Seroprevalence detection of *Coxiella burnetii* antibodies in milk and serum of dairy cattle by recent methods

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**ABSTRACT**

This study aimed to detect *C. burnetii* antibodies in milk and serum of dairy cattle using PCR compared with other immunological techniques namely IFAT and ELISA. These samples were collected from apparently healthy cows (*n=300*) from Giza, Fayoum and Beni Suef Governorates. Real-time PCR for detection of *C. burnetii* DNA was performed on samples using two individual PCR assays with specific primers and probes to increase specificity of the result using Roche kits. IFAT revealed detection of 6% IgM antibodies in milk and 7.3% in sera. While IgG was 10.6% of milk and 24% in sera. The main value of positive samples by ELISA test were 25.7%; in serum 34% and in milk 17.3%. These results showed that sensitivity and specificity of the ELISA test and IFAT were highly comparable. The result of Real time PCR from 77 positive samples of *C. burnetii* was 45.4%. The highest results for real time PCR recorded 57.1% in Beni Suef followed by 44% in Giza and 33.3% in Fayoum. It was concluded that the apparently healthy dairy cows are an important reservoir of *C. burnetii* infection. Investigations on *C. burnetii* using PCR as well as serological surveys of animals are important methods for diagnosis and control of Q-fever. Awareness is needed for animal owners, veterinarians, physicians and authorities.

**1. INTRODUCTION**

Q fever is a zoonotic and prevalent disease in most countries of the world, it caused by gram negative bacteria called *Coxiella burnetii*. This organism has many hosts include mammals, birds and arthropods like ticks (Angelakis and Raoult, 2010). *Coxiella burnetii* – a Gam-negative intracellular bacterial pathogen is the causative agent of Q fever – a widespread zoonotic disease (Boroduske, et al., 2017). Dairy cattle have been the main source of human infections during latest outbreaks of Q fever (Wallensten, et al., 2010).

Q fever has been recognized since 1952 when *C. burnetii* was first isolated from ticks collected at the Cairo Abattoir on infested camels and bulls imported from Sudan (Taylor, et al., 1952). The shedding of *C. burnetii* into the environment happens primarily by birth products, especially placenta, are heavily infected with *C. burnetii* also gained from milk and other excreta of infected animals (Schmeer, et al., 1987). *Coxiella* can persist in the environment for long periods and carried for a long distance through the wind (Kirkan et al., 2008). In dairy cattle, late abortion, mastitis and infertility are the major clinical symptoms related to Q fever (Arricau, et al., 2003).

Q fever can also be spread by ticks which pass the bacteria from an infected to a susceptible animal, and whose feces contain the bacteria thus also contaminating the environment. Since it is also shed in the milk of an infected animal, it can be contracted by drinking unpasteurized infected milk (OIE, 2018).

Animal birth products either from abortions or normal deliveries were the highest risk for animal and human infections due to a high load of *C. burnetii* in placental tissues. Aerosols containing airborne particles from dairy farms, especially animal birthing places and farms with high cow’s abortion rates are considered to be the highest risk for human infection (Kersh et al., 2013). Increased risk of *C. burnetii* infections in animals associated with local many factors such as regional herd density, herd size, animal housing system, animal movements, hygiene practice as well as climatic and geographical characteristics of the region (Nusinovici et al., 2015).

Molecular genotyping data does not exclude cattle as a possible reservoir of *C. burnetii* strains infectious to humans (Roest et al., 2013). The seroprevalence is associated with cattle farming has revealed seropositive individuals, suggesting that cattle-human contact can be a common route of infection (Schimmer et al., 2016).

The Real Time Polymerase Chain Reaction (R-PCR) and Immune Fluorescence Antibody technique (IFA) are considered the main method for diagnosis of *C. burnetii* infection (Meekelenkamp et al., 2012; Anati-Piraouz et al., 2015).

So, this study aimed to detect *C. burnetii* antibodies in milk and serum of dairy cattle using PCR compared with some
immunological techniques as IFAT and ELISA in some Egyptian Governorates.

2. MATERIAL AND METHODS

2.1. Samples:
Milk and serum samples were collected from apparently healthy cows from farms (n=300) consisted of pooled milk and serum (n= 150) from Giza, Fayoum and Beni Suief Governorates. Serum samples were collected in Vacutainer tubes under aseptic conditions from dairy farms cows. Pooled milk and sera samples were transferred in sterile screw capped bottles and stored at - 20°C until processed.

2.2. Indirect fluorescent technique:
The detection of IgM and IgG antibodies in serum and milk was done using commercial Kit’s manufacturer VIRCELL*, (SPAIN) according to Soriano et al. (1993).

2.3. ELISA test:
Commercial ELISA test kits (IDEXX Laboratories, USA) were used to detect anti- C. burnetii IgG antibodies. The sample optical densities (OD) were measured by a microplate ELISA reader (Biomed, USA) at 450 nm according to Schelling et al. (2003).

2.4. Rt-PCR tag Man:
DNA from milk or serum samples was extracted using the Roche kit (High Pure PCR Template Preparation Kit) according to the manufacturer’s instructions. An assay based on the use of Taq-Man probe was performed to screen the samples. Isolated DNA samples were tested using primers and a probe targeting the repetitive transposon like region targeting CB IS1111 element were performed of the C. burnetii genome (Hoover et al. 1992).

3. RESULTS

Laboratory diagnosis for detection of Coxiella burnetii antibodies (IgM and IgG) in dairy cattle in some governorates in Egypt has been doing by IFA test. Examination of 300 samples; IgG antibodies in serum were detected 36% in Beni Suief followed by Giza and 30% in Fayoum and Beni Suief (Table 2). The Seroprevalence of Coxiella burnetii was 34% in serum and 17.3% in milk by ELISA test. The highest results in serum were detected 36% in Beni Suief followed by 34% in Giza and 32% in Fayoum. While the results of Coxiella burnetii in milk were recorded 20% in Beni Suief and 16% in Fayoum and Giza (Table 2). The seroprevalence of Coxiella burnetii was 34% in serum and 17.3% in milk by ELISA test.

Table 1 Primer and probe

| Primer | Target | NTs | Sequence | Amplicon dimension (bp) | TAQ | Melt | M  |
|--------|--------|-----|----------|-------------------------|-----|------|----|
| Tag    | Trans- | 418-438 | 5'-gggtacagcttggacacta-3' | 101 | 85.1 | 4.08 |
| M1a    | RT-    | 496-516 | 5'-ctacccgcaaggcgtaa-3' | 40 | 68.1 |
| M1a    | RT-    | 446-471 | 5'-ctacccgcaaggcgtaa-3' | 40 | 68.1 |
| M1a    | Probe  | 446-471 | 5'-ctacccgcaaggcgtaa-3' | 40 | 68.1 |

M: melting temperature

Table 2 Seroprevalence of Coxiella burnetii in dairy cattle by IFA test

| Governorates | No of samples | No of positive samples IFA | IgG | Milk n=50 | Serum n=50 |
|-------------|---------------|---------------------------|-----|-----------|------------|
| Fayoum      | 100           | 22/100                     | 4   | 8 (4%)    | 20 (8%)    |
| Beni Suief  | 100           | 26/100                     | 4   | 8 (4%)    | 20 (8%)    |
| Giza        | 100           | 24/100                     | 4   | 8 (4%)    | 20 (8%)    |
| Total       | 300           | 72/300                     | 11  | 9 (6%)    | 26 (16%)   |

Table 3 Seroprevalence of Coxiella burnetii in dairy cattle by ELISA

| Governorates | No of samples | No of positive samples ELISA | IgG | Milk | Serum |
|-------------|---------------|------------------------------|-----|------|-------|
| Fayoum      | 100           | 24/100                       | 16/50 | 8/50 |
| Beni Suief  | 100           | 28/100                       | 16/50 | 10/50 |
| Giza        | 100           | 25/100                       | 17/50 | 8/50 |
| Total       | 300           | 77/300                       | 51/150 | 26/150 |

Table 4 Comparison between IFAT and ELISA test for detection of Coxiella burnetii antibodies in dairy cattle

| Governorates | No of samples | No of positive samples | IFAT | No of positive samples | PCR | % |
|-------------|---------------|------------------------|------|------------------------|-----|---|
| Fayoum      | 100           | 24/100                 | 22/100 | 22/100 |
| Beni Suief  | 100           | 28/100                 | 26/100 | 26/100 |
| Giza        | 100           | 25/100                 | 24/100 | 24/100 |
| Total       | 300           | 77/300                 | 72/300 | 72/300 |

Table 5 Detection of Coxiella burnetii antigen by using Rt-PCR in dairy cattle

| Governorates | No of samples | No of positive samples by PCR |
|-------------|---------------|------------------------------|
| Beni Suief  | 100           | 12/100                       |
| Giza        | 100           | 15/100                       |
| Total       | 200           | 27/200                       |

ELISA test for detection of Coxiella burnetii antibodies in dairy cattle revealed 77/300 (25.7%) positive samples compared to IFAT which detected 72/300 (24%) positive samples (Table 4). ELISA test gives specificity 100% and sensitivity 98% more than other serological tests used to diagnose coxiellosis (Table 6).
which all strains examined showed >99% homology. Molecular diagnostics have vastly improved the early and accurate diagnosis of *C. burnetii*, rapid and accurate diagnostic methods. PCR has also allowed the detection of Q fever disease from a variety of different sample types including serum, tissue samples from infected organs which highly loaded with bacteria (Lockhart, et al. 2011). Rt-PCR for the detection of *C. burnetii* was performed using two individual PCR assays with specific primers and probes targeting two different gene targets in order to increase specificity of the result (Jennifer, et al. 2002). The first gene was that of the repetitive transposon-like element IS1111 of the transposase gene, repeated up to 20 times throughout the *Coxiella* genome. The second gene target was the *Coxiella* Outer membrane (com1) gene, coding for the 27kD protein (Klee et al. 2006a). The results of Real time PCR from 77 positive samples of *Coxiella burnetii* recorded 45.4%. The highest results recorded 57.1% in Beni Suief followed by 44% in Giza and 33.3% in Fayoum. In this study, we selected for Rt-PCR assays the repeated sequence IS1111, increasing consequently the PCR sensitivity (Kim et al. 2005).

5. CONCLUSION

We concluded that dairy cows should be the focus of any further research to establish their role in the transmission of *C. burnetii* to humans and to identify any potential risk factors for exposure. Using PCR as well as serological surveys of animals are important methods for diagnosis and control of Q-fever. Awareness rising is needed for animal owners, veterinarians, physicians and authorities.

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