**Coxiella burnetii** infections among small ruminants in Ismailia Governorate

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**Abstract**

In a survey of Q fever in small ruminants in Ismailia Governorate, a total of 182 animals (91 sheep and 91 goats) of different ages were selected from different farms and veterinary clinics in Ismailia Governorate during the period from December 2010 to December 2011. Two blood samples (one without anticoagulant and the other with EDTA anticoagulant) were collected from each animal and subjected to serologic examination using ELISA (CHECKIT, IDEXX) and PCR assay. The overall *Coxiella burnetii* seroprevalence rate was 14.3% with occurrence rate of 12.1% in sheep and 16.5% in goats. Infection was more common in female animals and especially in those aged 1-2 years old. Seropositive cases were reported from 5 (62.5%) out of 8 sheep farms and 6 (66.7%) out of 9 goat farms examined, in comparison to 9.1% and 21.4% seropositivity among individual cases of sheep and goats, respectively, that had been collected from different veterinary clinics. Regarding the distribution of the disease in different districts in Ismailia Governorate, the highest occurrence of *C. burnetii* seroprevalence in sheep was observed in El Mahsama (37.5%) followed by Abu Balah (33.3%), Ein Ghosein (12.5%) and Wadi El Eshra (9.1%), while the lowest occurrence was observed in El Mananeif (5.6%). In goats, highest infection rates were in Wadi El Eshra (30.8%), El Mahsama (14.3%) followed by El Dabaeia (12.5%), while the lowest rate was in El Manaef (7.7%). PCR performed on 26 seropositive whole blood samples failed to detect *C. burnetii* genome in all of them. The study clarified that Q fever is widely distributed among small ruminants in Ismailia Governorate and they may represent an important source for human infections.

**Introduction**

Q fever is a zoonosis caused by obligate intracellular bacteria *Coxiella burnetii*. The disease is endemic worldwide, occurring in different geographic regions and climatic zones except in New Zealand (Maurin and Raoult, 1999).
The interest for Q fever is increasing worldwide as indicated by the rising number of reviews published (Arricau Bouvery and Rodolakis, 2005) even in countries where its incidence is supposed to be very low. Since the importance of Q fever became apparent, serologic surveys have been performed to trace the prevalence of the disease in both humans and animals (Kilic et al, 2005). Because of the polymorphism of the clinical picture, the potential hazards of the microorganism and because the diagnosis is based exclusively on serology, the prevalence of C. burnetii infection among animals is largely unknown (Rey et al, 2000) and the studies were mostly depending on serological investigation, worldwide (Kalender, 2001). Several studies concluded that ruminants are important reservoir for C. burnetii (Fishbein and Raoult, 1992; Ceylan et al, 2009; Rahimi et al, 2010 and Vaidya et al, 2010), especially sheep and goats are implicated as an important reservoirs for C. burnetii in Egypt (Nahed and Khaled, 2012). The organism is shed in the urine, feces and milk of infected animals. In general, infected animals remain asymptomatic and they often serve as the source of infection for humans via infective aerosols or contaminated dust (Maurin and Raoult, 1999). Reliable detection of C. burnetii shedders is a critical point for the control of the spread of this bacterium among animals and from animals to humans (Guatteo et al, 2006). In view of lack of available information about the prevalence of Q fever among small ruminants, this study was designed to assess the prevalence and distribution of Q fever infection among these animals in Ismailia Governorate.

Material and Methods
1. Animals:
During the period from December 2010 to December 2011, a total of 182 small ruminant animals (91 sheep and 91 goats) were selected randomly from different farms and veterinary clinics in Ismailia Governorate for examination for C. burnetii infection.

2. Sampling:
Two blood samples were collected from each animal, one with anticoagulant (EDTA) and the other without anticoagulant for separation of serum. Each sample was collected with a detailed data about animal species, age, sex, tick infestation, previous history of any reproductive problems and date of sample collection. All specimens were transferred immediately on ice packs to the Zoonoses lab, in the Department of Hygiene, Zoonoses and Animal Ethology, Faculty of Veterinary Medicine, Suez Canal University for processing. Sera were obtained from blood samples without anticoagulant by centrifugation at...
2000-3000 rpm for 5 min after allowing the blood to clot naturally at 37°C for 30-60 minutes. Serum samples were separated from the collected blood and poured into clean sterile labeled Eppendorf tubes. EDTA blood and serum sample form each examined animal was labeled and kept at -20°C until assayed.

3. Serological examination:
All sera were assayed using ELISA. The test was performed using ready-to-use commercially available kits (CHECKIT, IDEXX laboratories, Switzerland) according to the manufacturer instructions which can detect both C. burnetii anti-phase I and II antibodies.

4. Polymerase Chain Reaction.
PCR was performed on 26 seropositive (11 sheep and 15 goat) whole blood samples.
(i) DNA extraction was done using spin-column whole blood DNA extraction Mini Kit (BioTeke®, Beijing, China) according to the manufacturer instructions.
(ii) DNA concentration and purity were measured by NanoDrop spectrophotometer (ND-1000 UV/Vis spectrophotometer, USA, www.nanodrop.com) (Sambrook and Russel, 2001).
(iii) Oligonucleotide primers (Berri et al, 2000):
The sequences were retrieved from the GenBank (accession number M80806), where each C. burnetii Nine Mile Creek strain chromosome contains at least 19 copies of this sequence, and every C. burnetii isolate tested so far has multiple copies of this element (Berri et al, 2000 and Hoover et al, 2002).
The oligonucleotide primers were designed by alignment of published DNA sequence of Berri et al (2000). Primers were purchased form Alpha DNA, Montreal, Quebec. The sequences and nucleotide positions of both forward and reverse primers are summarized in Table (1).
(iv) Polymerase Chain Reaction (Berri et al, 2000):
The Trans-PCR thermal program is performed in a thermal cycler (Eppendorf Vapoprotect Mastercycler Pro S, Germany) in a total reaction volume of 25 µl, containing 12.5 µl of PCR Master Mix (BioTeke, Beijing, China P.R.), 2 µl of each primer, 3.5 µl of sterile nuclease free water and 5 µl of template DNA. PCR tubes were marked, tapped gently and mixed gently then transferred to the thermal cycler. Water was used as negative control, while positive control was C. burnetii NineMile RSA493 strain supplied by Dr. Carsten Heydel, (Giessen University, Germany). The PCR amplification program includes 5 cycles consisting of denaturation at 94°C for 30 s, annealing at 66 ± 61°C (the temperature will be decreased by 1°C between consecutive steps) for 1 min and the extension at 72°C for 1 min and then 40 cycles consisting of denaturation at 94°C for 30 s,
annealing at 61°C for 30 s and extension at 72°C for 1 min followed by final extension at 72°C for 2 min. The amplified products were detected by electrophoresis (DYY-6C, Beijing, 75 ampere) using Agarose gel with ethedium bromide (1.5% Agarose, Biotechnology, China; stained with 0.5 µg/ml ethedi, BioShop, Canada), TBE buffer 0.5X (Sambrook and Russel, 2001) and 100bp DNA molecular weight marker (BioTeke, China), 100 to 1500 log scale, and the amplified product was visualized using automated UV photo documentation system (UVP, BioSpectrum®, UK). Specific C. burnetii band was detected at 687 bp.

Results and discussion
Several studies conducted in many areas confirmed that C. burnetti is widely distributed in several countries and concluded that sheep and goats are important reservoirs for Q fever infection (Asmaa, 1993; Çetinkaya et al, 2000; Hatchette et al, 2002; Parisi et al, 2006 and Mazyad and Hafez, 2007).
The overall seroprevalence of Q fever in sheep and goats was 14.3%, Table (2). This finding was lower than that reported by Parisi et al (2006) (21.5%); while it was higher than that reported by Cekani et al (2008) (9.8%). The highest prevalence of C. burnetii antibodies was reported among goats (16.5%). This result was nearly similar to those reported by Hatchette et al, (2002) (15.6%) and (Mazyad and Hafez, 2007) (16.8%). While, it was lower than that previously recorded in different studies in Egypt by Asmaa (1993) (80.3%) and Nahed and Khaled (2012) (23.3%). On the other hand; lower rate of Q fever seroprevalence in goats was reported by Ruiz-Fons et al, (2010) (8.7%). This high occurrence of positive reactor goats for Q fever is of utmost importance to pay attention to the role of such animals as a natural reservoir of C. burnetii in the study area. The occurrence rate of Q fever antibodies in sheep was 12.1% (Table, 2). Nearly similar results were obtained by Çetinkaya (2000) (10.5%) and Ruiz-Fons et al (2010) (11.8%). On the other hand; lower result was reported by Kilic et al (2005) (3%). Meanwhile; higher results were reported by Asmaa (1993) (67.3%); Mazyad and Hafez (2007) (22.5%) and Nahed and Khaled (2012) (32.7%) in Egypt. Animal miss-care including the free movement of the flocks, poor fencing, insufficient confinement, housing at lambing and indiscriminate buying without adequate quarantine are additional factors in spreading of the infection among sheep (Asmaa, 1993). Such high frequency of C. burnetii amongst small ruminants may be attributed to keeping those animals indoor for a long period and living in crowded and unhygienic pens
that increase the occurrence and spreading of contagious diseases (Karaca et al, 2009).

In this study, both nannies (20%) and ewes (14.3%) showed a higher seroprevalence of Q fever than bucks (9.7%) and rams (8.6%). There was no statistically significant difference in the distribution of C. burnetii antibodies between males and females of the examined farm animals, (P >0.05). This finding was in agreement with Kilic et al (2005). This higher occurrence of C. burnetii seropositivity in female animals could be explained by the fact that after becoming infected, female animals shed large quantities of Coxiella into the environment during abortion or normal delivery through birth fluids, placenta and foetal membranes. Moreover, following parturition, these infected animals excrete the bacteria via the urine, faeces, vaginal discharges, and milk for several months (Arricua-Bouvery et al, 2003).

In the present study, older goats showed higher occurrence rate for C. burnetii antibodies (19.2%) than those aged 1-2 years old (15.6%), while those younger than one year old hadn't any C. burnetii antibodies. While in sheep, the highest occurrence of C. burnetii antibodies was reported in those aged 1-2 years (12.9%) followed by those ≤ 1 year old (11.1%), while older sheep had the lowest rate of C. burnetii antibodies (8.3%). Variability of different age groups of sheep and goats to Q fever infections was not statistically significant (P>0.05). These findings disagreed with García-Pérez et al (2009) who stated that older ewes showed a significantly greater prevalence (17.5%) compared with yearlings (7.5%) or replacement lambs (1.5%). Also our results varied from those reported by Kennerman et al (2010) who mentioned that primiparous ewes (1-year old) had higher antibody rates than newborn sheep (aged less than 10 months) or biparous ewes (2 years old). This wide variation among different age groups in sheep and goat may be due to exposure to a common source of infection, where goats shed C. burnetii in feces before and after kidding and the mean duration of excretion is 20 days when they are experimentally infected at 90 days of gestation (Arricau-Bouvery et al, 2003). Also, Berri et al (2001) mentioned that naturally infected ewes shed C. burnetii in feces during 8 days after lambing. Moreover, McQuiston et al (2001) stated that C. burnetii infection may persist in ruminants for years, and may be lifelong.

Concerning that matter, Ruiz-Fons et al (2010) observed that pathogen contact rate tends to increase with age in sheep and goats, simply as a consequence of a higher probability of contact with life span.

Out of 8 sheep farms examined, in the present study, 5 (62.5%) showed occurrence of Q fever antibodies, while occurrence rates among the
individual cases collected from Ismailia veterinary clinics was 9.1%, as shown in Table (3). The most highest C. burnetii antibody occurrence rate was reported in El Mahsama (37.5%) followed by Abu Balah (33.3%), Ein Ghosein (12.5%) and Wadi El Eshra (9.1%), while the lowest occurrence was observed in El Manaeif (5.6%).

In this study, occurrence of C. burnetii antibodies was proved in 6 (66.7%) out of 9 goat farms examined, while the overall occurrence rate among the individual cases examined from veterinary clinics was 21.4%, Table (4). The highest area with Q fever distribution in goats was Wadi El Eshra (30.8%) and El Mahsama (14.3%) followed by El Dabaeia (12.5%), while the lowest rate was in El Manaeif (7.7%). The previously declared findings confirm that the collections of these animals in large or small herds are important for disease transmission (Angelakis and Raoult, 2010). Data obtained in Table (3) and Table (4) revealed that in two (Wadi El Eshra and El Manaeif) of four (Abo Atwa, Wadi El Eshra and El Manaeif, 2 farms) combined farms of sheep and goats there was one seropositive sheep and goat in each of them. This variation in the occurrence of C. burnetii antibodies among the examined farm animals in different locations mentioned before, may be attributed to difference in the animal species, age, number, susceptibility and primary sources from which the examined animals were purchased, in addition to special farming conditions like breeding of more than one type of animals in the same place without fencing (Hirai and To, 1998 and Nahed and Khaled, 2012).

On interviewing the data obtained from the examined animals, it was revealed that there was no obvious clinical manifestation seen among the serologically positive sheep and goats except for one goat which was suffering from abortion 3 weeks before sampling. Meanwhile; Q fever in goats has attracted a considerable attention by Berri et al (2007) and de Bruin et al (2012) after reporting large outbreaks in dairy goat farms and implicated them as the primary source for transmission of C. burnetii to humans. Such silent nature of studied herds in the present study should motivate us to overlook this disease under any conditions.

PCR assay performed on 26 seropositive (11 sheep and 15 goats) whole blood samples failed to detect any C. burnetii DNA in any of them (Fig., 1). This was in agreement with Maurine and Raoult (1999) who mentioned that Q fever diagnosis is based upon serological methods because culture and molecular biology techniques have low sensitivity. In this regard, Lorenz et al (1998) and Berri et al (2005) observed that there is no relationship between the antibody response to C. burnetii and
excretion. Our finding was explained by Fritz et al (1995) who stated that PCR may be far more difficult when working with clinical material. Moreover; Stein and Raoult (1992) attributed this finding to low numbers of *C. burnetii* genomes in some clinical samples. The results obtained in this study indicate that apparently healthy sheep and goats may be an important source of *C. burnetii* infection in our study area. Therefore, much more attention should be paid to these animals as an important source for human and animal infection.

**Table (1):** Primer sequence

**Statistical analysis:** data obtained were analyzed by non-parametric Chi-square test ($\chi^2$) using M-stat computer software (Corder and Foreman, 2009).

<table>
<thead>
<tr>
<th>Name of Oligonucleotide primer</th>
<th>Primer sequence</th>
<th>Primer length (bp)</th>
<th>Product length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trans 1</td>
<td>5’-TAT GTA TCC ACC GTA GCC AGT C-3’</td>
<td>22</td>
<td>687</td>
<td>Hoover et al (1992)</td>
</tr>
<tr>
<td>Trans 2</td>
<td>5’-CCC AAC AAC ACC TCC TTA TTC-3’</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2):** Frequency distribution of Q fever serologically positive goats and sheep in relation to their age and sex.

<table>
<thead>
<tr>
<th>Age Animal Species</th>
<th>Total No. Examined</th>
<th>Up to 1 year</th>
<th>1-2 years</th>
<th>&gt;2 years</th>
<th>Total Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>24 3 (12.5)</td>
<td>3 0 (0.0)</td>
<td>12 (20)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>24 3 (12.5)</td>
<td>3 0 (0.0)</td>
<td>12 (20)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>24 3 (12.5)</td>
<td>3 0 (0.0)</td>
<td>12 (20)</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
<td>27 2 (7.4)</td>
<td>1 0 (0.0)</td>
<td>3 (8.6)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>43 7 (16.3)</td>
<td>11 1 (9.1)</td>
<td>8 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>70 9 (12.9)</td>
<td>12 1 (8.3)</td>
<td>11 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Grand Total</td>
<td>182</td>
<td>102 14 (13.7)</td>
<td>64 11 (17.2)</td>
<td>26 (14.3)</td>
<td></td>
</tr>
</tbody>
</table>
Table (3): Frequency distribution of Q fever serologically positive cases among examined sheep according to their locations in Ismailia Governorate.

<table>
<thead>
<tr>
<th>Seropositive Location</th>
<th>No. of animals /Farm</th>
<th>No. examined</th>
<th>Total positive No.</th>
<th>Total positive cases /location No. ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ein Ghosein</td>
<td>12/1 25/2</td>
<td>5 11</td>
<td>0 2</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Abu Balah</td>
<td>25/1</td>
<td>6</td>
<td>2 33.3</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>El Mahsama</td>
<td>23/1</td>
<td>8</td>
<td>3 37.5</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Abo Atwa</td>
<td>25/1</td>
<td>10</td>
<td>0 0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Wadi El Eshra</td>
<td>30/1</td>
<td>11</td>
<td>1 9.1</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>El Manaef</td>
<td>29/1 15/2</td>
<td>12 6</td>
<td>0 0.0</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Ismailia veterinary clinic cases</td>
<td>---</td>
<td>22</td>
<td>2 9.1</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>91</td>
<td>11</td>
<td>12.1</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Frequency distribution of Q fever serologically positive cases among examined goats according to their locations in Ismailia Governorate.

<table>
<thead>
<tr>
<th>Seropositive Location</th>
<th>No. of animals /Farm</th>
<th>No. examined</th>
<th>Total positive No.</th>
<th>Total positive cases /location No. ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Dobaiea</td>
<td>18/1 25/2 12/3</td>
<td>8 11 5</td>
<td>2 1 0</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>El Mahsama</td>
<td>20/1</td>
<td>7</td>
<td>1 14.3</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Abo Atwa</td>
<td>20/1</td>
<td>6</td>
<td>0 0.0</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Wadi El Eshra</td>
<td>16/1 15/2</td>
<td>7 6</td>
<td>1 3 14.3 50</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>El Manaef</td>
<td>15/1 23/2</td>
<td>6 7</td>
<td>0 1 0.0 14.3</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>Ismailia veterinary clinic cases</td>
<td>---</td>
<td>28</td>
<td>6 21.4</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>91</td>
<td>15</td>
<td>16.5</td>
<td></td>
</tr>
</tbody>
</table>
Fig (1): Trans-PCR assay performed on ELISA seropositive goat and sheep blood samples. Lane 1, 100-bp DNA ladder; Lane 2, positive control, Lane 3; negative control and Lane (4-12) blood samples.

References


animals in Albania. The Veterinary Journal 175(2): 276–278.


fever in domestic ruminants in semi-extensive grazing systems. BMC Veterinary Research. 6:3.


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 Mellash el-arabi

الإصابة بكوكسيللا بيرنرتي في المجترات الصغيرة في محافظة الإسماعيلية

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في دراسة عن مدى انتشار الكوكسيللا بيرنرتي الميكروب المسبب للحمي المجهول في المجترات الصغيرة في محافظة الإسماعيلية، تم فحص 91 حيواناً من الماعز و91 حيواناً من الأغنام من مزارع ومحللة في الفترة من ديسمبر 2010 إلى ديسمبر 2011. تم تجميع عينات دم من كل حيوان، أحدهما على مضاد تجلط لاستخدامها في تفاعل عديد البلمرة التسلسلى والآخر بدون مضاد تجلط لفصل المصل وفحصه باستخدام اختبار الألبأرا. بلغت نسبة الإصابة الكلية 14.3%، حيث بلغت 12.1% في الأغنام و16.5% في الماعز. كانت نسبة الإصابة أعلى في الإناث عن الذكور خاصة في عمر من عام الى عامين. كما ارتفعت نسبة الإصابة في الحيوانات المتواجدة في تجمعات صغيرة أو كبيرة مقارنة بالحالات الفردية التي تم تجميعها من العيادات البيطرية المختلفة. تبينت الإصابة في مختلف المناطق التي تم فحصها حيث ارتفعت نسبة الإصابة الأغنام في منطقة المحسمة (37.5%) وابو بلع (33.3%) وعين غصنين (12.5%) وحارپا منطقة وادي العشري (9.1%). في حين ان منطقة المنايف شهدت أقل معدلات للإصابة (5.6%). بينما سجلت منطقة وادي العشري (30.8%) ومحسمة (14.3%) وضبعة (12.5%) أعلى المناطق إصابة بالنسبة للماعز. بإجراء تفاعل عديد البلمرة التسلسلى على عينات الدم التي ثبت تواجد الأجسام المناعية بها، لم يتم تعين الحمض النووي للكوكسيللا بيرنرتي في أي منها، وأوضحت الدراسة أهمية الدور الذي قد تلعبه هذه الحيوانات كمصدر لعدوى الكوكسيللا بيرنرتي لكل من الإنسان والحيوان في منطقة الدراسة.