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SEROPREVALENCE DETECTION OF ANTIBODIES OF *COXIELLA BURNETII* IN SHEEP, GOATS AND HUMAN IN SOME GOVERNORATES IN EGYPT

MONA M. SOBHY [1]; FATHI, A. [2]; IBRAHIM, E.M.M. [3]; ABOU-GAZIA, KH.A. [1]; NOHA A. HELMY [4] and ASMAA GAHLAN YOUSEEF [5]

[1] Reproductive Dis. Res. Dept. ARRI. ARC. Giza, Egypt.
[2] Immunity Unit, ARRI, ARC. Giza, Egypt.
[3] Sheep and Goats Res. Dept. APRI, ARC, Giza, Egypt.
[4] Veterinary Sera and Vaccines Research Institute (VSVRI), ARC, Giza, Egypt.
[5] Zoonosis Dept. Fac. of Vet Med. South Valley Univ.

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ABSTRACT

Q fever is an important public health concern throughout the world and infection can lifelong illness in the host. It is caused by the bacterium *Coxiella burnetii*. The most frequent source of infection for human is domestic ruminants. This survey was carried out from April 2017 to May 2018 in some governorates in Egypt. A total of 1200 samples; 740 blood samples and 405 milk samples were collected from sheep and goat flocks also 55 serum samples were taken from workers of the farms. The overall seroprevalence of *C.burnetii* in serum and milk was detected in 25.5% & 22.7% and 23.1% & 23.7% in sheep and goats, respectively. While the seroprevalence of *Coxiella burnetii* in human in this study was 54.5%. Sera and milk were screened using indirect fluorescent technique for detection of IgM & IgG and a Commercial Q fever antibody indirect ELISA test kits (IDEXX Laboratories, USA) to detect anti- *C. burnet*i IgG antibodies.

Key words: Q fever; *Coxiella burnetii*; Prevalence; Human; Sheep; Goats.

INTRODUCTION

*Q fever i*s a worldwide zoonotic disease caused by an obligate intracellular pathogen *Coxiella burnetii* (Tissot- Dupont and Raoult, 2008). *C. burnetii* has a wide range of animal reservoirs including rodents, ruminants, carnivores, lagomorphs, ticks and even birds and some wild animals (Sawyer *et al.,* 1987 and Gardon *et al.,* 2001). Ruminants were considered to be the main reservoirs for human infections (Muskens *et al.,* 2007). Infected animals excrete *C. burnetii* in milk, urine, feces, and birth by products especially the placenta (Guatteo *et al.*, 2006 and Parker *et al*., 2006). The primary mode of transmission is inhalation of pathogen contaminated aerosols from excreta of birth products (Komiya *et al.,* 2003). All animal hosts for Q fever secrete *C. burnetii* in milk; thus, consumption of raw or unpasteurized milk or milk products could be a source of infection to humans (Maurin and Raoult 1999). Clinical manifestations of Q fever in humans includes acute, chronic to fatigue syndrome. The main characteristic of Q fever is clinical polymorphism. Acute Q fever is defined as primary infection with *C. burnetii*, and <60% of infected patients may be asymptomatic (Anderson *et al.,* 2013). However, acute Q fever can manifest as a flu-like and self-limited illness, and major clinical presentations of these patients are fever, headache, coughing, atypical pneumonia, hepatitis, myalgia, arthralgia, cardiac involvement, skin rash and neurologic signs, and 2% of patients with acute disease are hospitalized. The case fatality rate of acute Q fever is reported up to 1–2% (Angelakis and Raoult 2010, Parker *et al.,* 2006 and Frankel *et al.,* 2011).

Approximately 5% of acute Q fever cases go on to develop chronic Q fever. People may become chronically infected without having being previously diagnosed with acute disease and may manifest months or years after an acute infection (Fenollar *et al.,* 2001). Chronic Q fever is accompanied with endocarditis, vascular infection, prosthetic joint arthritis, osteoarticular infection and lymphadenitis (Angelakis and Raoult 2010, Raoult 2012 and Eldin *et al.,* 2017). Endocarditis and vascular infection caused by Q fever are fatal if untreated (Anderson *et al.,* 2013).

The aim of this study was to survey sheep, goats and human in some governorates in Egypt to estimate

Corresponding author: Dr. Mona M. Sobhy
E-mail address: monagabr17@yahoo.com
Present address: Reproductive Dis. Res. Dept. ARRI. ARC. Giza, Egypt

prevalence of *C.burnetii* infection in 4 seasons of the year.

MATERIALS AND METHODS

This survey was carried out from April 2017 to May 2018 in some governorates in Egypt. A total of 1200 samples; 740 blood samples and 405 milk samples were collected from sheep and goat flocks also 55 serum samples were taken from workers of the same farms. Sera were obtained by centrifugation at 1500 rpm for 10 minutes and kept at -20°C until tested. Sera and milk samples were screened by using a commercial Q fever indirect fluorescent technique for detection of IgM & IgG also applied ELISA test.

Table 1: Samples collected from sheep, goats and farm workers in some governorates in Egypt.

locality	No. of samples	Sheep samples		Goats samples		Farm Workers samples
		serum	milk	serum	milk	
Giza	**150**	50	45	25	25	5
Fayoum	**158**	70	25	40	15	8
Beni Suef	**117**	40	15	30	25	7
Menia	**198**	70	30	60	30	8
Mansoura	**109**	40	20	25	15	9
Sharkia	**178**	60	40	45	25	8
Assuit	**190**	60	30	60	35	5
Qena	**100**	30	20	35	10	5
Total	**1200**	420	225	320	180	55

Table 2: Samples from sheep, goats and farm workers in different season.

Season	No. of samples	Sheep samples		Goats samples		Farm Workers samples
		serum	milk	serum	milk	
Spring	**308**	120	70	65	40	13
Summer	**315**	110	45	90	55	15
Autumn	**287**	100	60	70	40	17
Winter	**290**	90	50	95	45	10
Total	**1200**	420	225	320	180	55

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

ELISA test:

Commercial Q fever antibody indirect ELISA test kits (IDEXX Laboratories, USA) were used to detect anti-*C. burneti*i 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).

RESULTS

Table 3: Seroprevalence of C. burnetii among sheep, goats and worker farms samples in some governorates in Egypt by ELISA test.

locality	No. of samples	Sheep +ve samples		Goats +ve samples		+ve Farm Workers samples
		serum	milk	serum	milk	
Giza	150	10/50 20%	7/45 15.5%	9/25 36%	3/25 12%	3/5 60%
Fayoum	158	18/70 25.7%	5/25 20%	8/40 20%	2/15 13.3%	5/8 62.5%
Beni Suef	117	12/40 30%	3/15 20%	9/30 30%	5/25 20%	4/7 57.1%
Menia	198	20/70 28.5%	9/30 30%	12/60 20%	4/30 13.3%	4/8 50%
Mansoura	109	12/40 30%	6/20 30%	9/25 36%	6/15 40%	5/9 55.5%
Sharkia	178	15/60 25%	8/40 20%	11/45 24.4%	10/25 40%	4/8 50%
Assuit	190	12/60 20%	7/30 23.3%	10/60 16.7%	7/35 20%	3/5 60%
Qena	100	8/30 26.7%	6/20 30%	6/35 17.1%	2/10 20%	2/5 40%
Total	1200	107/420 25.5%	51/225 22.7%	74/320 23.1%	39/180 21.7%	30/55 54.5%

Table 4: Positive cases for Q fever among examined sheep, goats and worker farms samples by ELISA test in relation to seasons.

Season	No. of samples	No. of +ve samples	Sheep samples		Goats samples		
			serum	milk	serum	milk	
Spring	308	70/308 22.7%	28/120 23.3%	12/70 17.1%	17/65 26.1%	5/40 12.5%	8/13 61.5%
Summer	315	82/315 26%	32/110 29.1%	12/45 26.7%	21/90 23.3%	9/55 16.4%	8/15 53.3%
Autumn	287	86/287 29.9%	27/100 27%	14/60 23.3%	20/70 28.6%	16/40 40%	9/17 52.9%
Winter	290	63/290 21.7%	20/90 22.2%	13/50 26%	16/95 16.8%	9/45 20%	5/10 50%
Total	1200	301/1200 25.1%	107/420 25.5%	51/225 22.7%	74/320 23.1%	39/180 21.7%	30/55 54.5%

Table 5: Seroprevalence of *C. burnetii* among sheep, goats and farm worker samples in some governorates by IFA test.

locality	No. of samples	Sheep +ve samples				Goats +ve samples				+ve farm workers samples	
		serum		milk		serum		milk			
		IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Giza	**150**	**3/50 6%**	**6/50 12%**	**2/45 4.4%**	**5/45 11.1%**	**3/25 12%**	**5/25 20%**	**1/25 4%**	**2/25 8%**	**1/5 20%**	**2/5 40%**
Fayoum	**158**	**4/70 5.7%**	**12/70 17.1%**	**2/25 8%**	**3/25 12%**	**2/40 5%**	**5/40 12.5%**	**0**	**2/15 13.3%**	**1/8 12.5%**	**4/8 50%**
Beni Suef	**117**	**3/40 7.5%**	**6/40 15%**	**0**	**2/15 8%**	**3/30 10%**	**5/30 16.6%**	**2/25 8%**	**2/25 8%**	**2/7 28.6%**	**3/7 42.8%**
Menia	**198**	**5/70 7.1%**	**14/70 20%**	**2/30 6.7%**	**5/30 16.7%**	**4/60 6.7%**	**7/60 11.7%**	**2/30 6.7%**	**2/30 6.7%**	**1/8 12.5%**	**2/8 25%**
Mansoura	**109**	**3/40 7.5%**	**8/40 20%**	**2/20 5%**	**3/20 15%**	**2/25 8%**	**4/25 16%**	**1/15 6.7%**	**2/15 13.3%**	**2/9 22.2%**	**4/9 44.4%**
Sharkia	**178**	**4/60 6.7%**	**10/60 16.7%**	**1/40 2.5%**	**5/40 12.5%**	**4/45 8.9%**	**6/45 13.3%**	**3/25 12%**	**4/25 16%**	**0**	**4/8 50%**
Assuit	**190**	**4/60 6.7%**	**6/60 10%**	**2/30 6.7%**	**3/30 10%**	**2/60 3.3%**	**6/60 10%**	**2/35 5.7%**	**4/35 11.4%**	**1/5 20%**	**3/5 60%**
Qena	**100**	**2/30 6.7%**	**4/30 13.3%**	**1/20 5%**	**3/20 15%**	**1/35 2.8%**	**4/35 11.4%**	**1/10 10%**	**2/10 20%**	**1/5 20%**	**2/5 40%**
Total	**1200**	**28/420 6.7%**	**66/420 15.7%**	**10/225 4.4%**	**24/225 10.7%**	**18/320 5.6%**	**42/320 13.1%**	**12/180 6.7%**	**20/180 11.1%**	**9/55 16.4%**	**24/55 43.6%**

DISCUSSION

In this study the overall occurrence of *Coxiella burnetii* in serum and milk was detected in (25.5% & 22.7%) and (23.1% & 23.7%) identified by ELISA in sheep and goats respectively (Table 3). Lower percentages of *Coxiella burnetii* specific antibodies were detected in 8.9% of sheep and 6.8% of goats' blood samples, respectively by Klemmer *et al.* (2018). Also Mohammed *et al.* (2014) reported that the percentage of *Coxiella burnetii* in goat's blood samples was 5.3%, but milk samples obtained from goats showed no positive samples, while none of the samples collected from sheep revealed positive for *C. burnetii*. However the overall seropositivity of Q fever detected in sheep and goats (25.5% & 23.1%) was in agreement with that reported (24.7% & 24.2%) in Iran and Sudan by Hussein *et al.* (2012) and Mobarez *et al.* (2017), respectively.

In contrast, a study on farm animals from the Giza, Cairo and Fayoum governorates showed remarkably high seroprevalence in sheep 32.7% but seroprevalence in goats was (23.3%) which agreed with Nahed and Abdel-Moein (2012). This difference could be explained by the high small ruminant density of this rural region and the fact that infected small ruminants may shed bacteria in high numbers (Dijkstra *et al*., 2012 and Abdel- Moein and Hamza, 2017).

Klemmer *et al*. (2018) examined sheep and detected *C. burnetii* specific antibodies in 8.3%, 16.7%, 8.3%, 0%, 25%, 0% and 11.1% from Menia, Sharkia, Qena, Giza, Fayoum, Beni Suef and Assuit governorates. Their results are in agreement with the data of this study corresponding to 25.7% in Fayoum governorate. But their results are lower than the result of this study corresponding to 28.6%, 25%, 26.7%, 20%, 30% and 20%in Menia, Sharkia, Qena, Giza, Beni Suef and Assuit governorates. The high seroprevalence of Q fever among sheep and goats highlighted the potential role which may be played by these animals in the epidemiology of Q fever which being important reservoirs for *C. burnetii* and its zoonotic implications in Egypt (Nahed and Abdel-Moein, 2012). However Klemmer *et al*. (2018) failed to detect C. *Burnetii* specific antibodies in goats from Sharkia, Qena, Giza, Assuit governorates, while C. *Burnetii* was detected in goats from Menia and Beni Suef in 8.3% and 12.5%, respectively. The differences in prevalence rates may be attributed to local ecological factors, type of management and practices, flock size... etc. that might influence the transmission rates and infection with *C. burnetii* (Hussein *et al*., 2017).

The seroprevalence of *Coxiella burnetii* in farm workers in this study was 54.5% which is greater than that obtained by Mazyad and Hafez (2007) (3.3%), Botros *et al*. (1995) who found a seroprevalence 25% among cattle workers in Egypt, Nahed and Abdel-Moein (2012) who recorded the seroprevalence 16.3% in the examined persons and Vilibic-Cavlek *et al.* (2012) detect IgG antibodies of *Coxiella burnetii* in Sera from 27.5% patients. Human seroprevalence was reported from 1–32% in Africa (Vanderburg *et al.,* 2014). Human seroprevalence of Q fever were reported 3 to 35.8% in Kenya (Njeru *et al.*, 2016), 12.3–32% in Turkey (Kilic *et al*., 2008 and Gozalan *et al.,* 2010). The differences between

countries could be due to varieties in ecologic, social, cultural, behavioral and economic conditions and also levels of animal's infections, which affect the exposures of people in each region of the world (Mobarez *et al.,* 2017). Also these differences can be attributed to the variations in the proportion of population involved in farming activities (Vilibic-Cavlek *et al.,* 2012).

The IgG of Q fever reported in this study by IFA test in sheep (15.7%) was higher than in goats (13.1%) in serum but was reverse in goats (11.1%) higher than in sheep (10.7%). While, IgG in farm workers was recorded 43.6% (Table, 5). Transmission of *C. burnetii* through milk is also possible (Kruszewska *et al*., 1997). Consumption of raw or unpasteurized milk or milk products could be a source of infection milk borne pathogens (Hussien *et al*., 2017).

Similar to other studies, the present results showed that the prevalence of *C. burnetii* antibodies tends to increase in summer and autumn months. A majority of cases were recorded in summer and autumn (Vilibic-Cavlek *et al.,* 2012). Also data from the European Union have shown a seasonal pattern of Q fever with more cases reported during the summer months (Coulombier, 2010).

CONCLUSION

It is concluded that consumption of raw milk and milk products from all domestic species should be avoided to reduce the risk of infection with Q fever as well as with other milk and milk products borne pathogens. The IFA and ELISA tests proved to be sensitive and specific methods for the detection of *Coxiella* antibodies. Both assays have a high positive and negative predictor value, ensuring a high correlation with previous exposure to *Coxiella.*

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إكتشاف نسبة التواجد السيرولوجى لميكروب الكوكسيلا برنتى فى الاغنام والماعز والانسان فى بعض المحافظات بمصر

منى محمد صبحى ، أحمد فتحى ، عيد محمد محمد ابراهيم ، خالد عبد السميع ابو جازية ، نهى عارف حلمى ، أسماء جهلان غضبان

E-mail: monagabr17@yahoo.com Assiut University web-site: www.aun.edu.eg

تعتبر حمى المجهولة أحد الامراض الهامة التى تؤثر على الصحة العامة في جميع أنحاء العالم ، ويمكن الإصابة بالعدوى للمصاب بالمرض مدى الحياة. ويسببه بكتيريا تسمى كوكسيلا برنتى Coxiella burnetii. وتعتبر المجترات الصغيرة المصدر الرئيسى للإصابة فى الانسان. تم إجراء هذا المسح من أبريل ٢٠١٧ إلى مايو ٢٠١٨ في بعض المحافظات في مصر. تم تجميع عدد ١٢٠٠ عينة عبارة عن: ٧٤٠ عينة دم و ٤٠٥ عينة لبن من قطعان الأغنام والماعز ، كما تم أخذ ٥٥ عينة مصل من عمال المزارع. وكانت نسبة تواجد الاجسام المضادة IgG لميكروب الكوكسيلا برنتى في السيرم (٢٥.٥ ٪ و ٢٢.٧ ٪) وفى اللبن (٢٣.١ ٪ و ٢٣.٧ ٪) في الأغنام والماعز على التوالي بأستخدام أختبار القياسية المناعية الانزيمية (الاليزا) (شركة IDEXX). في حين أن نسبة تواجد الاجسام المضادة IgG للميكروب فى سيرم عمال المزارع فى هذه الدراسة كان ٥٤.٥ ٪ بأستخدام أختبار القياسية المناعية الانزيمية وتم فحص السيرم والالبان باستخدام تقنية الفلورسنت غير المباشرة للكشف عن الاجسام المضادة IgM و IgG للحمى المجهولة.