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The burden of *Coxiella burnetii* among aborted dairy animals in Egypt and its public health implications☆

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ABSTRACT

Q fever is a zoonotic disease of mounting public health implications. Dairy animals are major reservoir for such disease whereas abortion is the main clinical outcome. The current study was conducted to investigate the burden of *C. burnetii* abortions among dairy animals in Egypt to provide more knowledge for better control of such disease. For this purpose, placental cotyledons and vaginal discharges from 108 aborted dairy animals (27 sheep, 29 goats, 26 cattle, 26 buffaloes) were examined for the presence of *C. burnetii* by nested PCR. Serum samples from 58 human contacts were examined for the presence of *C. burnetii* IgG antibodies using ELISA. Out of the 108 examined animals only one goat yielded positive result in both placental tissue and vaginal discharges with an overall prevalence 0.9% while that among goats is 3.4%. Moreover, the seroprevalence of *C. burnetii* IgG antibodies among the examined individuals was 19% whereas the prevalence in farmers is significantly higher than that among veterinarians and veterinary assistants. In conclusion, *C. burnetii* may play a role in dairy goat abortions rather than other dairy animals in Egypt while its public health implications cannot be ruled out.

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1. Introduction

Q fever is a zoonosis with great public health consideration throughout the world. The disease was firstly identified in 1935 after a large outbreak of febrile illness with respiratory manifestations among abattoir workers in Australia (Georgiev et al., 2013). The disease is caused by *Coxiella burnetii* which is a Gram negative obligatory intracellular bacterium that infects both humans and animals. *C. burnetii* is a dangerous infectious agent and has been classified by Centers for Disease Control and Prevention (CDC) as category B biological weapon a matter which highlights the global concern about this pathogen (Tissot-Dupont and Raoult, 2008). However, *C. burnetii* has wide range of animal reservoirs including ruminants, dogs, cats, birds and ticks, only sheep, goats and cattle are considered to be the major reservoirs for human infections. The main route for human infections is inhalation of aerosols contaminated with the pathogen while ticks may play crucial role in the circulation of the disease among animals especially wild ones (Maurin and Raoult, 1999). In animals, the disease is usually subclinical but abortion is a serious clinical outcome of such infection among ruminants (Guatteo et al., 2011). On the other hand, the disease in man is usually acute and characterized by fever, flu-like symptoms, pneumonia, malaise, headache and some cases may suffer from hepatitis while endocarditis is a serious complication of the chronic form of the disease (Hartzell et al., 2008). In spite of the great public health implications of *C. burnetii*, much remains unknown about its epidemiology in many countries. Since, ruminants are the main source for environmental contamination with such pathogen and consequently for human infections, to understand the disease in ruminants is the cornerstone in the control plan of *C. burnetii* in animals, environment and humans. Therefore, it is imperative to know more about the prevalence of *C. burnetii* related abortions in dairy animals which are responsible for the release of huge number of *C. burnetii* (10^9 organism per gram placenta) in the environment to infect both humans and animals (Roest et al., 2011). In Egypt, there is no available data about the burden of *C. burnetii* linked abortions among dairy animals. Therefore, the current study was carried out to investigate the prevalence of *C. burnetii* among aborted dairy animals as well as the seroprevalence of *C. burnetii* antibodies among humans occupationally in contact with such animals in order to provide more data essential for combating this pathogen.

☆ This work was carried out in department of zoonoses, Faculty of Veterinary Medicine, Cairo University.

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2. Materials and methods

2.1. Samples

Placental cotyledons as well as vaginal discharges were concomitantly collected from 108 aborted dairy animals (27 sheep, 29 goats, 26 buffaloes (*Bubalus bubalis*), 26 cattle). All samples were inserted in sterile screw capped tubes, placed in ice box and transported to the laboratory where they were kept at −20 °C till processing. All animal samples were collected from veterinary clinics at rural districts of Giza governorate, Capital Cairo, Egypt. Moreover, blood samples were collected from 58 individuals occupationally in contact with dairy animals (veterinarians, veterinary assistants, farmers) resided rural and urban districts of Giza governorate. Blood samples were collected through expert medical staff at private medical laboratories after participants gave their consents. Blood samples were received in sterile tubes (without anticoagulant) and transported to the laboratory in icebox. Upon arrival, blood samples were centrifuged to obtain sera which then stored at −20 °C till use.

2.2. Molecular detection of C. burnetii by nested PCR in animal samples

2.2.1. DNA extraction

Extraction of DNA from placental and vaginal samples were performed using DNaesy Blood &Tissue Kit (Qiagen, Germany) according to the manufacturer. The extracted DNAs were stored at −20 °C till use.

2.2.2. Nested polymerase chain reaction step

PCR amplification was performed using EmeraldAmp GT PCR master mix (Takara, Japan) with the following sets of primers which target the *htpAB*-associated repetitive element: For the first amplification cycle the primers IS111 F1 (5′TACTGGGTGTTGATATTGC-3′) and IS111 R1 (5′- CCGTTTCATCCGCGGTG-3′) were used, which amplify 485-bp fragment while The second (nested) PCR was performed with the IS111 F2 (5′- GTAAAGTGATCTACACGA-3′) and IS 111 R2 (5′- TTAACAGCGCTTGAACGT-3′) to amplify 260 bp fragment. All primers were synthesized by (Metabion, Germany) and according to Boden et al. (2012). PCR amplification for the first step was carried out using the following conditions: 95 °C for 3 min, followed by 40 cycles of: 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min then final extension at 72 °C for 4 min. Specific bands were appeared after electrophoresis step at 485 bp (Fig. 1). DNA products of the first step were used as templates for the nested PCR step with the following amplification conditions: 95 °C for 3 min, then 30 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s with final extension step at 72 °C for 4 min. After completion of the nested PCR step, electrophoresis was done to detect specific bands at 260 bp.

2.3. Sequencing of PCR products and sequence analysis

PCR product of positive vaginal sample from the first reaction was purified using Qiaquick purification kit (Qiagen, Germany) according to the manufacturer instructions and sequencing was conducted using Big Dye Terminator V3.1 sequencing kit (Applied Biosystems) with forward primer IS111F1.

2.4. Genbank accession number

The sequence has been deposited in the GenBank database under accession number: KU977532.

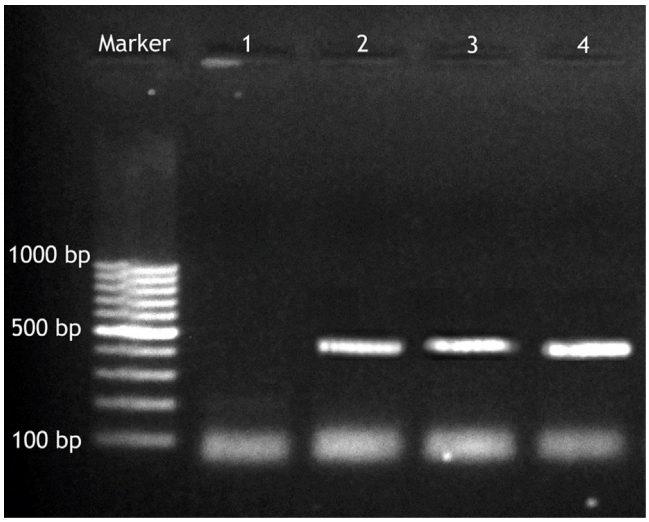

Fig. 1. Molecular detection of *C. burnetii* in placental tissues and vaginal discharges of aborted dairy animals.
Marker: DNA ladder 100 bp; lane 1 negative control; lane 2 positive control (Genekam, Germany); lanes 3, 4 positive samples with specific bands at 485 bp.

2.5. Phylogenetic analysis

The obtained nucleotide sequence was compared with those available in public domains using NCBI, BLAST server. Sequences were downloaded and imported into BioEdit version 7.0.1.4 for multiple alignments using the Clustral W program of the BioEdit.

Phylogenetic analysis was performed with MEGA version 6 using the neighbor-joining approach. The bootstrap consensus tree was inferred from 500 replicates (Fig. 2).

2.6. Detection of C. burnetii IgG antibodies among examined persons

Serum samples from examined persons were tested for the presence of *C. burnetii* IgG antibodies using Enzyme Linked Immuno-Sorbant Assay (ELISA) with *Coxiella burnetii* phase II IgG ELISA kit (Vircell, Spain). The protocol of the test was conducted according to manufacturer directions.

2.7. Statistical analysis

Statistical analysis of the human results was done using Chi-Square test by SPSS 16 software. P value less than 0.05 was considered statistically significant.

3. Results

Out of 108 examined aborted dairy animals only one was positive for *C. burnetii* giving an overall prevalence 0.9%. The positive case was goat among 29 examined goats (3.4%) and yielded *C. burnetii* in both placental tissues and vaginal discharges that passed directly after abortion while none of the other examined ruminant species showed positive result (Table 1). On the other hand, the seroprevalence of *C. burnetii* IgG antibodies among examined humans was 19% with the following distribution among veterinarians and veterinary assistants (9.4%) and 30.8% for farmers (Table 2).

4. Discussion

Q fever is a disease of both veterinary and public health concern throughout the world. The current study reports the occurrence of *C. burnetii* abortion among dairy animals in Egypt. Our results

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