SEROPREVALENCE AND MOLECULAR DETECTION OF 
COXIELLA BURNETII AMONG SHEEP IN EGYPT

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Summary


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-abortion 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 (Arriau-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. 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 pre-coated 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:

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

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-abortion ones (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 °C for 5 min; 5 cycles of: denaturation of 30 s at 94 °C, followed by a primer annealing at 66 to 61 °C for 1 min; extension at 72 °C for 1 min; these cycles were followed by 35 cycles consisting of denaturation of 30 s at 94 °C, followed by a primer annealing at 61 °C for 30 s; extension at 72 °C for 1 min and a final extension of 10 min at 72 °C. 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).
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 no 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 (Abass 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.
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-

| Table 2. C. burnetii DNA detected by PCR in examined sheep (n=50) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Factor                     | No. tested | Positive n (%) | Negative n (%) | Odds ratio 95% CI | P-value |
| **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 | 0.279 |
| >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 | 0.8811 |
| 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 |     |
| 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 |     |
| Farm 3                     | 17         | 2 (11.8)       | 15 (88.2)      | 0.42           | 0.08 – 2.23 | 0.461 |
| Total                      | 50         | 10 (20.0)      | 40 (80.0)      |                |         |     |
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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. As a result, detecting shedding animals, serological diagnosis of 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. Garcia-Pérez et al. (2009), Kinnerman et al. (2010), Klaassen 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 (Arsirim 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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REFERENCES


From Q fever to *Coxiella burnetii* infection: A paradigm change. *Clinical Microbiology Reviews*, 30, 115–190.


Kirkkan, Ş., Kay, O., Tekbıyık, S. & U. Parin, 2008. Detection of *Coxiella burnetii* in...
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Muskens, J., E. Van Engelen, C. Van Maanen, C. Bartels,& T. Lam, 2011. Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. *The Veterinary Record*, 168, 79.


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