

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.803.045>

Detection of *Coxiella burnetti* Antibodies among Workers and Butchers at Dhamar Slaughter House, Yemen

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ABSTRACT

Keywords

Coxiella burnetti,
Butchers,
Slaughter house,
Immunoblot

Article Info

Accepted:
04 February 2019
Available Online:
10 March 2019

The Q-fever considered as occupational disease of persons dealing with livestock and their products, although, almost no data have been identified on the situation of this disease in Yemen, where 70 % of their population depending on agriculture and raising animals. An investigation for detection of antibodies against *Coxiella burnetti* in serum samples collected from about 50 veterinarian and butchers who worked in Dharma's slaughterhouse carried out using commercial immunoblot test. Of 50 sample examined, three samples were positive (6%), one sample was Phase-1 positive, six samples were equivocal. The presence of *Coxiella burnetti* antibodies in processing Slaughter's house workers may give in part preliminary indication of distribution of this disease in livestock raising community and the necessity of enactment of publicly and occupational actions, measures the limit, and prevent the devastating effects of this zoonotic disease on human and animals.

Introduction

Q fever/ Query fever/ Balkan influenza/ abattoir fever was first described in Queensland, Australia, during an outbreak of a febrile illness of unknown origin among abattoir workers (Shakespeare, 2009). It's ubiquitous zoonotic disease caused by an extremely resistant intracellular bacterium, *Coxiella burnetti* (Anderson *et al.*, 2013). This disease cause severe economic losses and can be considered severe public health problem in certain areas (Porter *et al.*, 2011).

This may attributed in part to the fact that the disease has long been considered an underreported and underdiagnosed illness because symptoms frequently are nonspecific, making diagnosis challenging (Anderson *et al.*, 2013). Further, Q fever is widespread in domestic ruminants and its sero-prevalence thought to have increased in recent often neglected in the differential diagnosis. The domestic ruminants represent the main source of infection and considered the main reservoir for pathogen that infect wide variety of hosts, mammals (humans, ruminants, small rodents,

dogs, cats), birds, fish, reptiles and arthropod (Porter *et al.*, 2011). Transmission of disease is by direct contact with contaminated materials, especially the afterbirth or material contaminated with amniotic fluid. There is some evidence that inhalation of dust from infected straw or bedding and even soil may also cause infection. Contaminated milk or milk products are also a possible route of infection, and transmission via ticks, lice or fleas has been demonstrated (Shakespeare, 2009).

The people at highest risk are abattoir workers, veterinarians, individuals working with hides, fleece or bones of infected animals. As result of that the disease considered as occupational zoonosis of agricultural and other workers closely involved with cattle and sheep (Shakespeare, 2009). The acute form of disease characterized by incubation period last a few days to several weeks, with less than 1% of fatalities. The main manifestation includes fever, severe headache, and chills are the symptoms most commonly seen. Fever usually peaks at 40°C and lasts approximately days. Fatigue and sweats also frequently found. Cough, nausea, vomiting, myalgia, arthralgia, chest pain, hepatitis, and occasionally, splenomegaly, osteomyelitis, and meningoencephalitis are also associated with acute Q fever. In chronic Q fever the endocarditis, primarily of the aortic and mitral valves, are the most common manifestation of chronic Q fever; although chronic hepatitis and infection of surgical lesions have been seen. Approximately 90% of Q fever endocarditis patients have preexisting valvular heart disease (Waag and Fritz, 2012).

The clinical manifestation of disease in animals, include stillbirth, delivery of weak lambs, calves, or kids, are the most frequent clinical signs of the disease. The abortion occurs at the end of gestation without specific

clinical signs and pathognomic pathological findings, the intercotyledonary fibrous thickening and discolored exudates, may observed (Shakespeare, 2009). There are different serological tests available for Q fever, including Indirect Fluorescent Antibody Tests (IFAT), Enzyme linked Immunosorbent Assays (ELISA) and Complement Fixation Tests (CFT) (Wegdam-Blans *et al.*, 2012).

Materials and Methods

Study area and samples collection

This study was conducted at Slaughterhouse of Dhamar governorate. Uncoagulated blood samples collected from 50 veterinarian and butchers who worked in Dhamar city slaughter house. The samples collected from appropriate vein under aseptic manner in Plain blood collection tubes. The blood samples transported in icebox into laboratory, Faculty of Agriculture & Veterinary Medicine.

Samples preparation

The serum separated from blood by incubation the tubes in an upright position at room temperature for 30 minutes and then centrifuged at 1200g for 10 minutes. The serum samples aspirated into Eppendorf 1.5 tubes labeled and stored at -20 until Q- fever testing.

Immuno DOT assay

The ImmunoDOT assay, utilizing an enzyme-linked immunoassay (EIA) dot technique for the detection of IgG and IgM antibodies to *C. burnetti* (GENBIO, San Diego, CA, USA). The Immuno DOT test procedure and results interpretation performed according to the procedure of Manufacturer's instructions. The aluminum blocks (GENBIO, San Diego, CA, USA) and water bath (Memmert, Germany)

protocol applied to performing the test procedures in the required optimal temperature. Briefly, the kit left in room temperature for about 30 minutes. Four reaction vessels (cuvette) per serum samples inserted into appropriate slots in aluminum blocks, which in turn placed in water bath. 2mL of diluent, enhancer, conjugate and developer were placed into their corresponding vessel (1), (2), (3) and (4), and the thermometer used for calibrating the temperature inside the vessels to 48 °C. After adjusting the temperature in the vessels (10 minutes waiting), ten microliter of each serum sample were pipetted into reaction vessels 1 and then demonized water wetted strips were, inserted into the that vessels, moved up and down for about 10 seconds, and stood in vessels (1) for 15 minutes. The strips then washed with deionized water (Clarifier), by swishing the entire strip windows by swift back and forth motion for about 10 seconds. The same steps repeated for each strip with remaining vessels but with different incubation time, which was 5 minutes in vessels (2), 15 minutes in vessels (3), 5 minutes in clarifier, and 5 minutes in vessels (4). The strips wetted by slight pressing against filter paper. To ensure the good quality of test procedure, only strips with

clear blue dot in positive control window and without dot in negative control widow considered. The strips with clear blue dot in four test windows (Phase 1, Phase 2 dilution 3, Phase 2 dilution 2 and Phase 2 dilution 1) were considered as positive, If strips with only Phase 1 positive the results were interpreted as phase 1 positive (only if associated with consistent symptomatology that indicated chronic infection).

Results and Discussion

The results of this study shows that the presence of antibodies against *Coxiella burnetti* in serums from workers and veterinarian of Dhamar slaughter house. Only three positive serum samples (one for veterinarian and tow for butchers) that were reactive to *Coxiella burnetti* antigens (Phase-I and II) in all the windows of kit strip. The samples that show reaction to strip window containing *Coxiella burnetti* Phase-I antigen were three of which one were accompanied with clinical signs claim so it was reported as Phase-I antigen positive, the two other samples reported as equivocal. Other samples were negative or weakly reactive (4 samples) which show reaction with Phase-II antigen dilution-1 as showed in table 1.

Table.1 Percentages of Positive, Phase 1 positive and suspected Q-fever slaughterhouses' workers

Categories	Positive	Phase-I Positive	Equivocal	Negative
Veterinarian	1	1	3	1
Butchers	2*	-	3	39
Total	3	1	6	40
%	6	2	12	80
Result interpretation according to manufacture	Reported as "positive" when Phase-I and Phase-II dots were Positive	Reported as "Phase-1 Positive" when accompanied with clinical signs	Reported as "equivocal" when there were no claims of clinical signs	Reported as "Negative " When no reactions were recorded in all windows of test strip

* No claims for the preexisting clinical signs

The results of this study showed that the antibodies against *C. burnetti* in some veterinarian and butchers who works in Dhamar city Slaughterhouse were present. The presence of Q-fever antibodies in occupational highly risk people have been confirmed by many studies (Wilson *et al.*, 2010; Wade *et al.*, 2006; Brouqui *et al.*, 2004; McQuiston and Childs, 2002; Carrieri *et al.*, 2002; Cracea, 1987; CDC, 1986; Haas and Hacks, 1971; Topping *et al.*, 1947; Irons *et al.*, 1947; Irons and Hooper, 1947; Cox *et al.*, 1947).

The occupation and the duration of exposure to infectious agent affect the persistence of antibodies in blood and exacerbate the devastating effects of the disease (Nakladalova *et al.*, 2014). The results of this study indicated that the Slaughterhouse workers have been previously exposure to the *C. burnetti* most possibly from the slaughtered animals. These may imply that the workers exposed to causative agent mostly from the reservoir host (cattle, sheep, and goats) which brought into slaughterhouse. The slaughtered animals are mostly local breeds belonging to, whether farmers reside in city or villagers neighbouring the city or butchers, who bought local and imported breeds of animals and residing them in the city.

The presence of antibodies against like occupational disease, in highly risk people it may be guided to imagine, the extent to which this air-born disease may distribute in community and the size of unrealizable problems. Further studies, using doubtless diagnostic test, should intended to determine the epidemiological situation of Q-fever in both human and animals and then the suitable measure could take. This may supported by the study that indicated the detection of Q-fever in hepatitis suffered people reside in rural areas of Yemen (Gray *et al.*, 1999).

Acknowledgment

This research partially supported by grants in aid from Relief international, we are grateful to the Dhamar slaughterhouse administration, workers and veterinarians for their help in carrying out this study. We wish to thank Mr. Fred alder (GenBio Company, USA) for providing the discount of ImmunoDot kits and aluminum blocks.

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How to cite this article:

Badi A. Fateh, Hameed Ahmed Golah, Ahmed Y. Al-Qudari, Maged Ahmed AL-Garadi and Alhothy, H.A. 2019. Detection of *Coxiella burnetii* Antibodies among Workers and Butchers at Dhamar Slaughter House, Yemen. *Int.J.Curr.Microbiol.App.Sci*. 8(03): 361-365.
doi: <https://doi.org/10.20546/ijcmas.2019.803.045>