Prevalence of *Coxiella burnetii* infection in Dairy Cattle in Egypt and Associated Risk Assessment

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1. Abstract

Query fever (Q fever) is a disease caused by *Coxiella burnetii*, an obligate intracellular bacterium that affects humans and other animals. This organism is uncommon, but may be found in ruminant, and other domestic mammals. This study aimed to detect *Coxiella burnetii* (*C. burnetii*) in cattle and record the risk assessment associated with coxiellosis. Total of 100 bulk tank milk (BTM) samples were collected from dairy cattle located in different governorates in Egypt. *C. burnetii* antibodies were detected in the collected milk using ELISA. In addition, 15 samples were subjected to PCR targeting *ISIIII gene* specific to *C. burnetii*. All farms in the investigated area had a positive case for *C. burnetii* infection. The overall seroprevalence of *C. burnetii* was 51% among the examined farms. However, significant higher seropositive samples were observed in Alexandria, Menoufia, Giza, Faiyum, Ismailia, Beheira, followed by Gharbia, Sharqia, then Dakahlia, and BeniSuef. Using questionnaire some risk factors such as region, breed type, age, season herd size, water sources, feeding management, abortion, mastitis, presence of arthropods or rodents were investigated among the study area. In conclusion, the study revealed a wide distribution of infections over the study area and further epidemiological study is recommended to prevent and control of coxiellosis.

Keywords: *C. burnetii*, Cattle, ELISA, Milk, PCR, Risk assessment.

2. Introduction

Query fever, (Q fever) was first described as a febrile illness of abattoir workers in Australia in 1937 [1]. Infections caused by *Coxiella burnetii,* commonly referred to as coxiellosis when occurring in animals and Query fever when occurring in humans [2]. *C. burnetii* is an obligate intracellular biosafety level 3 agent and antibodies to this organism have been reported in a wide range of animals [3]. Q fever is of great significance with special reference to human public health [4]. Ruminants are considered as the main reservoir for human infections [5, 6]. The bacteria are shed in urine, feces, milk and within birth products [7, 8]. Consumption of unpasteurized milk and its products is associated with the infection [9, 10, 5]. *C. burnetii* can remain in the environment over long periods of time and is transported by winds over long distances [11, 12, 13]. The accurate diagnosis of coxiellosis in animals is of great

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importance to identify the infected flocks and to determine the public health significance of the disease in human [14]. CFT, ELISA and IFAT are equal in diagnosis of acute Q fever but more IgG and IgM antibodies were detected by IFAT than ELISA or CFT [15]. ELISA preferred for large scale screening of livestock [16] and of good agreement with IFAT results. Negative ELISA samples may show low CFT titres, possibly detecting IgM [17]. ELISA can be used on serum, plasma and milk of ruminants. Sensitivity is estimated to be 85% with specificity of 95% [18].

PCR generally indicates presence of DNA from live or dead bacteria, from infection or contamination [19]. Sensitivity and specificity of the test depend on the gene target of the assay. A number of commercial PCR kits are available for *C. burnetii* DNA detection. A commonly used target gene is IS*1111*, which is present in multiple copies on the genome, but varies in the exact copy number by genotype [8, 20]. A commercial European kit uses the *GAPDH* gene target [21]. However, a combination of serological testing and PCR helps in accurate identification of Q fever cases.

The risk factors of *C. burnetii* include free movement of animals across the borders, management systems e.g., extensive vs. intensive, lack of quarantine of newly introduced animals, abortion, age, sex and absence of vaccination. Other factors include: environmental factors, contact with other herds, history of infection in the herd, and presence of dogs, rodents and cats [22].

Therefore, the present study was conducted to detect *C. burnetii* infection using ELISA and PCR and to identify the risk factors associated with the infection among 12 governorates in Egypt

3. Materials and Methods

3.1. Ethical approval

The samples were collected from animals according to ethical guidelines of the Institutional Animal Care & Use Committee (IACUC) at the Faculty of Veterinary Medicine Cairo University (No: Vet CU 03162023648)

3.2. Bulk Tank Milk (BTM) testing and samples collection

A total of 100 BTM samples were collected from 12 cattle flock and farms located in 12 Egyptian governorates namely, Alexandria, Beheira, BeniSuef, Dakahlia, Faiyum, Gharbia, Giza, Ismailia, Matruoh, Menoufia, Sharqia, and Minya**.** The milk samples were centrifuged at 4000 g for 20 minutes. The middle layer of liquid was taken up by means of a glass Pasteur pipette inserted through the upper layer of cream, without touch the underlying cell sediment. The undiluted skimmed milk samples were used in the ELISA test plate wells.

3.3. Serological investigation

The samples were subjected to ELISA, using a commercial provided kit *C. burnetii* (Virion\Serion, Würzburg, Germany). Milk samples were examined for IgG antibodies using phase I and phase II ELISA and assessed according to the manufacturer's instructions. The cut off values of the tested sample were recorded [5].

3.4. Molecular investigation

Fifteen samples (11 ELISA positive and 4 negative samples) were subjected to

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PCR targeting IS*IIII* gene using specific primers (F: 5'-TAT GTA TCC ACC GTA GCC AGT C-3' and; R: 5'-CCC AAC AAC ACC TCC TTA TTC-3') from Metabion, Germany [23]**.** The QIAamp DNA Mini Kit (Catalogue no.51304) was employed for the DNA extraction. The kit provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes. Briefly milk samples were centrifuged at 2200 rpm for 5 minutes to be concentrated, and the sediment was re-suspended in 200µl TE buffer and centrifuged at 3000 rpm for 10 minutes to avoid the interference with casein. The milk pellet was diluted in 200 µl of PBS. Using QIAamp DNA Mini Kit DNA was extracted from the examined samples and according to Sambrook *et al*. [24]. Agarose gel electrophoreses was photographed by a gel documentation system and the data was analyzed to detect the amplified product at 687 bp.

3.5. Risk assessment

The risk factors analyzed within the current study such as region, breed type, age, season herd size, water sources, feeding management, abortion, mastitis, presence of arthropods or rodents were investigated using questionnaire

3.6. Statistical analysis

Analyses were performed using SPSS software® version 20 (IBM Corp, USA) to analyze the relationship between the demographic data, animal reproductive status, risk factors and seropositivity [25].

4. Results

4.1. ELISA screening results

Out of 100 examined BTM samples, 51 (51%) were positive for coxiellosis with ELISA. As shown in Table 1, high prevalence rates were recorded in Alexandria (80 %), Menoufia (70 %), Giza (66 %), Faiyum (55 %), Ismailia (50 %), Beheira (50 %), followed by Gharbia (40 %), Sharqia (40 %), Dakahlia (33 %), and Banished (33%). One BTM from Matruoh and one from Minya governorates were both positive.

4.2. Detection of C. burnetii IS1111 gene in milk samples by PCR

The data presented in Figure 1 illustrate that 11 out of 15 DNA extracted BMT samples (73.3 %) were positive for amplification of 687 bp segment which is expected product of IS*1111*gene specific for *C. burnetii*

4.3. Risk factors associated with C. burnetii infection in cattle

Tables 2 and 3 illustrate that all investigated farms had mastitis cases except of BeniSuef government. Meanwhile abortion cases were recorded in Gharbia, Giza, Matruoh, Minya, Menoufia, and Alexandria governorates. Arthropods were found on animals of flocks in Ismailia Gharbia Alexandria Beheira, Faiyum, Dakahlia, BeniSuef and Sharqia governorates. Rodents were found in farms of BeniSuef, Menoufia, Alexandria, Beheira and Sharqia governorates.

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5. Discussion

Serological testing of bulk tank milk (BTM) has been evaluated as a means to evaluate the likelihood of previous infection and the risk of shedding bacteria in healthy dairy herds. As such, BTM ELISA is a useful test for large-scale screening programs to detect flocks that have been previously infected [2].

In the present study, a total of 100 BTM samples were collected from apparently healthy cattle belonging to herds and flocks in many Egyptian governorates to detect the prevalence of *C. burnetii* at flock level by ELISA and PCR. All the surveyed flocks showed positive for coxiellosis with both tests. These results confirm that Q fever is endemic throughout Egypt in dairy cattle.

The overall prevalence of *C. burnetii* antibodies among the examined BTM was 51%. Based on previous reports Q fever sero/prevalence in livestock species in Africa ranges from 1–55% [22]. Seroprevalence at governorates ranged from 30 to 37.3%in cows [26, 27]. Klemmer *et al*. [28] recorded 4.2 to 36.4% seroprevalence in cattle, Jarelnabi *et al*. [29] in Saudi Arabia reported 30.7%, Johnson *et al*. [30] in Ghana reported 21.7%, Abbass *et al*. [31] reported 45.3% seroprevalence in cattle and Elhofy *et al*. [32] reported, 10.6% in milk. The higher positivity of *C. burnetii* may be attributed to the extensive husbandry system, and the fact that the cattle were in close contact with others species (sheep and goat) and wildlife [33].

These big differences among prevalence in different governorates investigated in this study could be attributed to differences in geographical scope or time and season of sample collection. A high prevalence was recorded in Alexandria (80%), Menoufia (70%), Giza (66 %), Faiyum (55 %), Ismailia (50%), Beheira (50%), followed by Gharbia (40%), Sharqia (40%), then Dakahlia (33%), BeniSuef (33%). In his study, Elhofy *et al*. [32] concluded that the highest anti-coxiella IgG seroprevalence was in Giza (30%) followed by Beni-Swif and Fayoum with 24 and 20%, respectively. Location of the farm is critical as *C. burnetii* organisms can be transmitted in airborne particles for 5 km or more, so it is suggested that a herd with low-risk status be located far from small ruminant operations [34, 35]. Klemmer *et al*. pointed out that animal species and geographical location are potential risks associated with seropositivity for *C. burnetii* infection [28].

As an alternative to serology, PCR can be used as a screening test [2]. In dairy cattle, PCR based BTM testing results for a given herd generally provide consistent results overtime and more sensitive for detection of actively infected/shedding flocks [21, 36].

A total of 15 BTM samples (11 ELISA positive and 4 negative) were examined using PCR. The IS*1111* gene of *C. burnetii* was detected only in 11 BTM samples (73.3%). Interestingly, one ELISA positive BTM sample was PCR negative. In a similar study, approximately 65% of cows with PCR-positive milk samples remained seronegative [37].

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The data illustrate that all the investigated farms had mastitis cases except that of BeniSuef governorate. It has been reported that *C. burnetii* has a high affinity for the mammary glands and uterus [38]. Depending on the sex of slaughtered animals, the seroprevalence of coxiellosis in males and females was 4.58 % and 26.62 %; respectively [33].

In the current study, BMT samples belonged to cow aging between 3 to 6 years. Animal age has been found a factor associated with exposure to Q fever in dairy cattle [29, 39]. The older animal is the more likely susceptible to the pathogen at some point in his life [40]. Age, sex, and breed, are important factors. However, animal age was strongly but not significantly associated with *C. burnetii* seropositivity in dairy cattle in Tanzania [22].

Q fever has been associated with bovine abortion [21]. Clinical signs of coxiellosis in ruminants are usually characterized by reproductive problems [16]. In the present investigation abortion cases were recorded in Gharbia, Giza, Matrouh, Minya, Menoufia, and Alexandria governorates. Coxiellosis is a disease manifesting most commonly in small ruminants as late-term abortion, stillbirth, and birth of weak offspring and rarely as abortion or reproductive failure in cattle [2]. With cows' milk samples tested for coxiellosis, only abortion showed significant elevation of IgG over IgM [41]. After abortion, bacterial shedding can be detected by PCR in vaginal mucus, feces, and milk, but patterns of shedding are different among species [2]. Nevertheless, Ruiz-Fons *et al*. [36] stated that no correlation was found between abortion and seroprevalence of *C. burnetii* in domestic ruminants.

Rodents were seen in and around farms investigated in this study in BeniSuef, Menoufia, Alexandria, Beheira and Sharqia. A study in northern Tanzania reported *C. burnetii* in 3.1% of tested rodents [42].

The presence of tick arthropods on animals of investigated farms in Ismailia, Gharbia, Alexandria, Beheira, Faiyum, Dakahlia, BeniSuef and Sharqia was elucidated. Mammals, both wild and domestic, birds, and ticks can act as reservoirs of *Coxiella burnetii* infection and also potential vehicles of transmission [2]. Concerning the climatic conditions, with average temperature of 21.8^{o}C and rainfall favor the development of pest like ticks, in this environment [33].

There was no significant correlation between breed and the seropositivity to *C. burnetii* in area investigated in this study. Concerning cattle farming system, cattle kept under extensive feeding management systems had significantly increased odds of exposure compared to cattle under intensive (zero-grazed) feeding management systems [22]. The epidemiology and risk factors of Q fever exposure in domestic ruminants in Africa reported across-sectional seroprevalence range of 3–89.7% in cattle in East Africa [22]. However, there are variations in these estimates due to different diagnostic tools with each having different specificity and sensitivity, making it difficult to compare the results across studies [20, 43].

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6. Conclusion

The detection of antibodies to Q fever in all examined regions, suggests the need for active surveillance employing a "One Health" approach to understand the epidemiology and distribution among people and animals. Therefore, further studies are necessary to fully understand the environmental and management issues within dairy cattle farms

Conflict of interest

Nothing to declare

7. References

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Table 1: Q fever prevalence in dairy cattle across 12 governorates in Egypt using ELISA on bulk tank milk samples.

Governorates	Examined number of BTM*	ELISA	
		Positive No.	%
Ismailia	4	2	50
Gharbia	15	6	40
Menoufia	7	5	70
Alexandria	5	4	80
Beheira	24	12	50
Faiyum	18	10	55
Dakahlia	6	2	33
Giza	3	2	66
BeniSuef	6	2	33
Matrouh	1	1	100
Minya	1	1	100
Sharqia	10	4	40
Total	100	51	**51**

BTM*: (60-300) cows ranging of each of the 100 farms.

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Table 2: Risks Factors associated with *Coxiella burnetii* infection cattle in six Egyptian governorates

Variables	**Governorates**					
Region	**Ismailia**	**Gharbia**	**Menoufia**	**Alexandria**	**Beheira**	**Faiyum**
Breed type	Friesian					
Age	3-6 y					
Season	Summer					
Herd size	60-300 each					
Abortion	no	yes	yes	yes	no	No
Mastitis	yes	yes	yes	yes	yes	yes
Presence of Arthropods	yes	yes	no	yes	yes	yes
Presence of rodents	no	no	yes	yes	yes	No
Water sources	Mixed with Tap & Ground					
Feeding management	Mixed with intensive & extensive					
Samples tested	4	15	7	5	24	18
ELISA positive samples	2	6	5	4	12	10
%	50	40	70	80	50	55

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Table 3: Risks Factors associated with *Coxiella burnetii* infection in another six governorates in Egypt

Variables	**Governorates**					
Region	**Dakahlia**	**Giza**	**BeniSuef**	**Matrouh**	**Minya**	**Sharqia**
Breed type	Fresian					
Age	3-6 y					
Season	Summer					
Herd size	60-300					
Abortion	No	Yes	No	Yes	Yes	No
Mastitis	Yes	Yes	No	Yes	Yes	Yes
Presence of Arthropods	Yes	No	Yes	No	No	Yes
Presence of rodents	No	No	Yes	No	No	Yes
Water sources	Mixed with Tap & Ground					
Feeding management	Mixed with intensive & extensive					
Number tested	6	3	6	1	1	10
Positive	2	2	2	1	1	4
%	33	66	33	100	100	40

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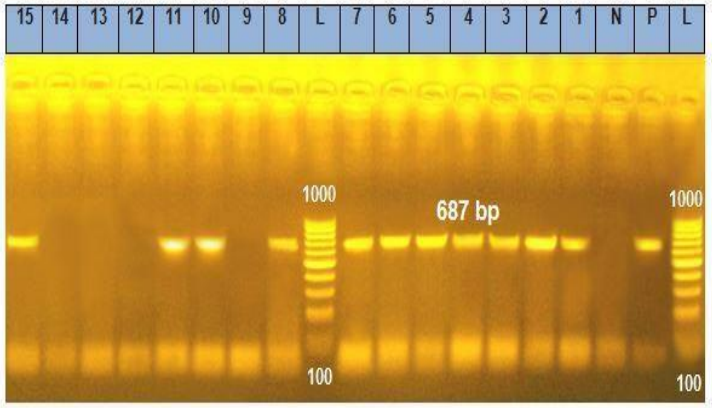

Fig. 1: *C. burnetii* IS*1111* PCR-positive samples: Lane L: DNA marker (cat. no. SM0243) from Fermentas (100-1000 bp), lane P: positive control, lane N: negative control, lanes 1-11: seropositive milk samples and lane 12-15 seronegative milk samples.

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