Seroprevalence of *Coxiella burnetii* (Q fever) in Cows and Buffaloes in Egypt

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Article History: 21-310

Received: 02-May-21

Revised: 25-May-21

Accepted: 27-May-21

ABSTRACT

Q fever is a zoonotic disease of public health significance caused by *Coxiella burnetii* affecting animals' productive and reproductive capabilities. This study aimed to compare seroprevalence of anti *C. burnetii* IgG and IgM antibodies (Abs) in cows and buffaloes in Egypt. Total of 314 serum and 282 milk samples were collected from 546 cows, and 50 buffaloes located in Assuit, Bani-Swif, Dakahlya, Fayoum, Giza, and Minya Governorates from 2015 to 2017. Animal reproductive status was recorded. Anti-*Coxiella* Abs were detected using Immuno-Fluorescent Antibody assay (IFA). In addition, seropositive samples were subjected to qPCR targeting isocitrate dehydrogenase-deficient (icd) gene. Differences in seropositive IgG and IgM in serum and milk samples among animal species, Governorates and animal reproductive status were investigated. In this study, overall seroprevalence of *C. burnetii* Abs was 31.5% (188/596). Considering sample type, IgG positive exceeds that of IgM in both sera and milk. IgG seroprevalence was significantly higher in serum (25.5%) than in milk (14.2%). In addition, within serum, IgG was significantly higher than IgM (12.1%). No significance was found between Governorates in IgG or IgM neither in serum nor in milk. However, significant higher seropositive IgG over IgM was observed in Assuit, Beni-Swif and Minya with 21.7, 24.5 and 23.9%, respectively. Regarding animal species, seroprevalence in cows (20.9%) was higher than in buffaloes (12.0%). IgG seroprevalence of cows was significantly higher in serum (26.0%) than milk (14.8%). In addition, within serum, seropositive IgG was significantly higher than IgM (12.5%). Both IgG and IgM seropositive were significantly the highest in abortion. Also, IgG seropositive was significantly higher in repeat breeder than apparent healthy. In buffaloes, only IgM seropositive was significantly the highest in abortion. In cows' sera, significant higher IgG over IgM was observed in all reproductive status. While in cows' milk samples, only abortion showed significant higher IgG over IgM. In buffaloes, no significant difference was observed between IgG and IgM neither in sera nor in milk. The icd gene of *C. burnetii* was detected only in 6 milk (8.6%) and 2 serum samples (1.7%). (In conclusion, the study revealed a wide distribution of infections over the study area. To set a preventive and control strategies of Q fever, further epidemiological studies are recommended. The study recommends using IgG in studying Q fever seroprevalence).

Key words: *Coxiella burnetii*, IFA, IgG, IgM, Cows, Buffaloes, Egypt.

INTRODUCTION

Q fever is a zoonotic disease caused by *C. burnetii*, a Gram negative obligate intracellular bacterium (Van Leuken et al. 2016; Sherry et al. 2019; Peter 2020). Coxiellosis affects various hosts including humans, ruminants, pets, cats, dogs, and wildlife may also serve as reservoirs for *C. burnetii* (Eldin et al. 2017). *C. burnetii* Phase I is highly infectious and can be isolated from human or animal (Gürtler et al. 2014). The infectious agent may be shed in milk, urine, and feces and found in high concentration in placenta and birth fluid (Eldin et al.

2017). Bacterial reservoirs in herds were capable of transmitting and maintaining infection in herds (Angen et al. 2011). Clinical signs of coxiellosis in animals vary, usually asymptomatic with subclinical presentation except in ruminants, where it is usually characterized by reproductive problems such as abortion, premature birth, stillbirth, weak offspring, endometritis and infertility (Agerholm 2013; Carbonero et al. 2015; OIE 2015; Chochlakis et al. 2018). Q fever is a neglected zoonosis in Egypt, and limited data on coxiellosis in human, and animals are available (Abbass et al. 2020). The accurate diagnosis of coxiellosis in animals is of great importance

Cite This Article as: Mo'awad HFM, Sobhy MM, Ismail TF and El-Enbaawy M, 2022. Seroprevalence of *Coxiella burnetii* (Q fever) in cows and buffaloes in Egypt. International Journal of Veterinary Science 11(1): 16-22. <https://doi.org/10.47278/journal.ijvs/2021.068>

to identify the infected flocks and to determine the public health significance of the disease in human (OIE 2016). *C. burnetii* does not grow using standard routine laboratory culture methods, so specific direct, and indirect diagnostic techniques have been used in diagnosis for detection of anti-*Coxiella* Abs and DNA (Eldin et al. 2017). IFA, ELISA and CFT are equal in diagnosis of acute Q fever but more IgG and IgM antibodies were detected by IFA than ELISA or CFT which make IFA more suitable for screening programs (Wegdam-Blans et al. 2012). This study aimed to compare the seroprevalence of anti *C. burnetii* Abs among sample types, Governorates and animal reproductive status in cows and buffalos in Egypt.

MATERIALS AND METHODS

Animal Ethics

All animal related procedures in the study were approved by Animal Reproduction Research Institute' research committee. The study was applied on adult animals healthy or suffering from reproductive problems.

Samples Collection

Total of 314 blood and 282 milk samples were collected from 546 cows and 50 buffaloes located in Assuit, Bani-Swif, Dakahlya, Fayoum, Giza, and Minya governorates during January 2015 to December 2017. Briefly, 5ml blood or milk were collected aseptically into plain vacuoners (Vaccutest Kima, Italy), samples were transported on ice to the laboratory. Blood samples were centrifuged at 3000rpm for 5min (Heal Force, Shanghai Lishen Scientific Equipment Co. Ltd. China), collected serum was pipetted into 1.5ml Eppendorf tubes (Eppendorf, Germany) and stored at -20°C until tested. Milk samples were centrifuged at 10000rpm at 10°C for 10min, separated milk or serum was collected in 1.5ml Eppendorf tubes. Reproductive status and geographical distribution data were recorded.

Serological Investigation

Serum and milk samples were subjected to IFA, using commercially provided kits *Coxiella burnetii* II IFA IgG or IgM (Viracell S.L., Spain) according to manufacturing instructions. Sera and milk samples were tested for IgG and IgM Abs against *C. burnetii* phase II using slides coated with *C. burnetii* antigen phase II. The reaction was considered positive when apple green fluorescence of coco-bacillary morphology was observed (Vaidya et al. 2008; OIE 2016).

Molecular Investigation

Samples showed seropositive for either IgG or IgM were subjected to quantitative real-time PCR targeting isocitrate icd gene (Maurin and Raoult 1999) Table 1. DNA extraction using DNA Miniprep Quick and Dirty Alkaline Lysis (Untergasser Lab, Germany). DNA from milk and sera samples was isolated using High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's instructions. PCR protocol consists of decontamination one cycle for 2min at 50°C, initial denaturation one cycle for 10min at 95°C, and 45 cycles of denaturation for 15s at 95°C and annealing-elongation for 30s at 60°C according to Kit's manufacturer (Roche Diagnostics, Germany).

Statistical Analysis

Analyses were performed using SPSS software® version 20 (IBM Corp, USA). The Chi-square or Fisher's exact test was used to determine differences in seropositive of IgG and IgM in serum and milk samples among governorates, species and animal reproductive status ($P < 0.05$) were considered significant.

RESULTS

Serological Investigation

Out of 596 examined samples, 188 (31.5%) were seropositive; 120 (20.1%) for IgG (80 from serum and 40 from milk) and 68 (11.4%) for IgM (38 from serum and 30 from milk). Percentage of positive IgG was significantly higher in serum 25.5% than in milk 14.2%. On the other side, no significant difference was observed between positive IgM in serum and milk; 12.1 and 10.6%, respectively. Within 314 samples of serum, IgG positive (25.5%) was significantly higher than IgM positive (12.1%). Within milk 282 samples, non-significant difference was observed between positive IgG and IgM; 14.2 and 10.6%, respectively.

Table 2 shows anti *Coxiella* IgG & IgM in sera and milk samples in different governorates. All six governorates showed seropositive in sera and milk samples. In sera, the highest IgG seroprevalence was detected in Beni-swif (27.9%), Minya (26.7%), then Assuit, Fayoum, Giza, and Dakahlya with 25, 25, 24.4 and 23.8%, respectively. For serum IgM, the highest seroprevalence of 14.3% was detected in Fayoum and Dakahlya followed by Giza (13.4%) then by Assuit, Beni-swif and Minya with 11.1, 10.3 and 6.7%, respectively. For milk IgG, the highest seroprevalence (18.8%) was detected in Minya followed by 16.7% in Beni-swif and Assuit then Giza, Dakahlya and Fayoum with 16, 13.3 and 12.1%, respectively. For milk IgM, the highest seroprevalence (13.3%) was detected in Dakahlya followed by Giza (12%) then Fayoum, Assuit, Beni-swif and Minya with 11.4, 8.3, 6.7 and 6.3%, respectively. No significance was found between Governorates in IgG or IgM either in sera or in milk. In general, the total positive IgG exceed that of total IgM within all governorates. However, significant higher IgG seropositive percentages over IgM was observed only in Assuit, Beni-swif and Minya with 21.7, 24.5 and 23.9%, respectively.

Fig. 1 displays anti *Coxiella* Abs in serum and milk samples of buffalo and cow. In buffaloes, total anti *Coxiella* Abs for sera and milk was 22.2 and 15.7%, respectively. No significant difference was observed between positive IgG or IgM in serum and milk. In cows, total anti *Coxiella* Abs for serum and milk was 38.5 and 26.0%, respectively. IgG was significantly higher in serum (26%) than in milk (14.8%). In addition, within cows' serum, seropositive IgG (26%) was significantly higher than seropositive IgM (12.5%). No significant difference was detected between positive IgM in serum and milk samples.

Table 3 shows seroprevalence of anti-*Coxiella* Abs in different reproductive status of buffaloes and cows. For buffaloes, no significant difference was observed in IgG seroprevalence between different reproductive status group. While in cows, IgG seropositive was significantly

Table 1: PCR protocol: *Coxiella burnetii* qPCR

Reagents	<i>C. burnetii</i> icd gene 5'-3' Sequence	End conc.	μl/1x	38Wells 42x
Water			7.5	315
Master mix	Maxima probe qPCR	2X	12.5	525
Icd 439F	CGTTATTTTACGGGTGTGCCA	7.5μM	1	42
Icd 439 R	CAGAATTTTCGCGGAAAATCA	7.5μM	1	42
Icd- 464 TMCoxbur	FAM-CATATTCACCTTTTCAGGCGTTTGTACCGT	2.5μM	1	42
	Master mix		23	966
	Template		2	
	Total		25	

Table 2: Anti Coxiella IgG and IgM in sera and milk samples of different Governorates

Governorate		Serum		Milk		Total	Total
		IgG	IgM	IgG	IgM	IgG	IgM
Assuit	Count/Total	9/36	4/36	4/24	2/24	13/60	6/60
	% within governorate	(25)	(11.1)	(16.7)	(8.3)	(21.7)*	(10)
Beni-swif	Count/Total	19/68	7/68	5/30	2/30	24/98	9/98
	% within governorate	(27.9)	(10.3)	(16.7)	(6.7)	(24.5)*	(10)
Fayoum	Count/Total	14/56	8/56	16/132	15/132	30/198	23/198
	% within governorate	(25)	(14.3)	(12.1)	(11.4)	(15.2)	(11.6)
Giza	Count/Total	20/82	11/82	8/50	6/50	28/132	17/132
	% within governorate	(24.4)	(13.4)	(16)	(12)	(21.2)	(12.9)
Dakahlya	Count/Total	10/42	6/42	4/30	4/30	14/72	10/72
	% within governorate	(23.8)	(14.3)	(13.3)	(13.3)	(19.4)	(13.9)
Minya	Count/Total	8/30	2/30	3/16	1/16	11/46	3/46
	% within governorate	(26.7)	(6.7)	(18.8)	(6.3)	(23.9)*	(6.5)

* Indicates significance within row at P<0.05. Values in parenthesis indicate %.

Table 3: Seroprevalence of anti Coxiella IgG and IgM in different reproductive status of buffaloes and cows

Reproductive Status		Buffaloes		Cows	
		IgG	IgM	IgG	IgM
Abortion	Count/Total	1/3	2/3	13/20	6/20
	% within status	(33.3)	(66.7) ^a	(65) ^a	(30) ^a
Repeat	Count/Total	1/8	0/8	53/227	26/227
	% within status	(12.5)	(0) ^b	(23.3) ^b	(11.5) ^b
Apparent	Count/Total	4/39	1/39	48/229	33/229
	% within status	(10.3)	(2.6) ^b	(16.1) ^c	(11) ^b

Different super scripts indicate significance within column of species at P<0.05.

Fig. 1: Anti Coxiella IgG & IgM in sera and milk samples of buffalo and cow. *Indicates significance at P<0.05.

Table 4 and 5 present reproductive status IgG and IgM crosstabulation in serum and milk samples of buffaloes and cows. For buffaloes' reproductive status, no significance was observed between seropositive IgG and IgM in milk or serum samples. For cows' reproductive status, in serum samples, significant higher seropositive IgG over IgM was observed in abortion, apparently healthy and repeat breeder; 72.7, 21.5 and 26%,

respectively. In milk samples, only abortion showed significant higher seropositive IgG (55.6%) over IgM (33.3%).

Molecular Investigation

Photo 1, out of 188 (70 milk and 118 serum) seropositive samples, icd gene of *C. burnetii* was detected only in 6 milk (8.6%) and 2 sera samples (1.7%).

Fig. 2: Q PCR detection of *C. burnetii* icd gene at seropositive milk and sera samples in cow and buffalo

Table 4: IgG and IgM crosstabulation in serum samples in relation to reproductive status

Animal species					IgM		Total
					Negative	Positive	
Buffalo	Apparent Healthy	IgG	Negative	Count	14	1	15
				% of Total	(77.8)	(5.6)	(83.3)
			Positive	Count	3	0	3
				% of Total	(16.7)	(0)	(16.7)
Cow	Abortion	IgG	Total	Count	17	1	18
				% of Total	0	3	3
			Negative	Count	0	3	3
				% of Total	(0)	(27.3)	(27.3)
	Apparent Healthy	IgG	Positive	Count	8	0	8
				% of Total	(72.7)*	(0)	(72.7)
			Total	Count	8	3	11
				% of Total	92	14	106
	Repeat Breeder	IgG	Negative	Count	92	14	106
				% of Total	(68.1)	(10.4)	(78.5)
			Positive	Count	29	0	29
				% of Total	(21.5)*	(0)	(21.5)
		Total	Count	121	14	135	
			% of Total	91	19	110	
			Negative	Count	91	19	110
				% of Total	(60.7)	(12.7)	(73.3)
Repeat Breeder	IgG	Positive	Count	39	1	40	
			% of Total	(26)*	(0.7)	(26.7)	
		Total	Count	130	20	150	

* Indicates significance at $p < 0.05$

Table 5: IgG and IgM crosstabulation in milk samples in relation to reproductive status

Animal species					IgM		Total	
					Negative	Positive		
Buffalo	Abortion	IgG	Negative	Count	0	2	2	
				% of Total	(0)	(66.7)	(66.7)	
			Positive	Count	1	0	1	
				% of Total	(33.3)	(0)	(33.3)	
Cow	Abortion	IgG	Negative	Count	1	2	3	
				% of Total	(11.1)	(33.3)	(44.4)	
			Positive	Count	5	0	5	
				% of Total	(55.6)*	(0)	(55.6)	
	Apparent Healthy	IgG	Negative	Count	6	3	9	
				% of Total	(76.8)	(11.6)	(88.4)	
			Positive	Count	19	0	19	
				% of Total	(11.6)	(0)	(11.6)	
	Repeat Breeder	IgG	Negative	Count	145	19	164	
				% of Total	(75.3)	(7.8)	(83.1)	
			Positive	Count	58	6	64	
				% of Total	(75.3)	(7.8)	(83.1)	
				Negative	Count	13	0	13
					% of Total	(16.9)	(0)	(16.9)
				Positive	Count	71	6	77
					% of Total			

* Indicates significance at $P < 0.05$.

DISCUSSION

In this study, seroprevalence of *C. burnetii* Abs, in sera and milk samples from buffaloes and cows located in six Egyptian Governorates, was investigated using IFA. IFA test is the most specific and sensitive for phase II and phase I IgG Abs and, to a lesser extent, for the phase II and phase I IgM Abs (Setiyono et al. 2005). Seropositive IgG and IgM among sample type, Governorates, species and reproductive status of animal were studied. In general, positive IgG exceed that of IgM in both sera and milk. These results confirm that IgM results provide subsidiary evidence to IgG titers (CDC 2013). The overall seroprevalence of *C. burnetii* Abs in this study was 31.5% which is nearly the same (30.7%) as reported by Jarelnabi et al. (2018) in Saudi Arabia while disagree with the findings of Abbass et al. (2020) who reported 45.3% seroprevalence in cattle may be due to different period. Also disagree with Johnson et al. (2019) who reported 21.7% seroprevalence in cattle in Ghana. IgG seroprevalence was significantly higher in serum 25.5% than in milk 14.2%. In addition, within serum, IgG (25.5%) was significantly higher than IgM (12.1%). IgG results agree with the findings of Elhofy et al. (2020) who reported, IgG seroprevalence 24% in sera and 10.6% in milk. In this study, no significant difference was observed between positive IgM in serum and milk; 12.1 and 10.6%, respectively. Also Elhofy et al- (2020) reported no significant difference between IgM in sera (7.3%) and milk (6%). Significantly higher IgG over IgM, in this study, in serum and milk agree with the findings of Cekani et al- (2008) and Khalifa et al. (2016). Seroprevalence at Governorates ranged from 30 to 37.3% in cows and from 17.9 to 18.2% in buffaloes, disagree with the findings of Klemmer et al. (2018) who recorded 4.2 to 36.4% seroprevalence in cattle and 3.3 to 100% in buffaloes. This difference could be due to difference in geographical scope or time and season. The highest IgG seroprevalence was observed in Beni-swif with 24.5% followed by Minya with 23.9% then Assuit, Giza, Dakahlya and Fayoum with 21.7, 21.2, 19.4 and 15.2%, respectively. The highest IgM seroprevalence was observed in Dakahlya 13.9% followed by Giza, Fayoum, Beni-swif, Assuit and Minya with 12.9, 11.6, 10, 10 and 6.5%, respectively, which is comparable to the findings of Elhofy et al. (2020) where the highest IgG was in Giza (30%) followed by Beni-Swif and Fayoum with 24 and 20%, respectively. We both agreed in the seroprevalence of Beni-swif and Fayoum having the lowest seroprevalence. However, in our study, Beni-swif has the highest IgG seroprevalence. Our results differ again from the findings of Elhofy et al. (2020) who reported the highest IgM in Fayoum and Beni-swif (8%) followed by Giza (5%). No significant difference was found between Governorates in IgG or IgM neither in serum nor in milk. However, within Governorates, significantly higher seropositive IgG over IgM was observed in Assuit, Beni-Swif and Minya with 21.7, 24.5 and 23.9%, respectively. These results confirm Q fever is endemic throughout Egypt in ruminants as proposed by Klemmer et al. (2018).

In relation to animal species, total seroprevalence in cows (20.9%) was higher than in buffaloes (12%), which is in accordance with the findings of Klemmer et al.

(2018) who reported 19.3 and 11.2% in cows and buffaloes, respectively. Lower seroprevalence in buffaloes than cows may be due to lower susceptibility Nahed and Khaled (2012). Our results disagree with the findings of Gwida et al. (2020) who reported *C. burnetii* Abs 20.8% in buffalo and 16% in cattle. This difference could be attributed to different sample source and different serological test (ELISA), only animals with history of abortion. In this study, the general serum profile of IgG seroprevalence was replicated in cows' serum. IgG seroprevalence of cows was significantly higher in serum (26%) than milk (14.8%). In addition, within serum, seropositive IgG (26%) was significantly higher than seropositive IgM (12.5%). Klemmer et al. (2018) pointed that animal species and geographical location are potential risks associated with seropositive *C. burnetii*.

C. burnetii infection in ruminants is primarily characterized by reproductive disorders such as abortion, stillbirth, endometritis, mastitis, and infertility (Lang 1990; To et al. 1998). In this study, seroprevalence of anti-*Coxiella* IgG and IgM between and within different reproductive status of buffaloes and cows were estimated. Between different reproductive status, in cows, both IgG and IgM were significantly the highest in abortion. Also, IgG seropositive was significantly higher in repeat breeder than apparent healthy. For buffaloes, only IgM seropositive was significantly the highest in abortion. Within reproductive status, in cows' sera, significant higher IgG seropositive over IgM was observed in all reproductive status. While in cows' milk samples, only abortion showed significant higher IgG seropositive over IgM. In buffaloes, no significance was observed between seropositive IgG and IgM neither in sera nor in milk samples. This study revealed significant higher seroprevalence of anti-*Coxiella* Abs in cow serum and milk samples with reproductive problems specially abortion, either between or within, than apparent healthy animals. These results agree with the findings of Agerholm (2013), Hansen et al. (2011) and Scolamacchia et al. (2010) who stated that *C. burnetii* infection has been associated with bovine abortion, premature delivery and birth of weak neonates in dairy cattle. However, our results also disagree with the findings of Ruiz-Fons et al. (2010) and Kim et al. (2006) who observed no correlation between abortion and seroprevalence in domestic ruminants.

A total of 8 (4.2%) seropositive serum and milk samples were found PCR positive for *C. burnetii* using qPCR. The *icd* gene of *C. burnetii* was detected only in 6 milk samples (8.6%) and 2 sera samples (1.7%). These results agree with the findings of Kirkan et al. (2008), who recorded 4.3% in cattle serum and with To et al. (1998) who reported 3.9% in Japan. And nearly in line with the findings of Yadav et al. (2021) who reported positive 6.87% of milk and 1.55% sera samples from cattle using TaqMan real-time PCR.

Conclusion

In this study, IgG in serum was significantly higher between and/or within sample types, Governorates, species and animal reproductive status. The findings recommend the use of IgG Ab against *C. burnetii* to provide evidence of past contact (Natale et al. 2012). In

addition, we confirmed IFA is suitable for detection of *C. burnetii* prevalence in cattle and buffaloes that help in identifying risk factors in *C. burnetii* epidemiology.

Acknowledgment

This paper is based upon work supported by Animal Reproduction Research Institute, ARC, Egypt and FLI, Germany. The authors would like to thank Professors Hosney Eladawy, Cathia Martien, and H. Neubor for their technical help.

Authors' Contributions

HFM Mo'awad collected the samples, performed the practical part, data analysis, interpretation and writing the original draft. MM Sobhy supervised the study. TF Ismail analyzed the data, edited and reviewed the paper. M El-Elenbaawy organized of the whole paper in final form. All authors read and approved the final version of manuscript.

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