا لالتهاب الضرع على عينات الحليب، وتم استخدام تفاعل البلمرة المتسلسل لتشخيص عدوى الكوكسيلا بورينتي. بينت النتائج ان ٥%، ٩%، ١١%، ٧٥% من ١٠٠ عينة حليب لتكون CMT (++)، CMT (+)، مشكوك فيها، وسلبية، على التوالي. تم العثور على الحمض النووي للكوكسيلا بورينتي في حليب ستة أبقار حلابة. لم يكن هناك فرق معنوي (P<٠,٠٥) بين النسب المئوية لعدوى الكوكسيلا بورينتي والمكان والعمر والسلالة (السلالة البلدية والمختلطة) والإصابة بالقراد ونظام السكن والحالة الصحية للأبقار الحلابة التي تم اختبارها جزيئيًا. يسلط انتشارعدوى الكوكسيلا بورينتي في قطعان أبقار الحلابة الضوء على الأدوار الحاسمة التي تلعبها المراقبة وتدابير الأمن الحيوي الكافية في الوقاية والحد من انتشار حمى كيو في محافظة أسيوط.