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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 Suief 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 Suief 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.*, 2014).

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-Pirouz *et al.*, 2015).

So, this study aimed to detect *C. burnetii* antibodies in milk and serum of dairy cattle using PCR compared with some

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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 - 20º 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 taq 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).

Table 1 Primer and probe

Real-time	Name	Nucleotides	Sequence	Amplicons dimension (bp)	TM (ºC)
Taq-Man	Trans-RT-F	418–438	50 -gggtaaaacggtggaacaaca-3\	101	88.1
	Trans-RT-R	498–518	50 -acaaccccgaatctcattg-3\		± 0.3
	Trans-RT-Probe	446–473	FAM-aacgatcgcgtatctttaacagcgcttg-TAMRA		

TM: melting temperature

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; IgM antibodies in serum were 7.3% and 6% in milk. The highest IgM antibodies 8% in Fayoum and Beni Suief followed by Giza. While the results of IgG antibodies were recorded 24% in serum and 10.6% in milk. The highest IgG were detected 30% in Giza, 24% and 20% in Beni Suief and Fayoum, respectively Fig (1 & 2) and (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 3). The data showed that 35/77 (45.4%) positive samples of *C. burnetii* for Rt-PCR; the highest results recorded in Beni-Suief 16/28 (57.1%) followed by Giza 11/25 (445) and then 8/24 (33.3%) in Fayoum (Table 5).

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).

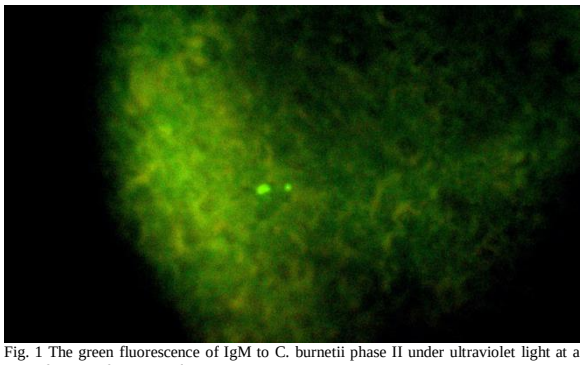

Fig. 1 The green fluorescence of IgM to C. burnetii phase II under ultraviolet light at a magnification of 400 X by fluorescence microscope

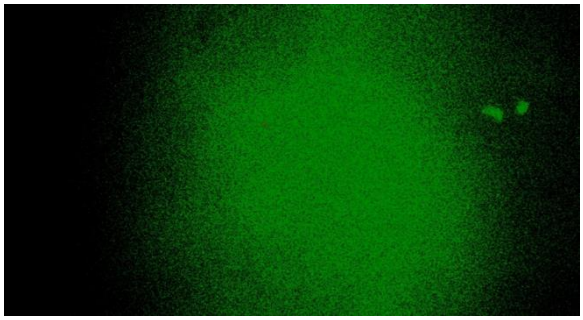

Fig. 2 The apple-green fluorescence of IgG to C. burnetii phase II under ultraviolet light at a magnification of 400 X by fluorescence microscope

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

Governorates	No of samples	No of positive samples IFAT	IgM		IgG	
			Serum n=50	Milk n=50	Serum n=50	Milk n=50
Fayoum	100	22/100 22%	4 (8%)	3 (6%)	10 (20%)	5 (10%)
Beni Suief	100	26/100 26%	4 (8%)	4 (8%)	12 (24%)	6 (12%)
Giza	100	24/100 24%	3 (6%)	2 (4%)	14 (30%)	5 (10%)
Total	300	72/300 24%	11 (7.3%)	9 (6%)	36 (24%)	16 (10.6%)

Table 3 Seroprevalence of *Coxiella burnetii* in dairy cattle by ELISA

Governorates	No of samples	No of positive samples ELISA	Type of samples	
			serum	milk
Fayoum	100	24/100 (24%)	16/50 (32%)	8/50 (16%)
Beni Suief	100	28/100 (28%)	18/50 (36%)	10/50 (20%)
Giza	100	25/100 (25%)	17/50 (34%)	8/50 (16%)
Total	300	77/300 (25.7%)	51/150 (34%)	26/150 (17.3%)

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 ELISA		No of positive samples IFAT	
		No.	%	No.	%
Fayoum	100	24/100	24%	22/100	22%
Beni Suief	100	28/100	28%	26/100	26%
Giza	100	25/100	25%	24/100	24%
Total	300	77/300	25.7%	72/300	24%

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

Governorates	No of	No of positive samples by PCR

	samples	No.	%
Fayoum	24	8/24	33.3%
Beni Suief	28	16/28	57.1%
Giza	25	11/25	44%
Total	77	35/77	45.4%

Table 6 Comparison between sensitivity and specificity of the serological tests used to diagnose coxiellosis.

Test	Sensitivity	Specificity
Immunofluorescence	84%	99%
ELISA	98%	100%
Rt-PCR	82%	97%

4. DISCUSSION

First report of Q-fever outbreak was recorded in Australia in 1935 (Setiyono *et al.,* 2005) and a zoonosis reported worldwide (Greenslade, *et al.*2003), *C. burnetii* is the causative agent, has a wide range of animal reservoirs (Li, *et al.,* 2005).

Diagnosis of Q fever is based on the detection of specific antibodies produced against the organism following exposure (Dupont, *et al.,* 1994). Serological tests have proven to be inadequate for detecting Q fever infections in the very early phase of the disease, when antibody levels are low or developing (Carrillo, *et al*.2009). The indirect immunofluorescence assay (IFA) is now considered the reference method or "Gold standard" for the diagnosis of Q fever by serology, the most sensitive and specific for the detection of *Coxiella* antibodies (Herremans, *et al.* 2013).

In the current study, IFAT revealed the presence of IgM antibodies for C. burnetii where the seroprevalence of *C. burnetii* were 6% of cow dairy milk, 7.3% of sera, IgG antibodies 10.6% of milk and 24% of dairy cow sera. The highest infection was recorded in Beni Suief 8% for IgM antibodies in both milk and sera. But the highest infection was recorded in Giza 30% for IgG antibodies in sera and 12% in dairy milk of Beni Suief. In this respect, Setiyono *et al.,* (2005) reported that IFA results are the most specific and sensitive for phase II and phase I IgG antibodies and, to a lesser extent, also for the phase II and phase I IgM antibodies in order to determine which animal represents a current risk for transmission, as animals may shedding or remain seropositive long after the acute infection.

The enzyme linked immunosorbant assay (ELISA) designed to measure antibody levels which have high sensitivity and specificity. This assay can be automated and reduce turnaround times and produces a measurable end point which allows for standardization of the assay results (Williams, *et al*. 1984). In the current study, positive samples by ELISA test were 25.7%; in serum 34% and in milk 17.3% respectively. The highest infection by ELISA test was in Beni- Suief 28% followed by Giza 25%. These results agreed with the previous results reported by Parker, *et al.* (2006). ELISA test gave specificity 100% and sensitivity 98% (Waag, 1995). The comparison between IFAT and ELISA test for detection of *Coxiella burnetii* antibodies in dairy cattle recorded 25.7% by ELISA test while, IFAT recorded 24%. The highest results were recorded in Beni Suief 28% by ELISA test and 26% by IFAT. These results demonstrated previously by Enoe, *et al.* (2000). These results showed that sensitivity and specificity of the ELISA test and IFAT were highly comparable, and supported the results previously published by Herremans *et al*. (2013).

C. burnetii is the only species belonging to the genus *Coxiella* based on the sequencing of the 16s rRNA gene in 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.

6. REFERENCES

1. Angelakis, E. and Raoult, D. (2010): Q- Fever. Veterinary Microbiology, 140 (3-4):297–309.
2. Arricau-Bouvery, N., Souriau, A., Lechopier, P., and Rodolakis, A. (2003): Experimental Coxiella burnetii infection in pregnant goats: excretion routes. Vet. Res. 34, 423-433.
3. Boroduske, P.D. Jay, P.; Manash, K. and Stayveer, K. (2017): Seroscreening of lactating cattle for coxiellosis by TRANS-PCR and commercial ELISA in Kerala, India. J. of India 5(3):377-383.
4. Carrillo, J. A., Gutierrez, J., Garcia, F., Munoz, A., Villegas, E., Rojas, J., Sorlozano, A. & Rojas, A. (2009): Development and evaluation of a multiplex test for the detection of atypical bacterial DNA in community-acquired pneumonia during childhood. Clin Microbiol Infect.J.1469-0691.
5. Dupont, H. T., Thirion, and X. & Raoult, D. (1994): Q fever serology: cutoff determination for micro-immunofluorescence. Clin Diagn Lab Immunol 1, 189–196.
6. Enoe C., Georgiadis M.P., Johnson W.O. (2000): Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown, Prev Vet Med. 45:61-81.
7. Greenslade, E., Jennings L, Woodward, A. & Weinstein, P. (2003): Has Coxiella burnetii (Q fever) been introduced into New Zealand? James A. Ferguson Emerging Infectious Diseases Fellowship. J. Clin. Microbiol. 9, 2001–2003.
8. Herremans, T., Hogema, B. M., Nabuurs, M., Peeters, M., Wegdam-Blans, M., Schneeberger, P., Nijhuis, C., Notermans, D. W., Galama, J., Horrevorts, A., van Loo, I. H. M., Vlaminckx, B., Zaaijer, H. L., Koopmans, M. P., Berkhout, H., Socolovschi, C., Raoult, D., Stenos, J., Nicholson, W. and Bijlmer, H. (2013): Comparison of the performance of IFA, CFA, and ELISA assays for the serodiagnosis of acute Q fever by quality assessment. Diagn. Microbiol. Infect. Dis. 75, 16–21.

9. Hoover, T. A., Vodkin, M.H. and Williams, J.C. (1992): *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. Bacterial. 174:5540–5548.
10. Jennifer, H. M.; Games, E.C.; Jon, G. A. and Peter, J.K. (2002): fever in Humans and Animals in the United States. Vector Borne and Zoonotic Diseases 2(3):179-91
11. Kersh G.J., Fitzpatrick K.A., Self J.S., Priestley R.A., Kelly A.J. and Lash R.R. (2013): presence and persistence of Coxiella burnetii in the environments of goat farms associated with a Q- Fever outbreak. Appl. Environ. Microbiol. 79: 1697-1703
12. Kim S.G., Kim E.H., Lafferty C.J. and Dubovi E. (2005): Coxiella burnetii in bulk tank milk samples. US Emerg Infect Dis 11(4):619–621.
13. Kirkan, S.; Kaya, O.; Tekbiyik, S. and Parin, U. (2008): Detection of Coxiella burnetii in cattle by PCR.Turkish Journal of Veterinary and Animal Sciences 32(3):215-220
14. Klee S.R., Judith T., Hainz E., Tatjana F., Sonja L., Georg Band Bernd A. (2006 a): Highly quantification sensitive real-time PCR for specific detection and of Coxiella burnetii. BMC Microbiology. 6:2.1-8
15. Li, Q., Niu, D., Wen, B., Chen, M., Qiu, L. and Zhang, J. (2005): Protective immunity against Q fever induced with a recombinant P1 antigen fused with HspB of Coxiella burnetii. Ann. N. Y. Acad. Sci. 1063, 130–42.
16. Lockhart, M. G., Graves, S. R., Banazis, M. J., Fenwick, S. G. and Stenos, J. (2011): A comparison of methods for extracting DNA from Coxiella burnetii as measured by a duplex qPCR assay. Lett. Appl. Microbiol. 52, 514–20
17. Meekelenkamp, M. N., Renvoise A, Nappez C and Raoult D. (2012): Immuno-PCR for the early serological diagnosis of acute infectious diseases: The Q fever paradigm. Eur J Clin Microbiol Infect Dis. 31(8):1951-60.
18. Nusinovici S, Hoch T, Brahim M L, Joly A and Beaudeau F. (2015): The Effect of Wind on Coxiella burnetii Transmission between Cattle Herds: a Mechanistic Approach. Trans boundary and Emerging Diseases, Apr;64(2):585-592
19. OIE, world organization for animal health (2018): Q fever. OIE, manual 2018.
20. Parker N.R., Barralet J.H., Bell A.M. (2006): Q fever. The Lancet,367(9511): 679-688.
21. Pirouz, H. A.; Gholamreza, M., Jalil M., Azizzadeh, M. & Mohammad H. N. (2015): Seroepidemiology of Q fever in one-humped camel population in northeast Iran. Tropical Animal Health and Production ISSN 0049-4747, 47. 5: 862-871.
22. Roest, H.J.; Conny, B. Jeroen, J.H.C. and Corne, K. (2013): Search for Possible Additional Reservoirs for Human Q fever, the Netherlands. Emerging Infectious Diseases 19(5) 834-835.
23. Schelling E., Diguimbaye C., Daoud S., Nicolet J., Boerlin P., et al. (2003): Brucellosis and Q-fever seroprevalence of nomadic pastoralists and their livestock in Chad. Prev Vet Med 61: 279–293.
24. Schimmer, B.; N. Schotten,1 E. van Engelen, J.L.A. Hautvast, P.M. Schneeberger, and Y.T.H.P. van Duijnhoven (2014): Coxiella burnetii Seroprevalence and Risk for Humans on Dairy Cattle Farms, the Netherlands, 2010–2011. Emerg Infect Dis. 20(3): 417–425.
25. Schmeer, N.; Krauss, H.; Lohrbach, W. and Wiegand, D. (1987): Differences in IgG1 and IgG2 responses of cattle infected with Coxiella. European Journal of Epidemiology, 5:420–424.
26. Setiyono, A.; Ogawa, M.; Cai, Y.; Shiga, S.; Kishimoto, T. and Kurane, I. (2005): New criteria for immunofluorescence assay for Q fever diagnosis in Japan. J Clin Microbiol; 43:5555–5559.
27. Soriano, F.R. Gil-Grande; J. M. Aguado; C. Pastor; M. García and C. Gómez-Pellico (1993): Conventional viral cultures and shell vial assay for diagnosis of apparently culture-negative Coxiella burnetii endocarditis. European Journal of Clinical Microbiology and Infectious Diseases, 14, (1): 64–67.
28. Taylor R.M., Mount E.A., Hoogstraal H., Dressler H.E. (1952): The presence of Coxiella burnetii (Q fever) in Egypt. J Egypt Public Health Assoc.27 (4): 123 -8.
29. Waag D.M., McKee K.T., Sandstorm J.R., Pratt L.L., Bolt C.R., England M.J., Nelson G.O. and Williams J.C. (1995): Cell-mediated and humeral immune responses after vaccination of human volunteers with the live vaccine strain of Francisella tularensis. Clin. Diagn. Lab Immunol. 2:143–148.
30. Wallensten, A., Moore, P., Webster, H., Johnson, C., van der Burgt, G., Pritchard, G., Ellis-Iversen, J. and Oliver, I. (2010): Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modeling to investigate the possibility of airborne spread. Euro. Surveill. J. 15: 19521- 32.
31. Williams, J. C., Johnston, M. R., Peacock, M. G., Thomas, L. A., Stewart, and S. & Portis, J. L. (1984): Monoclonal antibodies distinguish phase variants of Coxiella burnetii. Infect Immun 43: 421–428.