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Original article

SEROPREVALENCE AND MOLECULAR DETECTION OF *COXIELLA BURNETII* AMONG SHEEP IN EGYPT

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Summary

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Q fever has become one of the most common causes of abortion in sheep herds, resulting in significant financial losses for Egyptian farmers. The goal of this study was to establish *Coxiella burnetii* seroprevalence and molecular detection in three sheep farms in Egypt. A total of 184 sheep of various ages and sexes had their serum samples and vaginal swabs taken. All serum samples were checked for the presence of *C. burnetii* antibodies by using an ELISA. while 50 vaginal swabs were randomly chosen for molecular detection of the *C. burnetii IS1111* gene. The overall seroprevalence of Q fever in sheep was 37.5%, and it was more common in females (39.5%) than in males (8.3%). Antibodies to *C. burnetii* were found in more than half of pregnant ewes (47.7%). Antibodies to *C. burnetii* were found in 47.7% of pregnant ewes, compared to 31.4% in non-pregnant and abortive ewes (43.8%). Seroprevalence was observed to be significantly higher in sheep older than 3 years (71.2%). The presence of the *C. burnetii* IS1111 gene was found in 20% of the molecularly analysed vaginal swabs. Based on the abortion history and pregnancy state of the studied sheep, no statistical significance was identified, since the *C. burnetii* gene was present in equal percentages in both aborted and non-aborted ewes. A comparison of ELISA and PCR results for vaginal swab samples revealed a statistically non-significant link between the two procedures' results. These findings revealed sheep as an important reservoir for *C. burnetii* infection, implying that the role of *C. burnetii* in sheep should be studied further.

Key words: *Coxiella burnetii*, Egypt, ELISA, PCR, Q fever, sheep

INTRODUCTION

Coxiella burnetii (*C. burnetii*), an obligate intracellular proteobacterium that causes abortion in livestock and acute or chronic illness in humans, causes Query fever (Q fever), also known as coxiellosis, a worldwide contagious zoonotic bacterial disease. The disease's primary reservoirs include cattle, sheep, and goats. (Das *et al*., 2013; OIE, 2008, 2018). The agent's increased resistance to chemical and

physical conditions allows it to survive in the environment (Ceylan *et al*. 2009). Around 25% of domestic ruminants in many developing countries, particularly sheep and goats, show symptoms of current or previous *C. burnetii* infection, and are regarded as major sources of infection for their human contacts (Ruiz-Fons *et al*., 2010; Eldin *et al*., 2017; Mohabbati Mobarez *et al*., 2017; Johnson *et al*., 2019).

Although the condition in ruminants is mostly asymptomatic, reproductive issues such as late abortions, premature delivery, stillbirths, retained placenta, and delivery of weak or dead offspring, metritis, and infertility can occur in some cases (Arricau-Bouvery & Rodolakis, 2005; Ratmanov *et al*., 2013). Abortions are frequently followed by a quick recovery with no complications. The illness of Q fever can last for years, if not the entire life of the animal (Kirkan *et al*. 2008; OIE, 2008).

Infected females can shed *C. burnetii* into the environment persistently without showing any symptoms during abortion or regular parturition through vaginal fluids, placental fluids, and birth fluids. Furthermore, the bacterium can be shed in milk, faeces, and urine. The route and duration of shedding varies among ruminant species – sheep shed the bacteria primarily in vaginal fluid and for a long time, but goats shed the bacteria primarily in faeces and for a short time (OIE, 2008; Angelakis & Raoult, 2010; Keyvani Rad *et al*., 2014; Bauer *et al*., 2020). It is worth noting that asymptomatic persons and intermittent cattle shedders might test negative for the pathogen in serological testing while unknowingly shedding it into the environment for months or years (De Cremoux *et al*., 2012).

Serological procedures such as immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA), and complement fixation tests (CFT) are used to diagnose Q fever. Isolation of the pathogen is a reliable diagnostic procedure, but is time-consuming and hazardous, necessitating biosafety level 3 standards (Angelakis & Raoult, 2010). The World Animal Health Organization (OIE) recommended CFT and ELISA tests as more sensitive and specific (Sidi-Boumedine *et al*., 2010; Emery *et al*., 2014). The CFT is time-consuming and requires specialised laboratory equipment, whereas ELISA has ready-to-use kits and is thus the preferred diagnostic method (OIE, 2015). While serological approaches suggest prior exposure, molecular testing employing polymerase chain reaction (PCR) offers the benefit of detecting bacteraemia and continuing infection.

Few reports of seroprevalence of Q-fever in sheep have been found in Egypt. According to El-Mahallawy *et al*. (2012), the seroprevalence of Q fever in sheep in Ismailia province was 12.1%. Abushahba *et al*. (2017) found seroprevalence of 25.68% in El Minya Governorate, and disease seroprevalence was 8.9% in Egypt, and 22.7% in north Egypt, according to Klemmer *et al*. (2018) and Selim *et al*. (2018). In Assiut Governorate, Sobhy & Gahlan (2019) and Abbass *et al*. (2020) found 20% and 60% seroprevalence, respectively. According to these findings, Q fever has been present in Egyptian ruminants for several decades. However, the incidence of *C. burnetii* as an etiological cause of animal abortion has not been thoroughly investigated (Gwida *et al*. 2014; Abdel-Moein & Hamza, 2017). Therefore, the goal of this study was to use ELISA and PCR to investigate the prevalence of Q fever in sheep farms in two Egyptian provinces (Assiut and Sohag).

MATERIALS AND METHODS

Study area

This study was conducted in the Egyptian provinces of Assiut and Sohag. Assiut is 389 kilometers south to Cairo, Egypt's capital, while Sohag is 507 kilometers away. Sheep are raised on a small scale (2–100 animals), either individually or as part of a flock. Samples were taken from three sheep farms, two in Assiut and one in Sohag, between September 2020 and October 2021. Consent was obtained orally from all farm owners before the study.

Animals

A total of 184 sheep were randomly chosen for the investigation (172 female and 12 male). The average age of the animals chosen was 3.28 ± 1.19 years. The female participants were classified into three groups based on their pregnancy status: pregnant, non-pregnant, and aborted. Farm 1 yielded 41 animals, farm 2 yielded 81, and farm 3 yielded 62 sheep.

Ethics approval

Animal handling were conducted in line with animal welfare regulation and the guide for the care and use of animals. All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and animal ethics was approved by the Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt (No. 4 09 2020, Assiut, Egypt).

Samples

Blood samples. Approximately 5 mL of blood was taken from each animal via jugular venipuncture and injected directly onto the inner surface of a clean, dry, sterile plain vacutainer tubes using sterile syringes. Locality, age, sex, pregnancy status, and abortion history were all included in the labelling numbers and data. The tubes were maintained vertically in an ice box and promptly moved to the laboratory at the Department of Animal Medicine (Clinical Laboratory Diagnosis), Faculty of Veterinary Medicine, Assiut University. The blood samples were cooled in the refrigerator for 30 min to coagulate, then centrifuged at 3,000 rpm for 15 min to separate the clear blood serum, which was preserved in clean dry Eppendorf tubes and stored at –20 °C for future analysis. The blood serum sample was used in an indirect enzyme linked immune sorbent assay for serological analysis (ELISA).

Vaginal swabs. Swabs were taken by rubbing a sterile cotton swab against the inner vaginal wall to ensure capture of cells and intracellular microorganisms. Labelling numbers and associated data, such as location, age, pregnancy status, and abortion history, were included and then swabs transported to the laboratory, where they were stored at –20 °C until processing for *C. burnetii* molecular detection.

Serological detection

Serum samples were tested for the presence of IgG by using a commercially available indirect enzyme linked immune sorbent assay (ELISA) kit ID Screen® Q fever Indirect Multispecies (ID. Vet innovative diagnostics, Grables, France) following the manufacture instructions. The technique uses microtiter plates precoated with a purified *C. burnetii* antigen. The microtiter plate was read at a wavelength of 450 nm. The results were interpreted according to the producer equation:

$$S/P\ \% = 100 \times \frac{\text{Sample OD} - \text{Negative control OD}}{\text{Positive control OD} - \text{Negative control OD}}$$

where OD is the optical density.

Samples of S/P % ≥50% were considered positive for *C. burnetii* infection.

Molecular detection

A total of 50 vaginal swabs were selected for molecular detection of *C. burnetii* (25 swabs were from the seropositive animals for *C. burnetii* antibodies by the indirect ELISA, and 25 swabs from seronegative ones). Those swabs were collected randomly from Farm 2 and Farm 3 only for the molecular detection of *C. burnetii* (*IS1111* gene). Sixteen vaginal swabs were from aborted ewes, 34 from non-aborted ewes (20 from pregnant and 14 from non-pregnant ewes). Vaginal swabs from Farm 1 were not collected. DNA was extracted from swabs using a commercial QIAamp DNA mini kit (Qiagen, France) according to the manufacturer's instructions. DNA extracts were stored at –20 °C until tested using a conventional polymerase chain reaction for detection of *IS1111* gene of *Coxiella burnetii*. *C. burnetii* (*IS1111*) screening was carried out by PCR using primers (Sigma-Aldrich) Trans-1: 5′-TAT GTA TCC ACC GTA GCC AGT C-3′ and Trans-2: 5′-CCC AAC AAC ACC TCC TTA TTC-3′ (Hoover *et al*. 1992). The PCR reactions were carried out in total volume of 25 µL and the thermocycler was programmed with the following PCR cycling conditions: initial denaturation at 94 ºC for 5 min; 5 cycles of: denaturation of 30 s at 94 ºC, followed by a primer annealing at 66 to 61 ºC for 1 min; extension at 72 ºC for 1 min; these cycles were followed by 35 cycles consisting of denaturation of 30 s at 94 ºC, followed by a primer annealing at 61 ºC for 30 s; extension at 72 ºC for 1 min and a final extension of 10 min at 72 ºC. Twenty µL amplified PCR products of each sample, negative control, and positive control, along with a 100 bp DNA ladder, were loaded onto a 1.5% agarose gel stained with ethidium bromide in gel electrophoresis. The product size of the reaction was 687 base pairs (bp).

Statistical analysis

To measure the impact of each factor individually on the seroprevalence of *C. burnetii* in examined sheep (i.e., age, sex, pregnancy status, abortion status, and farm), relative risk and chi-square tests were calculated in IPM SPSS Statistics software (IBM Corp, USA, Version 26). To measure the association between ELISA seropositive and seronegative sheep with the molecular detection of *C. burnetii*, odds ratios and 95% confidence intervals were used. A probability value (P-value) of $P<0.05$ was considered statistically significant.

RESULTS

Seroprevalence of C. burnetii antibodies by ELISA

The overall seroprevalence of *C. burnetii* antibodies in sheep was 37.5% (69 out of 184). Most seropositive animals were older than 3 years, and the difference between age groups was very highly statistically significant ($P=0.0001$). Females were found to have significantly higher seropositivity (39.5%) than males (8.3%). There was no statistically significant link between abortion and the rate of Q fever infection in examined animals. Pregnant ewes had a significant greater seroprevalence of *C. burnetii* antibodies (47.7%) than non-pregnant and abortive ewes. Compared to the other farms in the study, Farm 2 had a statistically significant higher seroprevalence of *C. burnetii* (49.4%) (Table 1).

Table 1. Factors associated with *C. burnetii* seroprevalence among the examined sheep

Factor	No. tested	ELISA		Odds ratio	95% CI	P-value
		Positive n (%)	Negative n (%)			
Age						
1-2 years	55	13 (23.6)	42 (76.4)	1.857	0.73 – 4.76	0.0001
> 2-3 years	63	9 (14.3)	54 (85.7)	Reference		
>3 years	66	47 (71.2)	19 (28.8)	14.84	6.13 – 35.94	
Total	184	69 (37.5)	115 (62.5)	–	–	
Sex						
Female	172	68 (39.5)	104 (60.5)	7.19	0.91 – 56.99	0.064
Male	12	1 (8.3)	11 (91.7)	0.14	0.02 – 1.10	
Total	184	69 (37.5)	115 (62.5)	–	–	
Pregnancy						
Pregnant	86	41 (47.7)	45 (52.3)	1.99	1.07 – 3.71	0.04341
Non-pregnant	86	27 (31.4)	59 (68.6)	0.502	0.27 – 0.94	
Total	172	68 (39.5)	104 (60.5)	–	–	
Abortion						
Yes	16	7 (43.8)	9 (56.2)	1.21	0.43 – 3.42	0.925
No	156	61(39.1)	95 (60.9)	0.83	0.29 – 2.33	
Total	172	68 (39.5)	104 (60.5)	–	–	
Farm						
Farm 1	41	6 (14.6)	35 (85.4)	Reference		0.0001
Farm 2	81	40 (49.4)	41 (50.6)	5.691	2.16 – 15.00	
Farm 3	62	23 (37.1)	39 (62.9)	3.44	1.26 – 9.42	
Total	184	69 (37.5)	115 (62.5)	–	–	

Correlation coefficient: r^2: Age = 0.16, Sex = 0.03, Pregnancy = 0.02, Abortion = 0.00 and Farm = 0.02.

Molecular detection of C. burnetii

A total of 50 vaginal swabs were examined for the presence of *C. burnetii* DNA using a standard PCR assay (25 swabs from seropositive ewes and 25 swabs from seronegative ones). Positive samples showed specific, obvious bands of the 687-bp region, indicating that 20% of the swabs analysed were positive for *C. burnetii* infection (Fig. 1).

Comparing ELISA and PCR data, there was no statistically significant association between the two procedures' results (P=1.000). *C. burnetii* DNA was found in a larger percentage in non-pregnant ewes (26.7%) than in pregnant ones (only 10%). It was found in about identical percentage in both abortive (18.8%) and non-abortive ewes (20.6%). There was non-statistically significant difference in PCR results based on age, pregnancy, abortion, and farms (Table 2).

DISCUSSION

Q fever is a disease that has veterinary and public health implications all over the world (Georgiev *et al*., 2013). It mostly causes abortion and mastitis in sheep, resulting in significant financial losses for

animal producers and the country's economy (Cutler *et al*., 2007). Furthermore, financial losses may result from *C. burnetii* shedding in milk (Pexara *et al*., 2018). Q fever diagnosis in sheep is critical not only for identifying diseased flocks but also for determining the risk of disease transmission to people (OIE, 2015; Ullah *et al*., 2019). It has recently become more common in animals, particularly sheep and goats (Gwida *et al*., 2012). In most countries, including Egypt, the epidemiology and prevalence of Q fever have not been thoroughly investigated. As a result, most laboratory and veterinary practitioners do not consider Q fever in small ruminants as an abortive disease (Gwida *et al*., 2014). Therefore, the study aimed to investigate the disease prevalence in sheep by serological (ELISA) and molecular (PCR) methods.

The overall seroprevalence of Q fever in examined sheep was 37.5%. Ghoneim & Khaled (2012) reported similar seroprevalence (32.7%). Lower seroprevalence of *C. burnetii* in sheep was reported by Horton *et al*. (2014) and Klemmer *et al*. (2018) – 8% and 8.9% respectively. Also, El-Mahallawy *et al*. (2012) found 12.1% overall seroprevalence of Q fever in sheep in Ismailia province. According to Abushahba *et al*. (2017), the seroprevalence of Q fever in sheep in El Minya Governorate was 25.68%. According to Sobhy & Gahlan (2019), the total seroprevalence of *C. burnetii* in sheep in Egypt was 25.5%, with 20% in El Giza 25.7% in El Fayoum, 30% in Beni Sueif, 28.5% in El Menia, 30% in El Mansoura, 25% in El Sharkia, 20% in Assiut, and 26.7% in Qena. The seroprevalence in the current study was lower than that in previous reports from Egypt, with 60% in sheep in Assiut governorate (Abbass *et al*., 2020), 61.96% in Menofiya governorate (Byomi *et al*., 2019), and 50% in

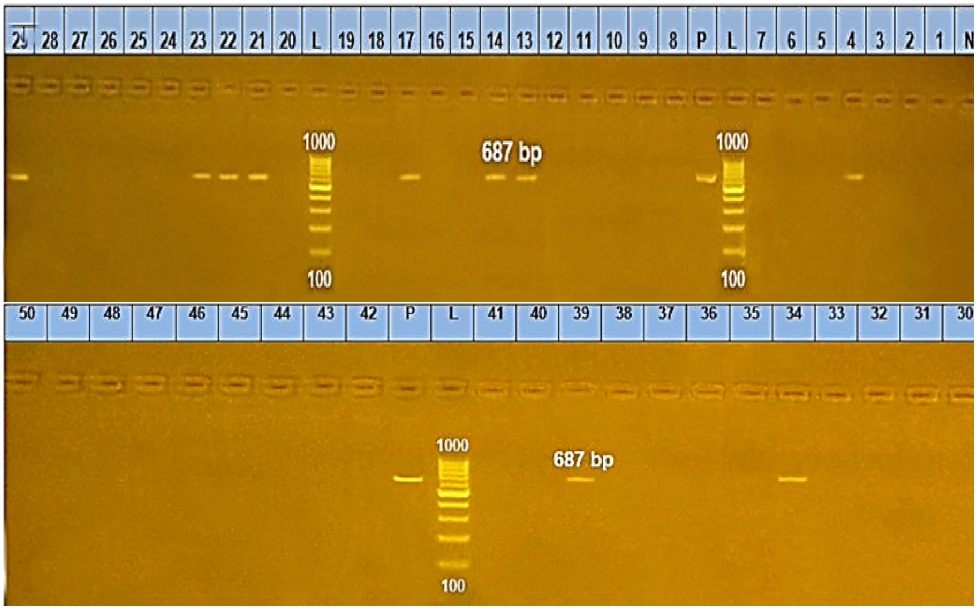

Fig. 1. PCR- product in the electrophoresis gel, the product size of the reaction was 687 bp. Lanes 1-50: examined DNA samples, L: 100 bp ladder, N: negative control and P: positive control.

Table 2. *C. burnetii* DNA detected by PCR in examined sheep (n=50)

Factor	No. tested	PCR analysis		Odds ratio	95% CI	P-value
		Positive n (%)	Negative n (%)			
ELISA test						
Positive	25	5 (20.0)	20 (80.0)	1.000	0.25 – 3.998	0.724
Negative	25	5 (20.0)	20 (80.0)			
Total	50	10 (20.0)	40 (80.0)	–	–	
Age						
1–2 years	16	4 (25.0)	12 (75.0)	2.333	0.45 – 12.23	0.423
>2–3 years	10	3 (30.0)	7 (70.0)	3.000	0.49 – 18.42	
>3 years	24	3 (12.5)	21 (87.5)	Reference		
Total	50	10 (20.0)	40 (80.0)	–	–	
Pregnancy						
Pregnant	20	2 (10.0)	18 (90.0)	0.306	0.058 – 1.62	0.279
Non-pregnant	30	8 (26.7)	22 (73.3)	3.273	0.62 – 17.39	
Total	50	10 (20.0)	40 (80.0)	–	–	
Abortion						
Yes	16	3 (18.8)	13 (81.3)	0.89	0.197 – 4.01	0.8811
No	34	7 (20.6)	27 (79.4)	1.12	0.25 – 5.06	
Total	50	10 (20.0)	40 (80.0)	–	–	
Farm						
Farm 2	33	8 (24.2)	25 (75.8)	2.4	0.45 – 12.83	0.461
Farm 3	17	2 (11.8)	15 (88.2)	0.42	0.08 – 2.23	
Total	50	10 (20.0)	40 (80.0)	–	–	

northern Egypt (Hegazy *et al*., 2021). Moreover, Ullah *et al*. (2019) recorded a seroprevalence of 15.3% in small ruminants in Pakistan. According to Ezatkhah *et al*. (2015), the seroprevalence of Q fever in small ruminants was 26.4% in five counties in Iran's southeast area, ranging from 5% in Sarbaz
to 39.2% in Rudan. In Ghana, Johnson *et al*. (2019) found 28.4% seroprevalence of Q fever in sheep. The obtained results are lower than the previously reported high frequency of 69.4% and 75% in Pakistan by Zahid *et al*. (2016). On the other side, the district of Rajanpur in Pakistan had a very low seroprevalence (5.8%), which could be related to superior hygienic measures. Geographical location, kind of animal husbandry, and animal age all influenced seroprevalences, not the animal's origin. As a result, Q fever is endemic in sheep all over Egypt (Hussein, 2021). This variation in the prevalence of infection of Q fever could be linked to farm hygiene, regular management techniques, and environmental factors such as vegetation, soil moisture, and the presence of infected animals in the surroundings (Rizzo *et al*., 2016). These management and environmental factors may be to blame for the higher seroprevalence of *C. burnetii* infection in sheep and animal miss-care including the free movement of the flocks, poor fencing, insufficient confinement housing at lambing and indiscriminate buying without adequate qua-

rantine are additional factors in spreading of the infection among sheep (El-Mahallawy *et al*., 2012). The high prevalence of Q fever disease in sheep may be due to the animals being kept indoors for long periods of time and living in crowded and unsanitary environments, which encourage the occurrence and spread of *C. burnetii* infection (Karaca *et al*., 2009).

Because immunological responses only indicate evidence of previous and/or current exposure to *C. burnetii*, but not shedding animals, serological diagnosis of *C. burnetii* antibodies in sheep is insufficient. As a result, detecting shedding animals requires more than a serological diagnosis (Muskens *et al*., 2011). PCR detected *C. burnetii* DNA in 20% of the examined vaginal swabs. Higher result was reported by Abiri *et al*. (2019), who found that 33.5% of vaginal swabs were positive for *C. burnetii* in sheep. *C. burnetii* DNA was detected in an equal percentage in both seropositive and seronegative samples. This may be due to the after-disease outbreak in sheep, where shedding continues while no clinical signs of the disease are present, as shedding of *C. burnetii* in the vaginal secretions persist for a long time after infection. In fact, vaginal secretion is the most important way of shedding *C. burnetii* in sheep (Ruiz-Fons *et al*. 2010; De Cremoux *et al*. 2012). *C. burnetii* bacteria have been detected for a long time after abortion and normal parturition in vaginal secretions of sheep. Infected sheep may shed high numbers of bacteria in their excretions. Animals may shed bacteria and/or remain seropositive long after the acute infection (Cong *et al*., 2015).

Although the prevalence of Q fever was 7 times higher (odds ratio = 7.19) in female sheep (39.5%) than in males (8.3%) it was statistically non-significant (P=0.064). The higher odds ratio may be due to females being exposed to more risk factors such as pregnancy, parturition, and abortion (Melenotte *et al*. 2018). This finding was in agreement with previous studies (Kilic *et al*., 2005; Sakhaee & Khalili, 2010; El-Mahallawy *et al*., 2012; Zahid *et al*., 2016; Abushahba *et al*., 2017). The higher occurrence of *C. burnetii* seropositivity in female animals might be explained by the fact that after becoming infected, ewes shed large quantities of *C. burnetii* into the environment during abortion or normal parturition through birth fluids, placenta, and foetal membranes. Moreover, following parturition, these infected ewes excrete the bacteria in urine, faeces, vaginal discharge, and milk for several months (Bouvery *et al*., 2003). Naturally infected ewes shed *C. burnetii* in faeces during 8 days after lambing (Berri *et al*., 2001), and *C. burnetii* infection persist for years and may be lifelong (McQuiston *et al*., 2002).

There was a highly significant relation between age of examined sheep and Q fever infection rate. Higher infection rate was recorded in sheep more than 3 years old (71.2%), followed by 23.6% in animals 1–2 years old, lastly 14.3% in animals older than 2–3 years of age. These findings are in line with those of Byomi *et al*. (2019) in Egypt's Menofia governorate and Hegazy *et al*. (2021) in northern Egypt. García-Pérez *et al*. (2009), Kennerman *et al*. (2010), Klaasen *et al*., (2014), Ezatkhah *et al*. (2015), and Rizzo *et al*. (2016) revealed that the age of studied sheep had a substantial impact on the frequency of Q fever occurrences. On the other hand, the results from this study contradict those of Abushahba *et al*. (2017), El-Mahallawy *et al*. (2012), Ullah *et al*. (2019), and Elelu *et al*. (2020), who found no significant association between age and Q fever infection rate. Infection in older sheep may be due to more fre-

quent exposure to the bacteria over the course of their lives (García-Pérez *et al*. 2009). On the other hand, the high seroprevalence of Q fever in sheep aged 1–2 years may indicate that natural exposure occurs in sheep population, particularly in the first year of life. However, control of *C. burnetii* infection at this age is critical because this period has a high risk of infection (Kennerman *et al*., 2010). *C. burnetii* shed in various sources (vaginal discharge, milk, urine, faeces, and birth products) survives in the environment for long periods of time while resisting many physical and chemical stresses such as elevated temperature and pressure, desiccation, osmotic shock, and several chemical disinfectants, which could explain the high significance among different age groups in the examined sheep. As a result, the amount of time sheep spend in contact with disease sources tends to grow as they get older (Byomi *et al*., 2019). The wide variation among different age groups in sheep may be due to exposure to common source of infection and disease emergence in the study area (El-Mahallawy *et al*., 2012). Moreover, *C. burnetii* contact rate tends to increase with age in sheep, simply as a consequence of a higher probability of contact with life span (Ruiz-Fons *et al*., 2010).

In the present study, pregnant ewes had a significantly higher seroprevalence of Q fever (47.7%) than non-pregnant ewes (31.4%). Similarly, Abushahba *et al*. (2017) found that the seroprevalence of *C. burnetii* was greater in pregnant (26.76%) than in non-pregnant (23.68%) ewes. This could be caused by the immunosuppressive effects of pregnancy (Rahman *et al*., 2016), as trophoblast cells of the chorioallantoic are the main primary target cells of *C. burnetii* (Van den Brom *et al*., 2015).

Abortive ewes had a non-significant higher seroprevalence of Q fever than non-abortive ewes. In sheep, Q fever is typically asymptomatic because abortion is the only clinical outcome. These findings suggest that non-abortive ewes may be infected with *C. burnetii* on a subclinical level (Van den Brom *et al*., 2015). Because native breeds, which are extensively reared in Egypt, are relatively immune to infection, the majority of *C. burnetii* infections among sheep in Egypt are subclinical (Ghoneim & Khaled, 2012). The presence of *C. burnetii* antibodies in sheep with a history of reproductive difficulties (abortion and stillbirth) does not rule out the possibility of infection with additional pathogens such as *Brucella melitensis* and *Toxoplasma gondii*. In addition, malnutrition in pregnant sheep could lead to abortion (Arserim *et al*., 2011).

The statistically significant high seroprevalence on Farm 2 (49.4%) could be related to the exposure of the investigated sheep to poor sanitary conditions, which might help *C. burnetii* to remain in the soil for a long time. Furthermore, overcrowding of sheep on Farm 2, is a major factor in the spreading of *C. burnetii* infection across the farm (Cong *et al*., 2015; Rizzo *et al*., 2016; Byomi *et al*., 2019). Farm 1 low seroprevalence (14.3%) could be attributed to the farm's stronger hygiene procedures.

CONCLUSION

The current study found that Q fever is prevalent among sheep in Assiut governorate, Egypt. It is the leading cause of reproductive issues in sheep, as well as of significant economic loss. Higher seroprevalence was found in pregnant ewes over the age of three years. PCR is a good test for detecting shedder animals.

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