J.Egypt.vet.med.Assoc 76, no 1. 79 – 87(2016)

THE PREVALENCE OF *COXIELLA BURNETII* (Q-FEVER) AS A CAUSE OF ABORTION AND INFERTILITY AMONG FARM ANIMALS IN SOME DELTA GOVERNORATES

By

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ABSTRACT

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. Its prevalence among farm animals is important to both public and animal health. The aim was to investigate the presence of *C. burnetii* in cattle, sheep and goats. Three hundred samples consisted of pooled milk and serum 180 of each from dairy cattle (n=180) dairy goats (n=60) and dairy sheep (n=120) from Qaliubia, Monofia, Gharbia and Kafr ElSheikh Governorates. All samples were examined by indirect immunofluorescent antibody technique (IFAT) for IgG antibodies against *C. burnetii* phase II antigen. The prevalence of antibodies in dairy herds was 22.5 % with large regional differences. The study revealed that antibodies against *C. burnetii* in cattle raw milk and sera were 14.44% and 31.11%, respectively, in goat raw milk and sera were 26.67% and 46.67%, respectively and in sheep raw milk and sera were 21.67% and 33.33%, respectively. These results denoted that, the apparently healthy cattle, sheep and goats are an important reservoir of *C.burnetii* infection. *Coxiella burnetii* is shed in milk of the infected animals; therefore, their milk should not be consumed raw or sold unpasteurized directly to the consumers. Pasteurization of milk is carried out at 145° F (63° C) for at least 30 minutes or at 161° F (72° C) for 15 seconds is sufficient to destroy *C. burnetii*, as well as other pathogens that can be present in raw milk. Finally, we conclude that it is of utmost importance to avoid contact with the placenta, birth products, fetal membranes, and aborted fetuses of sheep, cattle, and goats. On the other hand eating and drinking unpasteurized milk and milk products pose a great risk of infection to man and should be avoided.Further investigation is recommended concerning *C. burnetii* prevalence in the aborted cases of farm animals.

INTRODUCTION

Query or Queensland fever (Q fever) is a bacterial infection affecting a variety of animal species as well as human beings. Q fever is caused by *Coxiella burnetii*, an obligate, intracellular, rickettsia organism that can survive in a dried condition for extended periods **(Astobiza, *et al.,* 2010)**. The disease is found worldwide, causing reproductive problems in livestock and severe respiratory (lung) and liver disease in humans. Ruminants can contract Q fever when grazing contaminated pastures and from tick bites **(Arricau, *et al*., 2003)**. Other animal species and humans can be infected by inhaling contaminated dust. The microorganism can be found in the placenta, uterine fluid, and milk. Infected animals show no symptoms of the disease until aborting or having stillborn kids in late pregnancy **(Sanchez, 2006)**. After the initial abortions or infections, animals become immune to abortion but can remain subclinically infected. After the infection is established, the female can carry the organism indefinitely, sporadically shedding it in milk and at parturition **(Bottcher, *et al.,* 2011 and Anderson, *et al.*, 2013)**. The bacteria are very hardy in the environment and can survive for long periods. This can lead to infection by inhaling (aerosol) the bacteria from contaminated barnyard dust. Ticks (vector) can also spread infection between animals **(Berri, *et al.,* 2001)**. Although infection with *Coxiella burnetii* is often asymptomatic, it may lead to reproductive dysfunction and abortion usually late in gestation in goat, sheep and less often cattle **(CDC, 2009**). Ingestion has been proposed as a route of spread, particularly through the consumption of contaminated, unpasteurized dairy products **(Garcia-Perez, *et al*., 2008)**. Although direct exposure to parturient animals or their after-birth products poses the highest risk for infection, the organism's ability to persist in the environment may result in a continued risk for infection weeks to months after the giving-birthing event. The organism is killed by pasteurization but can be transmitted in unpasteurized milk **(Hackert, *et al.,* 2012)**. Diagnosis of Q fever abortion requires laboratory testing of aborted fetuses and placenta from aborting cows, does or ewes. Diagnosis is based on identification of the organism by non-culture methods. Culturing of C. *burnetii* in the laboratory is not feasible because of the particularly contagious potential of the organism in laboratory cultures to laboratory technicians **(Jones, *et al.,* 2006)**. There are two numbers of serological tests for Q fever infection in animals that identify the host immune response (antibodies) to *C. burnetii* infection as an indicative of a previous or current infection **(Rouss *et al*., 2007)**. Serological

testing methods available for the detection of *C. burnetii* in animals includes complement fixation (CF), enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) tests **(Kim, *et al*., 2005)**. IFA is more sensitive than CF and it is the preferred diagnostic tests. Serology is best utilized on a herd- or flock-wide basis to demonstrate *C. burnetii* infection or exposure and is one tool that can be used to determine if infection is likely present in the herd or flock **(Porter, *et al*., 2011)**. This study aimed to declare the prevalence of *C. burnetii* in Egyptian cattle, sheep and goats. In addition, the application of IFA technique for the detection of antibodies against *C. burnetii* in cattle, sheep and goats is reported in raw milk and sera. The potential risk of transmission of the organism to man is considered.

MATERIAL AND METHODS

Samples:

Pooled milk and serum samples were collected from apparently healthy cows, sheep and goats (n= 360) consisted of pooled milk and serum 180 of each from dairy cattle (n=180) dairy goats (n=60) and dairy sheep (n=120) from **Qaluobia, Monofia, Gharbia and Kafr El-Sheikh** Governorates. Samples were collected in test tubes under aseptic conditions from dairy farms cows, sheep and goats during one year. Pooled milk and sera samples were transferred in sterile screw capped bottles and stored at - 20^0 C until processed (Table 1).

Table (1): Samples collected from farm animals in some Delta Governorates

Localities	Cows samples		Sheep samples		Goats samples		Total
	Milk	Serum	Milk	Serum	Milk	Serum	
Qaluobia	25	25	10	10	8	8	86
Monofia	35	35	20	20	9	9	128
Gharbia	15	15	12	12	6	6	66
Kafr ElSheikh	15	15	18	18	7	7	80
Total	90	90	60	60	30	30	360

- ***Indirect Immunofluorescence technique (IFA):***

The reagents were commercially provided from VIRCELL*, (SPAIN). Milk serum and sera were tested for IgG antibodies against *C. burnetii* phase II by using slides coated with *C. burnetii* phase II. All samples were screened at an initial dilution of 1:32 in PBS; those with negative results were considered negative. Positive samples were further classified as indicative of past infections (IgG phase II titer >32). Milk serum and sera were prepared in a

phosphate buffered saline (PBS) with IgG-fluorescein isothiocynate (FITC) immunoconjugate, *C. burnetii* control positive serum and *C. burnetii*-control negative serum. The slides were examined under ultraviolet light at a magnification of 400x by fluorescence microscope. The reaction is positive when the apple-green fluorescence of coco-bacillary morphology can be observed. The reaction is negative when no fluorescence can be observed. The technique was applied according to **Rousset *et al*., (2009)**.

RESULTS

Table (2): *Coxiella burnetii* seroprevalence and seropositivity detected in dairy farm animals By IFA technique in some Delta Governorates.

Governorates.	Cows Samples		Sheep Samples:		Goats Samples		Total
	Serum	Milk	Serum	Milk	Serum	Milk	
Qaluobia	7 (28%)	3 (12%)	4 (40%)	2 (20%)	2 (25%)	1 (12.5%)	19 (22.09%)
Monofia	11 (31.43%)	5 (14.29%)	6 (30%)	2 (10%)	2 (22.22%)	1 (16.67%)	28 (21.88%)
Gharbia	5 (33.33%)	2 (13.33%)	4 (33.33%)	2 (16.67%)	3 (50%)	1 (16.67%)	17 (25.76%)
Kafr ElSheikh	5 (33.33%)	3 (20%)	3 (16.67%)	1 (5.56%)	3 (42.86%)	2 (28.57%)	17 (21.25%)
Total	28 (31.11%)	13 (14.44%)	17 (33.33%)	7 (21.67%)	10 (46.67%)	6 (26.67%)	81 (22.5%)

***Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre.18320 Santa Fé *GRANADA* SPAIN* http://www.vircell.com.**

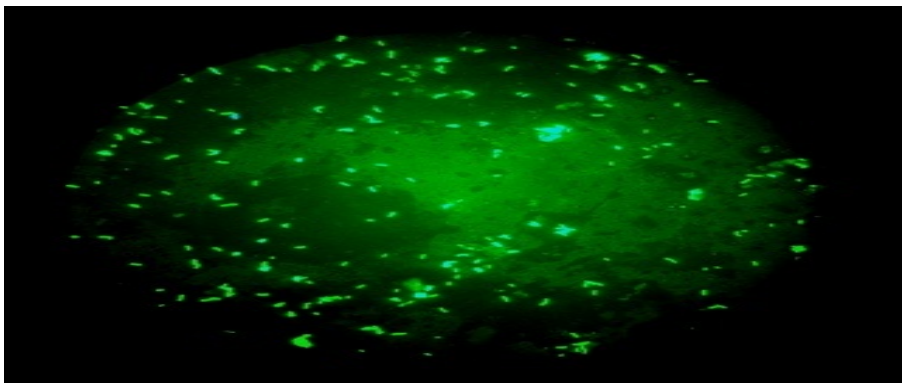

Fig. (1): C. *burnetii* phase II antibodies IgG by IFA under fluorescence microscope 400x

DISCUSSION

Q fever is an occupational zoonosis caused by *Coxiella burnetii*, a gram-negative bacterium **(Dorko, *et al*., 2012)**. Ruminant attendants, laboratory workers, dairy workers, and veterinarians are at particular risk for infection. Humans usually acquire Q fever by inhalation of *C. burnetii* aerosolized from contaminated materials originating from infected animals. The primary animal reservoirs responsible for human infections are cattle, sheep, and goats, which can shed *C. burnetii* in urine, feces, milk, and after-birth products **(Schimmer, *et al*., 2012).** Some animals may require two to three weeks to produce a serological response and others may have persistent serological titers that last for years. In addition, some animals (10%-20%) remain serologically negative while shedding *C. burnetii* on the contrary other animals that test positive may not be shedding the organism. Therefore, in the individual animal, negative serologic test results do not rule out infection, and positive serologic test results do not necessarily indicate shedding or risk of transmission **(Van der Hoek, *et al*., 2010)**. To determine the true status of the herd or flock, individual animal serologic testing of the entire breeding herd provides the best picture of the herd status. The sample size should, at a minimum assure the ability to detect disease at a 10% prevalence level **(McCaughey, *et al*., 2008**). In cattle, particularly dairy cattle, infection with *C. burnetii* is common, but abortion is uncommon. Serological testing of milk or serology of the adult herd is a reliable method for screening dairy herds for *C. burnetii* infection **(Wegdam*, et al.,* 2012).** Vaginal shedding is a short term in cattle; thus, vaginal secretions in cows should be collected within two weeks of calving or abortion **(Ryan, *et al*., 2011)**. The prevalence of antibodies in dairy herds by using IFA technique was 22.5 % with large regional differences. The prevalence of *C. burnetii* antibodies in cattle raw milk and sera were 14.44% and 31.11%, respectively. While in goats raw milk and sera were 26.67% and 46.67%, respectively and in sheep raw milk and sera were 21.67% and 33.33%, respectively. These finding agreed with **Mazyad and Hafez (2007)** who found that *C. burnetii* antibodies were detected in 22.5% and 16.8% of sheep and goats respectively in Egypt. The highest percentage of prevalence of *C. burnetii* antibodies was detected in Gharbia governorates was 25.76% and the low percentage was detected in Kafr ElSheikh governorate was 21.25%. The prevalence of *C. burnetii* antibodies in serum the sera of dairy herds by using IFA technique was 30.56% and in pooled milk samples was 14.44%. These results observed by **Hussein *et al,* (2012) and Nashwa, *et al*. (2016).**

The prevalence of *C. burnetii* antibodies was higher in dairy goat raw milk and sera were 26.67% and 46.67%, respectively than in dairy sheep raw milk and sera were 21.67% and 33.33%, respectively while in dairy cattle raw milk and sera were 14.44% and 31.11%, respectively. These findings were supported by those reported by **Rahimi *et al.*, (2010)** who found that caprine bulk milk samples from 20 goat breeding farms were positive for *C. burnetii*. The current study detected that *Coxiella burnetii* is shed in the milk of infected animals; therefore, their milk should not be consumed raw or sold unpasteurized directly to consumers. Pasteurizing milk at 145° F (63° C) for at least 30 minutes or at 161° F (72° C) for 15 seconds is sufficient to destroy *C. burnetii*, as well as other pathogens that can be present in raw milk **(FAD, 2011 and Guatteo, 2011).** Serological testing must be applied for the entire active breeding herd/flock using CF, ELISA or IFA. Testing should be performed at least annually. General Biosecurity measures and good animal husbandry performed to minimize risk of introduction of *C. burnetii* to the herd or flock **(Herrin *et al.*, 2011)**. Antibodies against *C. burnetii* are usually detected by indirect immunofluorescence (IFA). Prevalence data of C. *burnetii* infection in different ruminant species are important to support risk assessments or decisions on preventive measures regarding public and animal health **(OIE, 2010)**. Finally, we conclude that it is of utmost importance to avoid contact with the placenta, after-birth products, fetal membranes and aborted fetuses of sheep, cattle and goats. Those who are assisting the delivery of newborn animals should wear gloves, masks and eye protective glases. People with heart valve disease, who have had valve replacements or pregnant women, should be especially careful around pregnant sheep, cattle and goats. Eat and drink only pasteurized milk and milk products. The risk for spread can be decreased by:

1) Proper sanitation – good hygiene, especially when working with parturient animals.

2) Segregated kidding/lambing areas.

3) Removal of risk material from birthing areas (birthing products/fluids, contaminated bedding, manure).

4) Good manure management.

5) Control of ticks in livestock envirobment.

6) Restriction of moving peri-parturient animals (close to birthing or giving birth within the past two weeks) off the farm.

REFERENCES

Anderson, A, Bijlmer H, Fournier JE, Graves S, Hartzell J, Kersh G, Limonard G, Marrie T, Massung R, McQuiston J, Nicholson W, Paddock C and Sexton D. (2013): Diagnosis and Management of Q Fever- United States: Recommendations from the CDC and the Q Fever Working Group. MMWR*; 62; 1-23.*

Arricau Bouvery N., Souriau A., Lechopier P., and Rodolakis A. (2003): Experimental *coxiella burnetii* infection in pregnant goats: Excretion routes. *Vet. Res., 34* (4), 23-33.

Astobiza I, Barandika JF, Hurtado A, Juste RA and Garcia-Perez AL. (2010): Kinetics of *Coxiella burnetii* excretion in a commercial dairy sheep flock after treatment with oxytetracycline. Vet. J.184:172-175.

Berri M, Sourlau A, Crosby M, Crochet D, Lechopler P and Rodolakis A. (2001): *Relationships between the shedding of Coxiella burnetii, clinical signs* and serological responses of 34 sheep. Vet. Rec.; 148: 502-505.

Bottcher J. (2011): Insights into the dynamics of endemic *Coxiella burnetii* infection in cattle by application of phase –specific ELISA's in an infected dairy herd. Vet Microbiol 151: 291-300.

CDC. (2009): Case definitions for infectious conditions under public health surveillance. National Notifiable Diseases Surveillance System, CDC. Retrieved February 20, 2012. Available at:
http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/q_fever_htm

Dorko E, Rimarova K and Pilipcinec E. (2012): Influence of the environment and occupational exposure on the occurrence of Q fever. Cent Eur J Public Health. 20:208 -14.

(FDA) Food and Drug Administration (2012): List of Terms: Q. (October 26, 2011). Retrieved April 10, 2012: http://www.fda.gov/Food/ResourcesForYou/StudentsTeachers/Scienceand the food Supply/ucm 215846. htm.

Garcia-Perez AL, Astobiza I, Barandika JF, Atxaerandio R, Hurtado A and Juste RA. (2008): Short communication: investigation of *Coxiella burnetii* occurrence in dairy sheep flocks by bulk-tank milk analysis and antibody level determination. J Dairy Sci; 92:1581-1584.

Guatteo R, (2011): Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Vet Microbiol 149: 1-16, 2011.

Hackert VH, van der Hoek W, Dukers-Muijrers N, de Bruin A, Al Dahouk S, Neubauer H, Bruggeman CA and Hoebe JPA. (2012): Q Fever: Single-Point Source Outbreak with High Attack Rates and Massive Numbers of Undetected Infections across an Entire Region. Clin Infect Dis; 55:1591-9.

Herrin B, Mahapatra S, Blouin E and Shaw E. (2011): Growth of *Coxiella burnetii* in the *Ixodes scapularis*- Derived IDE8 Tick Cell Line. Vector Borne Zoonotic Dis; 11:917-922.

Hussien MO, ElFahal AM, Enan KA, Taha KM, Mohammed MS, Salih DA, Mohammadain SI, Saeed AA and El-Hussein AM. (2012): Seroprevalence of Q fever in Goats of the Sudan, Vet World; 5:394 -397.

Jones RM, Twomey DF, Hannon S, Errington J, Pritchard GC, Sawyer J. (2006): Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR. Vet Rec18; 167:965-7.

Kim S, Kim E, Lafferty C and Dubovi E. (2005): *Coxiella burnetii* in bulk tank milk samples, United States. Emerg Infect Dis; 11:619-621.

Mazyad SA and Hafez AO. (2007): Q fever (*Coxiella burnetii*) among man and farm animals in North Sinai, Egypt. J Egypt Soc Parasitol. 37:135-42.

McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ and O'Neill HJ, (2010): *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiol Infect. 138:21–7**.**

Nashwa O. Khalifa, Fatma I. Elhofy, Hanan A. Fahmy, Mona M. Sobhy and Agag, M. A. (2016): Seroprevalence and Molecular Detection of *Coxiella burnetii* Infection in Sheep, Goats and Human in Egypt. ISOI J. Microbiol. Biotechnol. Food Sci. 2:1-7.

Porter SR, Caplicki G, Mainil J, Guatteo R and Saegerman C.(2011): Q fever: current state of knowledge and perspectives of research of a neglected zoonosis. Int J Microbiol. 2011: 248-418.

Rahimi E, Doosti A, Ameri M, Kabiri E and Sharifian B. (2010): Detection of *Coxiella burnetii* by Nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Zoonoses Public Health; 57: 38-41.

Rousset E, (2007): Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. Vet Microbiol 124: 286-297.

Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A and Rodolakis A. (2009): *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl Environ Microbiol; 75:428-433.

Ryan ED, Kirby M, Collins DM, Sayers R, Mee JF and Clegg T. (2011): Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples. Epidemiol Infect. 139:1413 -7.

Sanchez, J., (2006): Experimental *coxiella burnetii* infection in pregnant goats: A histopathological and immunohistochemical study. *J Comp. Pathol.; 135*: 108- 115.

Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, (2012): Low seroprevalence of Q fever in the Netherlands prior to a series of large outbreaks. Epidemiol Infect. 140:27–35.

Van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellum P, Wijkmans C, ter Schegget R, Hackert V and Van Duynhoven Y. (2010): Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveillance 15, 2. Accessed: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19520

Wegdam-Blans MC, Wielders CC, Meekelenkamp J, Korbeeck JM, Herremans T, Tjhie HT, Bijlmer HA, Koopmans MP and Schneeberger PM. (2012): Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. Clin Vaccine Immunol; 19:1110-5.

World Organization for Animal Health. (2010): OIE Terrestrial Manual, Chapter 2.1.12, and NB: Versionadopted by the World Assembly of Delegates of theOIE http://www.oie.int/ fileadmin/ . Home/eng/Health_standards/tahm/2.01.12.