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Some associated risk factors with *Coxiella burnetii* in sheep, humans and ticks in Menoufiya governorate, Egypt

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Q fever is an emerging and re-emerging anthropozoonosis infecting humans, more than 30 species of domestic and wild animals, wild and domestic birds, reptiles, ticks and even marine mammals all over the world except in the Antarctic and New Zealand. The current study aimed to highlight the public health significance and risk factors of acquiring Q fever in sheep, humans and ticks in Menoufiya governorate. The seroprevalence of anti-*C. burnetii* phase II IgG antibodies was 61.96% (57/92) among sheep and 41.85% (77/184) among humans by using ELISA. A significant association was revealed between increasing *C. burnetii* seroprevalence rate and each of sheep male sex, sheep age, the warm climate during sampling of sheep and humans, human males, increasing human age, immuno compromised status of individuals, smoking behaviour and the history of contact with water bodies among patients. Furthermore, a significant association was revealed between increasing *C. burnetii* seroprevalence rate and presence of fever, cough, retrobulbar headache, anemia, thrombocytopenia, increased liver enzymes and absence of skin rash. *C. burnetii* DNA was found in 13.33% (4/30) of sheep samples, 6% (3/50) of human samples and 13.33% (8/60) of tick pools samples. A non-significant association was showed between PCR positivity and tick species, sex and the prevalent climatic condition during sampling of tick pools. In conclusion, the survival capability of *C. burnetii* for long periods in environment, spread by several ways and the asymptomatic nature of the disease with lack of cheap diagnostic tools enable Q fever to pass miss-diagnosed in Menoufiya governorate, Egypt.

Keywords: ***Coxiella burnetii*, ELISA phase II IgG, humans, PCR, sheep and ticks.**

INTRODUCTION

Query fever (Q fever) is a serious worldwide old recognized poorly understood disease infecting animals and man all over the world except in the Antarctic and New Zealand (Eldin et al. 2017). In the developing countries, Q fever is a major threat since it causes a significant loss of animal productivity, major economic losses and zoonotic risk to humans. Sheep were shown as the most frequent ruminant species in acquiring Q fever followed by goats and cattle. Although animal coxiellosis is usually asymptomatic in nature, clinical expression of *C. burnetii* infection in sheep includes decreased appetite and

depression 1-2 days, reduced reproductive efficiency expressed as outbreaks of abortion in late pregnancy, stillbirths, endometritis and infertility (Johnson et al., 2019).

The importance of *C. burnetii* among humans was represented firstly when used as a bioterrorism agent during World War II causing numerous epidemics of febrile illnesses among the German forces and during the biological warfare between USA and the former Soviet Union. Later in the 1980s, many Q fever human outbreaks estimated by hundred to thousand cases have been reported in different world countries as: Athens, Italy, USA, Spain, Switzerland, Great Britain, Germany, Scotland and finally in the Netherlands where more than 4,000 human cases had been recorded in the period between 2007 and 2010 due to the intensification of dairy goat husbandry (Roest et al., 2011). As a result, USA and some European countries implemented up-to-date surveillance systems for the notification of Q fever (CDC, 2002 and Sidi-Boumedine et al., 2010). In Africa, Q fever was considered the first causative agent of abortion followed by toxoplasmosis, neosporosis and chlamydiosis and also was considered the third causative agent of atypical pneumonia after *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* (Koulla-Shiro et al., 1997 and Abdelhadi et al. 2015) whereas in Egypt, Q fever was demonstrated as a potential risk in animals and man at 1995. However, the disease is an endemic neglected disease with a little attention is paid for the public education about the danger of it, modes of its spread and methods of control (Botros et al., 1995).

In humans, the disease has a public health potential since its ability for manipulation of the immune system and establishing a chronic infection after 6 months of infection resulting in serious damage of person's vital organs (heart, brain, liver and lungs). Spontaneous abortions and many adverse pregnancy outcomes may also develop in pregnant women (Rahaman et al., 2019). In ticks, 40 hard and 14 soft tick species are naturally *Coxiella* infected by transstadial or transovarian manner, harboring *C. burnetii* in the digestive tract and expelling the organism in saliva and faeces contaminating wool and skin of hosts during feeding (Eldin et al., 2017).

MATERIALS AND METHODS

2.1. Specimen collection:

A total of 92 serum samples were collected from the jugular vein of 92 adult sheep of Baladi breed of different ages and sexes from different localities in Menoufiya governorate. Serum samples were examined against anti-*C. burnetii* phase II IgG antibodies by ELISA. Furthermore, 30 blood samples were also collected and examined by using PCR. Sheep samples were collected in the period from February 2018 to October 2018. In addition, a total of 184 attendants of Shebein El Kom fever and chest hospitals (the main fever and chest hospitals in Menoufiya governorate) were examined in that study. 184 human serum samples were collected from human cephalic veins and examined for the presence of anti-*C. burnetii* phase II IgG antibodies by ELISA in the period from March 2018 to February 2019. 50 human blood samples were also collected and examined by PCR.

Approximately 5 ml of sheep and human blood samples were collected by using sterile disposable syringes in a sterile glass tube without anticoagulant. The blood was left to stand for about half an hour and then centrifuged at 3000 rpm for 10 minutes to obtain non haemolyzed clear serum. Moreover, another 3 ml of sheep and human blood samples were collected by sterile syringes with wide pores in tubes coated with an anticoagulant Ethylene Diamine Tetra Acetate (sodium salt of EDTA). The serum and whole blood samples were stored in labeled aliquots at -20 °C.

Moreover, during the period from May 2018 to May 2019, a total of 300 adult ticks (200 from cattle and 100 from dogs) were collected from different localities in Menoufiya governorate. They were properly noted and transferred on ice to the laboratory of department of Parasitology in Animal health research institute (AHRI), Giza, Egypt for immediately identification by using of stereomicroscope according to Walker (2003). After identification, ticks were divided into 60 pools (each pool contained 5 ticks) according to collection date, locality, size, sex, species and host as described by Noh et al., (2017). Ticks were washed in 70% ethanol for 10 minutes, air dried, rinsed twice with sterile distilled water and they were cut with sterile scalpel. Each tick pool was homogenized with 200 µl of PBS in 1.5 ml sterile micro-centrifuge tubes and kept frozen at -20 ˚C till DNA examination by PCR.

2.2. Detection of anti-*C. burnetii* phase II IgG antibodies by ELISA assay:

All collected serum samples were examined for phase II IgG antibodies by using the commercial ELISA kit (Vircell SL® Granada, Spain. G1001, 96 tests).

2.3. Detection of *C. burnetii* DNA by PCR:

2.3.1. Extraction of C. burnetii DNA:

Extraction of ticks DNA was performed using the commercial G-spin™ total DNA extraction kit (cat. no. IBT-QMS-GT1704 (R01-2012-01), intron biotechnology, Seoul, Korea) according to the manufacturer's instructions whereas extraction of DNA from sheep and human blood samples was done by using ABIOpure™ total DNA Blood/Cell Extraction kit (cat. no. M501DP100, Alliance Bio Inc., USA).

2.3.2. Amplification and detection of C. burnetii DNA:

The primers of (*com1*) gene were used: forward 5'-CCCTGCAATTGGAACGAAG-3' and reverse 5'-GTTCTGATAATTGGCCGTCGACA-3'. Final volume of PCR reaction was set up in a 25 µl. Each PCR tube contained 12.5 µl of master mix, 1 µl of each primer, 6.5 µl of distilled water and 4 µl of the extracted DNA. In a thermal cycler (model GS1, Thermal Cycler Base Unit GS00001, USA), cycling conditions were performed as: denaturation at 95 ˚C for 10 minutes followed by 35 cycles at 95 ˚C for 30 seconds, at 55 ˚C for 30 seconds and 72 ˚C for 1 minute for each cycle. The final cycle was followed by an extension step at 72 ˚C for 10 minutes. A volume of 10 µl of each PCR product was subjected to gel electrophoresis in 1.5% agarose gel electrophoresis (cat. no. PG-40005, 100 g, Genetix Biotech Asia Pvt. Ltd., New Delhi, India) with using a DNA ladder (Trans® 100 bp plus II DNA ladder, cat. no. BM321-01, Transgen biotech Co., Beijing, China). Expected PCR product size was 775 bp. Nine-Mile strain of *C. burnetii* which was used as positive control that was kindly obtained from department of molecular diagnostics and therapeutics, GEBRI, USC, Egypt.

2.4. Statistical analysis:

Data were analyzed using Chi-square analysis test (X^2) by using SPSS (Statistical Package for Social Science) version 17. P-value > 0.05 meant statistically insignificant while P-value ≤ 0.05 meant statistically significant and P-value < 0.01 meant statistically highly significant.

RESULTS

3.1. Seroprevalence of anti-*C. burnetii* phase II IgG antibodies in the tested sheep and patients in Menoufiya governorate by ELISA test.

The results presented in table (1) revealed the seroprevalence of anti-*C. burnetii* phase II IgG antibodies among the examined sheep was 61.96% (57 out of 92). Moreover, the seroprevalence of anti-*C. burnetii* phase II IgG antibodies was 41.85% (77 out of 184) among the human patients.

3.2. Demographic characteristics of the examined sheep.

As shown in table (2), the seroprevalence rate of anti-*C*. *burnetii* phase II IgG antibodies in the tested sheep in relation to sex was higher 81.82% (18 out of 22) among rams versus a seropositivity rate of 55.71% (39 out of 70) among ewes. The difference between groups was statistically significant whereas the highest seropositivity rate 76.09% (35 out of 46) was detected among sheep aging (> 2 years) in comparison with a seropositivity rate of 48.28% (14 out of 2) among sheep aging (1-2 years) and the least seropositivity rate of 47.06% (8 out of 17) among sheep aging (< 1 year). A significant association was declared between increasing the seroprevalence rate and increasing sheep age. Moreover, it was showed that the warm climate had a higher seroprevalence rate 69.49% (41 out of 59) versus a seroprevalence rate of 48.48% (16 out of 33) in cold climate. A significant association was shown between warm climate and increasing the seroprevalence rate.

3.3. Demographic characteristics, clinical symptoms and laboratory findings of the examined patients.

Table (3) revealed the seroprevalence of anti-*C. burnetii* antibodies in the examined patients in relation to their sex to be higher 49.53% (53 out of 107) among males versus 31.17% (24 out of 77) among females. The difference between two groups was statistically significant.

Regarding the human age, it was showed that the highest seroprevalence rate 55% (33 out of 60) was recorded among the age group (41-60 years) followed by seroprevalence rate 44.44% (20 out of 45) among the age group (61-80 years).

Table 1: Seroprevalence of anti-*C. burnetii* phase II IgG antibodies in the tested sheep and patients in Menoufiya governorate by ELISA test:

Species	Total no. tested	Positive phase II IgG		Negative phase II IgG	
		No.	%	No.	%
Sheep	92	57	61.96	35	38.04
Human	184	77	41.85	107	58.15

Table 2: Demographic characteristics of the examined sheep:

Demographic characteristics	Positive phase II IgG		Negative phase II IgG		Chi-square	P-value
	No.	%	No.	%		
Sex:						
-Males (Rams) (No. = 22)	18	81.82	4	18.18		
-Females (Ewes) (No. = 70)	39	55.71	31	44.29	4.84*	0.028
Total (No. = 92)	57	61.96	35	38.04		
Age:						
< 1 year (No. = 17)	8	47.06	9	52.94		
1-2 years (No. = 29)	14	48.28	15	51.72		
> 2 years (No. = 46)	35	76.09	11	23.91	7.80*	0.02
Total (No. = 92)	57	61.96	35	38.04		
Prevalent climatic condition during sampling:						
-Cold climate (No. = 33)	16	48.48	17	51.52		
-Warm climate (No. = 59)	41	69.49	18	30.51	3.96*	0.047
Total (No. = 92)	57	61.96	35	38.04		

* Statistically Significant

Table 3: Demographic characteristics, clinical symptoms and laboratory findings of the examined patients:

Items	Positive phase II IgG		Negative phase II IgG		Chi-square	P-value
	No.	%	No.	%		
1.Demographic characteristics:						
1.1.Sex:						
-Males (No. = 107)	53	49.53	54	50.47		
-Females (No. = 77)	24	31.17	53	68.83	6.21*	0.013
Total (No. = 184)	77	41.85	107	58.15		
1.2.Age:						
≤ 20 (No. = 48)	13	27.08	35	72.92		
21-40 (No. = 31)	11	35.48	20	64.52		
41-60 (No. = 60)	33	55	27	45		
61-80 (No. = 45)	20	44.44	25	55.56	9.21*	0.03
Total (No. = 184)	77	41.85	107	58.15		
1.3.Prevalent climatic condition during sampling:						
-Cold climate (No. = 83)	23	27.71	60	72.29		
-Warm climate (No. = 101)	54	53.47	47	46.53		
Total (No. = 184)	77	41.85	107	58.15	12.42**	0.0004
1.4.Immunological status (patients with HIV, HCV and HBV):						
-Immunocompromized individuals (No. = 96)	50	52.08	46	47.92		

-Immunocompetent individuals (No. = 88)	27	30.68	61	69.32	8.64**	0.003
Total (No. = 184)	77	41.85	107	58.15		
1.5.Smoking behavior: **-Smokers (No. = 39)**	22	56.41	17	43.59		
-Non-smokers (No. = 145)	55	37.93	90	62.07	4.31*	0.038
Total (No. = 184)	77	41.85	107	58.15		
1.6.Contact with water bodies (during washing animals, cleaning dishes and/or swimming in canals) **-Yes (No. = 31)**	19	61.29	12	38.71		
-No (No. 153)	58	37.90	95	62.10	5.79*	0.016
Total (No. = 184)	77	41.85	107	58.15		
<u>2.Clinical symptoms:</u> **2.1.Presence of acute febrile illness (more than 0.8 °C above the normal) of unknown cause for more than one week:** **-Present (No. = 115)**	56	48.70	59	51.30		
-Absent (No. = 69)	21	30.43	48	69.57	5.91*	0.015
Total (No. = 184)	77	41.85	107	58.15		
2.2.Skin rash: **-Present (No. = 38)**	9	23.68	29	76.32		
-Absent (No. = 146)	68	46.58	78	53.42	6.49*	0.011
Total (No. = 184)	77	41.85	107	58.15		
2.3.Cough: **-Present (No. = 116)**	57	49.14	59	50.86		
-Absent (No. = 68)	20	29.41	48	70.59	6.86**	0.009
Total (No. = 184)	77	41.85	107	58.15		
2.4.Retrobulbar headache: **-Present (No. = 97)**	48	49.48	49	50.52		
-Absent (No. = 87)	29	33.33	58	66.67	8.64**	0.003
Total (No. = 184)	77	41.85	107	58.15		
2.5.Oral congestion: **-Present (No. = 117)**	56	47.86	61	52.14		
-Absent (No. = 67)	21	31.34	46	68.66	4.78*	0.03
Total (No. = 184)	77	41.85	107	58.15		
<u>3.Laboratory findings:</u> **3.1.Anemia (Hemoglobin < 11 g/dl):** **-Present (No. = 108)**	51	47.22	57	52.78		
-Absent (No. = 76)	26	34.21	50	65.79	7.14**	0.008
Total (No. = 184)	77	41.85	107	58.15		
3.2.Thrombocytopenia (Platelets < 150x109 /L): **-Present (No. = 65)**	34	52.31	31	47.69		
-Absent (No. = 119)	43	36.13	76	63.87	4.52*	0.034
Total (No. = 184)	77	41.85	107	58.15		
3.3.Increased liver enzymes (ALT & AST > 40 IU/L): **-Present (No. = 86)**	46	53.49	40	46.51		
-Absent (No. = 98)	31	31.63	67	68.37	8.99**	0.003
Total (No. = 184)	77	41.85	107	58.15		

** Highly Significant * Statistically Significant

The statistical analysis of these results by using chi-square revealed that there was a significant variation between different age groups with obvious association was declared between increasing prevalence of anti-*C. burnetii* IgG antibodies with human age even if their total number decreased in comparison with a seroprevalence rate of 35.48% (11 out of 31) among the age group (21-40 years) and the least seroprevalence rate 27.08% (13 out of 48) was shown among the age group (≤ 20 years).

Concerning the prevalent climatic conditions during human sampling, warm climate was declared having a higher seroprevalence of anti-*C. burnetii* phase II IgG antibodies 53.47% (54 out of 101) in comparison with the seroprevalence rate noted during cold climate 27.71% (23 out of 83). Statistical analysis using chi-square revealed that the difference between the two groups was highly significant indicating that chances for acquiring Q fever increases in humans residing Menoufiya governorate during warm climate than during cold climate in the present study.

Concerning the immunological status of the examined patients, the seroprevalence of anti-*C. burnetii* phase II IgG antibodies was higher 52.08% (50 out of 96) among immunocompromized patients versus 30.68% (27 out of 88) among immunocompetent individuals. A highly significant association was observed between the two groups.

By studying the effect of smoking as a risk factor for acquiring Q fever, smokers were represented to be at risk for acquiring Q fever since the seroprevalence of anti-*C. burnetii* phase II IgG antibodies among them was 56.41% (22 out of 39) versus a seroprevalence rate of 37.93% (55 out of 145) among non-smokers. The difference between the two groups was statistically significant.

Furthermore, the current study represented the patients with history of contact with water bodies had a higher seroprevalence of anti-*C. burnetii* phase II IgG antibodies 61.29% (19 out of 31) versus a seroprevalence of 37.90% (58 out of 153) among patients who had no contact with water bodies. The difference between the two groups was statistically significant.

The current study also showed a significant association between Q fever infectivity and suffering from acute febrile illness, retrobulbar headache, oral congestion and absence of skin rash as higher seroprevalence rates of anti-*C. burnetii* phase II IgG antibodies were presented as: 48.70% (56 out of 115) among feverish patients, 49.48% (48 out of 97) patients with retrobulbar headache, 47.86% (56 out of 117) among patients had oral congestion and 46.58% (68 out of 146) among patients with absence of skin rash in comparison with the obtained seroprevalence rates of 30.43% (21 out of 69), 33.33% (29 out of 87), 31.34% (21 out of 67) and 23.68% (9 out of 38) among patients had no fever, no retrobulbar headache, no oral congestion but they suffered from skin rashes, respectively. Concerning presence of cough, a higher seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies was illustrated 49.14% (57 out of 116) among patients suffered from cough than a seroprevalence rate of 29.41% (20 out of 68) among patients without cough. The difference between the two groups was highly significant.

Moreover, studying of laboratory findings among the tested patients revealed that the seroprevalence of anti-*C. burnetii* phase II IgG antibodies was higher 47.22% (51 out of 108) and 53.49% (46 out of 86) among patients with anemia and increased liver enzymes versus seroprevalence rates of 34.21% (26 out of 76) and 31.63% (31 out of 98) among those with normal levels of hemoglobin and nomal liver enzymes, respectively. Statistical analyses using Chi-square revealed that the differences among the studied groups were highly significant. In addition, seroprevalence of anti-*C. burnetii* phase II IgG antibodies was higher 52.31% (34 out of 65) among patients who had thrombocytopenia than the seroprevalence rate of 36.13% (43 out of 119) among those who hadn't. The difference between groups was statistically significant.

3.4. Molecular detection of *C. burnetii* DNA in the examined sheep blood, human blood and tick pools samples by using PCR.

Molecular detection of *C. burnetii* DNA in the examined sheep blood, human blood and tick pools samples by using PCR was declared in table (4) and figures (2), (3) and (4). *C. burnetii* DNA was found in 13.33% (4 out of 30) of sheep whole blood samples while in the human whole blood samples, *C. burnetii* DNA was found in 6% (3 out of 50).In addition, a percentage of 13.33% (8 out of 60) of the examined tick pools were *C. burnettii* positive.

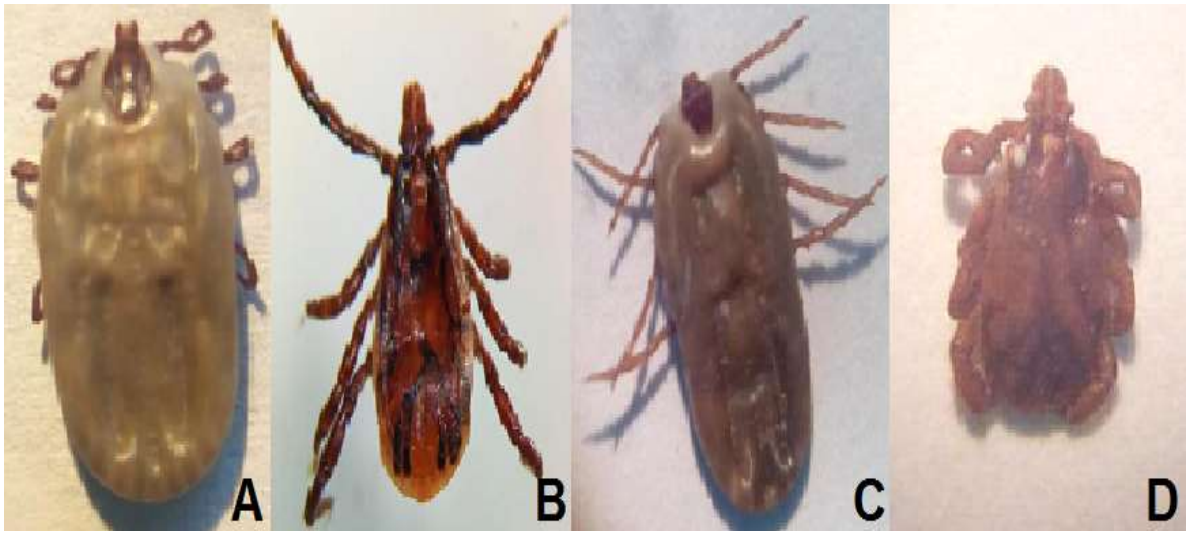

Figure 1: A representative picture of tick species (Phenotypic identification by stereomicroscope) showing A) female *R. sanguineus* B) male *R. sanguineus* C) female R. microplus and D) male R. microplus.

Table 4: Molecular detection of *C. burnetii* DNA in the examined sheep blood, human blood and tick pools samples by using PCR:

Species	Total no. tested	Positive PCR		Negative PCR	
		No.	%	No.	%
Sheep	30	4	13.33	26	86.67
Human	50	3	6	47	94
Tick pools	60	8	13.33	52	86.67

3.5. Demographic characteristics of the examined tick pools.

Studying species of examined tick pools in table (5) revealed that showing *Rhipicephalus (Boophilus) microplus* species (tick pools collected from cattle) were higher in *C. burnetii* prevalence rate 15% (6 out of 40) than *Rhipicephalus sanguineus* species (tick pools collected from dogs) 10% (2 out of 20). However, statistical analysis using chi-square revealed that the difference between the two groups was statistically non-significant.

Regarding sex of tick pools, the higher *C. burnetii* prevalence rates were among females of the examined tick pools 16% (8 out of 50) in comparison with a prevalence rate of 0% (0 out of 10) among males of the examined tick pools. Statistical analysis using chi-square revealed that the difference between males and females groups was statistically non-significant.

Concerning the prevalent climatic conditions during ticks sampling, the current study revealed that the examined tick pools showed a higher PCR positivity for *C. burnetii* 17.14% (6 out of 35) during warm climate versus the positivity rate of 8% (2 out of 25) during cold climate. However, Statistical analysis using chi-square revealed that the difference between the two groups was statistically non-significant.

Table 5: Demographic characteristics of the examined tick pools:

Demographic characteristics	Positive PCR		Negative PCR		Chi-square	P-value
	No.	%	No.	%		
Species: -Rhipicephalus sanguineus (No. of pools = 20)	2	10	18	90	0.288^{NS}	0.59
-Rhipicephalus (Boophilus) microplus (No. of pools= 40)	6	15	34	85		
Total pools (No. = 60)	8	13.33	52	86.67		
Sex: Males (No. of pools = 10)	0	0	10	100		
Females (No. of pools = 50)	8	16	42	84		
Total pools (No. = 60)	8	13.33	52	86.67	1.85^{NS}	0.174
Prevalent climatic condition during sampling: -Cold climate (No. of pools = 25)	2	8	23	92	1.06^{NS}	0.304
-Warm climate (No. of pools = 35)	6	17.14	29	82.86		
Total pools (No. = 60)	8	13.33	52	86.67		

^{NS} Non Significant

Figure 2: PCR amplification of (*com1*) gene of *C. burnetii* in sheep blood. Lane 1: DNA ladder, lanes 2, 3, 4 and 5: positive results, lanes 6, 7, 10, 11, 12, 13, 14, 15 and 16: negative results, lane 9: negative control and lane 8: positive control.

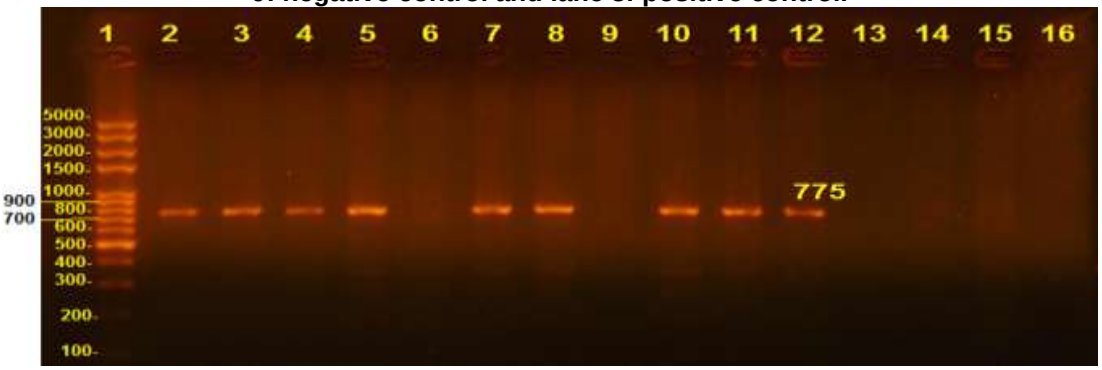

Figure 3: PCR amplification of (*com1*) gene of *C. burnetii* in examined tick pools. Lane 1: DNA ladder, lanes 2, 3, 4, 5, 7, 8, 10 and 11: positive results, lanes 6, 9, 14, 15 and 16: negative results, lane 13: negative control and lane 12: positive control.

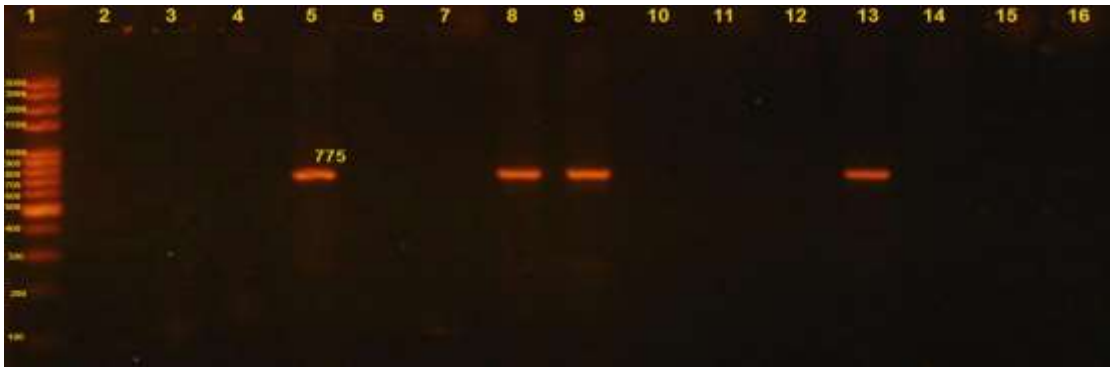

Figure 4: PCR amplification of (*com1*) gene of *C. burnetii* in human blood. Lane 1: DNA ladder, lanes 8, 9 and 13: positive results, lanes 2, 3, 4, 7, 10, 11, 12, 14, 15 and 16: negative results, lane 6: negative control and lane 5: positive control.

DISCUSSION

The obtained high seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies among the examined sheep was in agreement with Reinthaler et al. (1988) who reported a seroprevalence rate of 62.5% (20 out of 32) in Sudan and Dorko et al. (2010) who declared a seroprevalence rate of 58.43% (52 out of 89) in Slovakia. The obtained result still considered somewhat lower than the seroprevalence rate of 73.91% (34 out of 46) that was reported among sheep in Spain (Ruiz-Fons et al. 2010). On the contrary, lower seropositivity rates were detected among sheep in different Egyptian governorates as: 12.08% (11 out of 91) in Ismailia governorate (El-Mahallawy et al. 2012), 23% (23 out of 100) in Qaluobia governorate (Khalifa et al. 2016) and 25.69% (28 out of 109) in El Minya governorate (Abushahba et al. 2017). Furthermore, lower seropositivity rates were illustrated among sheep in other parts of the world as: 34% (214 out of 630) in Saudi Arabia (Jarelnabi et al. 2018), 12.35% (21 out of 170) in India (Gangoliya et al. 2019) and 28.48% (45 out of 158) in Ghana (Johnson et al. 2019).

The high seroprevalence of *C. burnetii* among sheep in the current study could be referred to the fact that the examined sheep were reared in flocks not in special farms so they were exposed to poor hygienic conditions involved a little attention was paid for tick eradication (high prevalence of ticks infestation). In addition, infected sheep could show no apparent symptoms but they could shed viable bacteria for long period of time in their milk, urine, feces and birth fluids especially at the lambing season with the bacterium *C. burnetii* has the ability to persist in the soil for a period exceeding 150 days. Grazing of sheep on the infected soil could be a way for transmitting infection through flocks. As well, sheep were noticed to be suffered from overcrowding that was found to be an essential factor in intensifying the infection through the flock.

Moreover, the obtained human seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies result was in a harmony with that of Dabaja et al. (2018) who reported a seroprevalence rate of 38.72% (163 out of 421) among Lebanese individuals. On the other hand, relatively higher seroprevalence rates were documented as: 74% (31 out of 42) in Behera governorate, Egypt (Samaha et al. 2012) and 89.47% (17 out of 19) in India (Dhaka et al. 2019). On the contrary in Egypt, lower seroprevalence rates were illustrated in different governorates as: 3.33% (1 out of 30) in North Sinai governorate (Mazyad and Hafez, 2007), 2% (2 out of 100) in Dakahlia governorate (Zaki and Goda, 2009), 23.33% (7 out of 30) in Qaluobia governorate (Khalifa et al. 2016) and 25.71% (9 out of 35) in El Minya governorate (Abushahba et al. 2017). Moreover, lower seroprevalence rates were shown in other parts of the world as: 2% (14 out of 747) in Nicaragua (Reller et al. 2016), 8.7% (32 out of 368) in Turkey (Cikman et al. 2017) and 24.38% (88 out of 367) in Iran (Mostafavi et al. 2019).

The relatively high result obtained in the current study suggested wide range of exposure to Q fever and this might be due to the agricultural nature of Menoufiya governorate that enables many individuals to have the behaviour of consumption of raw milk products (as: homemade butter and Kareish cheese) which can be sold immediately after milking by local milk sellers to the consumer without any heat treatment. Moreover, the relative high phase II IgG antibodies prevalence and lifelong persistence of

Q fever with the non-specific presentations of the disease among infected individuals within many geographical areas and also the lack of cheap, sensitive and specific laboratory diagnostic methods reflect the endemicity of the neglected Q fever in Menoufiya governorate (Oyston and Davies, 2011).

The obtained seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies in the tested sheep in relation to their sex was in agreement with Souza et al. (2018) who declared a higher seroprevalence of 6.67% (5 out of 75) among rams versus a seroprevalence of 1.52% (5 out of 330) among ewes in Brazil. On the other hand, El-Mahallawy et al. (2012) showed through their study in Ismailia governorate, Egypt that the seroprevalence of anti-*C. burnetii* phase II IgG antibodies was higher 14.29% (8 out of 56) among ewes than 8.57% (3 out of 35) among rams. In addition, Ezatkhah et al. (2015) reported a higher seroprevalence rate of 38.38% (38 out of 99) among ewes versus a seroprevalence rate of 17.86% (5 out of 28) among rams in Southeast Iran. The high rate of male seroprevalence in our work would be referred to the fact that any one of the examined flocks had a little number of males in relation to the number of females. All the examined males were old, old males have a greater opportunity to be exposed to *C. burnetii* than younger ones through more exposure to infection sources.

Regarding the sheep age, the obtained result was in agreement with Abushahba et al. (2017) who showed through their study in El Minya governorate, Egypt that sheep of the age group (> 2 years) had a higher seroprevalence of 26.44% (23 out of 87) than the seroprevalence rate of 22.73% (5 out of 22) among sheep of the age group (1-2 years) and Filioussis et al. (2017) who detected a higher seroprevalence rate of 19.51% (32 out of 164) among adult sheep (> 2 years) versus a seroprevalence rate of 11.11% (4 out of 36) among yearling sheep (< 2 years) in Greece. On the contrary, El-Mahallawy et al. (2012) illustrated the highest seroprevalence rate was 12.86% (9 out of 70) among sheep aging (1-2 years) followed by the seroprevalence rate of 11.11% (1 out of 9) among sheep aging (up to 1 year) and the least seroprevalence rate of 8.33% (1 out of 12) among sheep aging (> 2 years) in Ismailia governorate, Egypt. Furthermore, Edalati-Shokat et al. (2015) represented the age group (≤ 2 years) had a higher seroprevalence rate of 41.86% (18 out of 43) versus the age group (> 2 years) which had a seroprevalence rate of 23.57% (37 out of 157) in Iran. The wide variation among different age groups would be due to the the ability of *C. burntii* to be shed in various sources (as: milk, urine, feces and infected birth products), survive in the outdoor environment for long time periods and resist many physical and chemical stresses as elevated temperature and pressure, desiccation, osmotic shock and several chemical disinfectants. As a result, the more contact with the pathogen sources tends to be increased with sheep age.

Concerning the prevalent climatic conditions during sheep sampling, the obtained result was consistent with the findings demonstrated by Hellenbrand et al. (2001) who showed a higher seropositivity of *C. burnetii* IgG antibodies of 75.91% (167 out of 220) during warm climate versus a seropositivity of 24.09% (53 out of 220) during cold climate in Germany. On the other hand, Avbersek et al. (2019) presented that the prevalence of *C. burnetii* was higher in sheep manure 80% (24 out of 30) in cold climate versus a prevalence rate of 73.68% (14 out of 19) in warm climate in Italy because of the routine Q fever vaccination in the early of June.

Two reasons would become behind the increased seroprevalence of *C. burnetii* in warm climate versus in cold climate that was shown in the present study. Firstly, during warm climate (peak of activity in early and middle summer) occur abundance of activation for the parasitic stages of ticks and other arthropods (fleas and lice) that may share in transmitting *C. burnetii* between different sheep populations in Menoufiya governorate. Secondly, sheep are seasonal breeders (short day breeders i.e. in winter season) with the expected parturition time in end of spring-early summer and females shed huge amount of the bacterium *C. burnetii* around parturition through infected birth fluids that are rapidly desiccated to form infectious dust particles by dry hot climate and become a potential source of infection to other animals in the flock through aerosolization and may be transferred to sheep in other localities whereas increased rainfall in winter season could increase the soil humidity so, the amount of dust would become reduced decreasing the dispersion of bacteria through air.

The obtained seroprevalence of anti-*C. burnetii* antibodies in the examined patients in relation to their sex was consistent with Kilic et al. (2008) who reported a higher seroprevalence of anti-*C. burnetii* phase II IgG antibodies among males 92.35% (555 out of 601) versus a seroprevalence rate of 7.65% (46 out of 601)

among females in Turkey and Wardrop et al. (2016) showed a higher seroprevalence rate 3.37% (31 out of 919) among males in Kenya than 1.95% (21 out of 1078) among females. On the contrary, Samaha et al. (2012) found the seroprevalence was somewhat higher among females 70% (24 out of 34) than among males 69% (11 out of 16) among apparently healthy humans examined in Behera governorate, Egypt. Moreover, Abushahba et al. (2017) found a higher seroprevalence of phase II IgG antibodies 46.15% (6 out of 13) among females in comparison with a seroprevalence of 13.64% (3 out of 22) among males in El Minya governorate, Egypt. and Mostafavi et al. (2019) showed that the seroprevalence was higher among females 37.93% (33 out of 87) than among males 30.36% (85 out of 280). The slightly increased seroprevalence among men than among women would be explained by many facts. Firstly, there were more men performing livestock obstetrical work in farms and clinics than women. Secondly, there were more men involved in slaughter houses than women. Thirdly, the female sex hormone (17-beta-estradiol) showed a protective effect for women against *C. burnetii* (Chang et al., 2017).

Regarding the human age, the obtained result was supported by Kilic et al. (2008) who showed that the seroprevalence of anti-*C. burnetii* phase II IgG antibodies in Turkey was higher 39.16% (56 out of 143) among age group (40-61 years) than 30.13% (138 out of 458) among age group (18-39 years) and Abushahba et al., (2017) who presented a higher seroprevalence rate 31.25% (5 out of 16) among age group (40-63 years) versus 21.05% (4 out of 19) among age group (15-39 years) in El Minya governorate, Egypt. On the other hand, Samaha et al., (2012) declared a relatively high seroprevalence of phase II IgG antibodies 69% (9 out of 13) among the age group < 30 years versus a seroprevalence rate of 62% (13 out of 21) among age group (45- >60 years) in Behera governorate, Egypt. Furthermore, the highest seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies was 3.97% (34 out of 856) among age group (5-14 years) in comparison with a seroprevalence of 2.16% (8 out of 371) among age group (15-24 years) and the least seroprevalence of 1.22% (10 out of 822) among age group ≥ 25 years in Kenya (Wardrop et al. 2016).

The highest seroprevalence rate among age group (41-60 years) that was obtained in the current study could be explained by the fact that this age group was the most one with longer exposure time to sources of infection in slaughter houses and farms with higher contact with infected farm animals and soil during different rural activities followed by the age group of (61-80 years) where the aged un-employed individuals were located since they had changes in their inflammatory and immune systems that could produce reactivation of persistent Q fever inside their body cells (González-Quijada et al. 2015).

Regarding the prevalent climatic conditions during human sampling, the obtained result was consistent with Chen et al. (2008) who revealed that the incidence of acute Q fever, murine typhus and scrub typhus in Taiwan was higher 78.57% (22 out of 28) among patients in warm climate versus to the incidence of 21.43% (6 out of 28) among patients examined in cold climate and Lai et al. (2009) who showed that the incidence of Q fever in Taiwan was higher 77.94% (53 out of 68) during warm climate in comparison with the incidence of 22.06% (15 out of 68) that prevailed during cold climate. The increased infestation activity of ticks and other arthropods, increased field activities of humans and increased activity of animal reservoirs during warm seasons that was shown by the fact that sheep and goats (the main reservoirs of Q fever) are seasonal breeders where the infected pregnant females shed large numbers of the bacterium *C. burnetii* mainly around parturition time which become concentrated during late spring and early summer so, the risk of exposure of individuals to *C. burnetii* is higher during warm climate than during cold climate.

Concerning the immunological status of the examined patients, the obtained result was in a harmony with that of Boschini et al. (1999) who revealed through their study on risk factors of acquiring Q fever during two large outbreaks in Italy that the seroprevalence ratio for Q fever was higher 45.92% (107 out of 233) and 5.63% (17 out of 302) among HIV seropositive individuals in comparison with seroprevalence ratios of 25.14% (90 out of 358) and 4.72% (21 out of 445) among HIV seronegative individuals during 1987 and 1988, respectively. Furthermore, Lai et al. (2007) represented the Q fever seroprevalence ratio was higher 81.25% (13 out of 16) among individuals with both hepatitis B and hepatitis C viruses in Taiwan versus a seroprevalence ratio of 78.57% (33 out of 42) among individuals with neither of HBV nor HCV.

Current reports about HIV-associated Q fever pneumonia are somewhat limited and

underrepresented. However, HIV seropositive individuals were reported to acquire Q fever more frequently than the general healthy population according to the ability of *C. burnetii* to replicate in large numbers in the acidic phagolysosome of the immunosuppressed hosts (Madariaga et al. 2004). In addition, *C. burnetii* can produce hepatitis as it is or may share other causative agents leading to hepatitis. In general, replication of *C. burnetii* is shown to be elevated in patients with immunocompromised status (such as: HCV and HBV infections). However, the clinical manifestations of acute Q fever hepatitis in patients with and those without viral hepatitis are very similar (Melenotte et al., 2018).

By studying the effect of smoking as a risk factor for acquiring Q fever, the obtained result in the current study was consistent with the findings of Orr et al. (2006) who showed that the incidence of Q fever in Southwest England and Northern Ireland in the period from 2002 to 2004 was higher 29.41% (10 out of 34) among smokers than the incidence of 20.59% (7 out of 34) among humans who never smoked and Fenga et al., (2015) who illustrated through their study on Italian humans that the seroprevalence of phase II IgG antibodies against *C. burnetii* was higher 63.64% (28 out of 44) among smokers versus a seroprevalence rate of 62.5% (60 out of 96) among non-smokers. On the contrary, Karki et al., (2015) reported that the incidence of Q fever per 100,000 persons per year was 3.7% (27 out of 718,838) among humans who never tried smoking in comparison with an incidence rate of 3.4% (18 out of 529,243) among those who had the habit of smoking in Australia. Smoking causes respiratory distress since it causes adverse mechanical effects on the respiratory mucosa and it causes also adverse structural and functional effects on the individual immune response (immunomodulation) promoting the invasion of *C. burnetii* to respiratory macrophages (van der Hoek et al., 2012).

Furthermore, the obtained seroprevalence of anti-*C. burnetii* phase II IgG antibodies among patients with history of contact with water bodies was in agreement with the findings of Orr et al., (2006) who showed that the incidence of Q fever in Southwest England and Northern Ireland was higher 26.47% (9 out of 34) among humans in contact with water sources via swimming, water sport and other pictures of contact with rivers and lakes versus an incidence of 19.48% (15 out of 77) among humans with no contact with water sources. In addition, swimming in rivers in Nicaragua was illustrated as a risk factor for acquiring rickettsial diseases and Q fever since a higher percentage of humans with history of swimming in rivers 29.41% (10 out of 34) had antibodies against rickettsial diseases and Q fever versus a percentage of 11.06% (79 out of 714) of those with no rickettsial nor Q fever infections (Reller et al. 2016). Contact with water bodies was presented as a main risk factor for acquiring Q fever as a result of the fact that humans get rid of the waste water from animal farms, the infected animal placentas and other infected animal excrements by throwing them into stream water and rivers enabling the survival of biological *C. burnetii* in the free-living water amoebae for long time (Schets et al., 2013).

The role of doctors is no longer limited for waiting the disease occurrence and then giving medications (Singh, 2010). As a result, different apparent clinical and laboratory findings were noticed among the examined patients to help doctors for future suspecting, differential diagnosing or even excluding the presence of the disease among different patients.

Little reports are available worldwide about the clinical presentations and laboratory findings of acute Q fever. From these reports, Lai et al. (2009) was in agreement with the obtained findings of the current study since they noticed that the higher ratios of clinical signs were 98% (98 out of 100) for fever, 81% (81 out of 100) for headache and 35% (35 out of 100) for cough among patients with acute Q fever, murine typhus and scrub typhus diseases in Taiwan in comparison with ratios of 93.65% (118 out of 126) for fever, 69.05% (87 out of 126) for headache and 32.54% (41 out of 126) for cough among patients with other diseases. In addition, they showed a lower ratio of 16% (16 out of 100) for skin rash among patients with acute Q fever, murine typhus and scrub typhus diseases versus a ratio of 30.16% (38 out of 126) for skin rash among patients with other diseases. Moreover, they declared through their study in Taiwan that the ratios of laboratory findings such as thrombocytopenia and increased liver enzymes level were matched with the obtained findings of the current study when they showed relatively higher ratios: 69% (69 out of 100) of thrombocytopenia, 94.90% (93 out of 98) of increased ALT and 97% (97 out of 100) of increased AST among patients with acute Q fever, murine typhus and scrub typhus versus ratios of 57.94% (73 out of 126), 66.67% (80 out of 120) and 70.34% (83 out of 118), respectively among

patients with other diseases. On the contrary, Esmaeili et al., (2017) declared that the seroprevalence rate of anti-*C. burnetii* phase II IgG antibodies was higher 14.63% (12 out of 82), 25% (2 out of 8) and 17.91% (12 out of 67) among Q fever patients without cough, headache nor atypical pneumonia, respectively versus ratios of 11.76% (4 out of 34) for cough, 12.96% (14 out of 108) for headache and 8.16% (4 out of 49) for atypical pneumonia among Q fever Iranian patients.

Concerning the molecular detection of *C. burnetii* DNA in the examined sheep blood, human blood and tick pools samples by using PCR. From several researches available about *Coxiella* DNA in sheep blood, relatively higher rates were documented as: 69.33% (113 out of 163) in Canada (Hazlett et al., 2013), 36% (72 out of 200) in Turkey (Parin and Kaya, 2015) and 91.3% (21 out of 23) in Qaluobia governorate, Egypt (Khalifa et al. 2016). On the contrary, lower rates were reported about *Coxiella* DNA in sheep blood as: 4.38% (6 out of 137) in France (Cardinale et al. 2014) and 0% (0 out of 22) in Saudi Arabia (Mohammed et al. 2014).

In human individuals, the obtained PCR positivity was consistent with Melenotte et al., (2018) who showed a PCR positivity of 8.70% (142 out of 1,632) in France. Lower PCR positivity rates were declared in six African countries such as: 0.48% (6 out of 1,238) in Senegal, 0.37% (1 out of 268) in Algeria, 0% (0 out of 184) in Tunisia, 0% (0 out of 100) in Mali, 0% (0 out of 50) in Gabon, 0% (0 out of 48) in Morocco (Angelakis *et al.,* 2014). On the other hand, Zhang et al., (1998) presented a higher PCR positivity rate of 57.25% (146 out of 255) in Japan and Khalifa et al. (2016) reported a PCR positivity rate of 57.14% (4 out of 7) in Qaluobia governorate, Egypt. The available studies on *C. burnetii* DNA in sheep and humans are very low since most of the specific different antibodies against *C. burnetii* such as IgG-II, IgM-I and IgG-I antibodies appear with coinciding disappearance of circulating *C. burnetii* DNA showing the importance of serologic profiles for distinguishing the infection whereas PCR is time dependent and become non-valuable with time passes (Lucchese et al., 2015).

In ticks, the obtained PCR positivity was in agreement with the findings of Sulyok et al. (2014) who showed in their studies on ticks a prevalence rate of: 10.81% (32 out of 296) in Ethiopia. On the contrary, lower *C. burnetii* prevalence rates were illustrated as: 1.96% (20 out of 1,019) in Egypt (Loftis et al. 2006) and 5.78% (10 out of 173) in Malaysia (Nurkunasegran et al. 2017). On the other hand, relatively higher prevalence rates among ticks were documented as: 37.59% (50 out of 133) in Senegal (Mediannikov et al., 2010) and 89.09% (49 out of 55) in Malaysia (Khoo et al., 2016). In addition, Ghashghaei et al., (2017) reported a higher *C. burnetii* prevalence rate of 62.88% (83 out of 132) in Iran. In the enzootic cycle of *C. burnetii*, ticks are considered as important components. PCR was considered more sensitive and specific diagnostic tool for *C. burnetii* infection in ticks than serological techniques (Eldin et al., 2017). The relative low prevalence of *C. burnetii* among ticks in Menoufiya governorate could be referred to increased urbanization, application of highly improved hygienic measures in the animal husbandry through increasing the intensive treatment of infested animals with drugs as: (Ivermectin) in association with application of acaricidals as: (Deltamethrin 1%) to the surrounding environment at 14 days intervals for tick control under field conditions. As well, application of policy of destruction of stray dogs within whole the governorate.

The result of the present study concerning *R. microplus* was somewhat consistent with that of Mediannikov et al., (2010) who demonstrated a PCR positivity of 20% (1 out of 5) among pools of *R. microplus* in Senegal whereas Trinachartvanit et al., (2018) showed a *C. burnetii* prevalence rate of 0% (0 out of 14) among *R. microplus* species in Thailand. On the other side, the obtained result of *R. sanguineus* species (brown dog ticks) was in agreement with results of Bogunović et al. (2018) who illustrated a *C. burnetii* prevalence of 10.53% (24 out of 228) in Serbia and Khalili et al. (2018) who reported a *C. burnetii* prevalence of 12.5% (1 out of 8) in Iran. On the contrary, a high prevalence rate of *C. burnetii* was declared among brown dog ticks as: 59.09% (26 out of 44) in Malaysia (Watanabe et al., 2015). However, Fard and Khalili (2011) represented a lower prevalence of *C. burnetii* 2.86% (1 out of 35) among pools of brown dog ticks in Iran that still far away from the result presented by Oskam et al., (2017) who failed to found any *C. burnetti* among brown dog ticks examined in Australia.

The obtained non-significant association between prevalence of *C. burnetii* and the different examined tick species would be referred to the fact that the association between ticks and *C. burnetii* is universal across tick species and geographical boundaries. Ticks are natural *Coxiella* vectors that can transmit the infection to

different populations within different continents (Duron et al. ,2015).

Regarding sex of tick pools, the obtained result was consistent with Ghashghaei et al. (2017) who declared a higher *C. burnetii* prevalence rate 62.88% (83 out of 132) among female ticks in comparison with 50.4% (63 out of 125) among male ticks in Iran. On the other hand, Watanabe et al., (2015) showed the prevalence percentage of *C. burnetii* was the same among female ticks 80% (24 out of 30) and among males 80% (12 out of 15) in Malaysia. As well, Bogunović et al. (2018) noted that the male ticks had a higher prevalence rate of *C. burnetii* 22.37% (51 out of 228) versus a prevalence rate of 17.54% (40 out of 228) among female ticks in Serbia. The increased prevalence of *C. burnetii* among tick females than among tick males that was noticed in the current study could be referred to the fact that *C. burnetii* organisms has a clear tissue tropism to Malpighian tubules and ovaries of females and can also be maternally transmitted through tick generations. As well, female ticks can start feeding early on different animals even in the absence of male ticks (Watanabe et al. 2015).

Concerning the prevalent climatic conditions during ticks sampling, the obtained result was consistent with that of Fard et al., (2016) who represented through their studied on ticks in Iran that the majority of *C. burnetii* infested ticks 79.67% (239 out of 300) was collected in warm climate (from late March to early September) in comparison with 30.22% (68 out of 225) among ticks collected in cold climate (from late September to early March) and Ghashghaei et al., (2017) who detected also that the cattle ticks which collected in warm climate in Iran had a higher prevalence of *C. burnetii* 80.95% (119 out of 147) than the prevalence rate of 24.55% (27 out of 110) among ticks collected in cold climate. The higher prevalence of *C. burnetii* among ticks collected in warm climate than among those collected in cold climate could be referred to the fact that the life cycle of ticks involves stage of larvae that are naturally predominate from late summer to early winter (peack of activity in autumn) followed by stage of nymphs that are naturally predominate from early winter to early spring (peack of activity in middle Winter) and finally the adult stage (that were chosen in the current study) that predominates during spring and summer (peak of activity in early and middle summer) (Seo et al., 2016) so, warm climate provides a suitable condition for growth of adult ticks since increasing temperature and humidity stimulate the activity and prevalence of suckling adults.

CONCLUSION

We can conclude that Q fever is a potential zoonosis in Menoufiya governorate, Egypt and the risk of the disease transmission to humans couldn't be neglected. Prevention of Q fever in humans mainly depends on the prevention of the disease in animal populations and tick control. In addition, health education of the public about the danger of Q fever, its mode of spread, risk factors of acquiring it and methods of control are critical steps to eliminate the infection in the governorate.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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All procedures performed in this study including collection of animal, human and even arthropod (ticks) samples were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the blood samples, with the agreement that any identifying details of the individuals should not be published.

AUTHOR CONTRIBUTIONS

All listed authors have made substantial contributions to the research design, the acquisition, analysis, or interpretation of data; and to drafting the manuscript or revising it critically; and that all authors have approved the submitted version.

REFERENCES

Abdelhadi F, Abdelhadi S, Niar A, Benallou B, Meliani S, Smail N, Mahmoud D, 2015. Abortions in cattle on the level of Tiaret Area (Algeria). Global Veterinaria 14: 638-645.

Abushahba MF, Abdelbaset AE, Rawy MS, Ahmed SO, 2017. Cross-sectional study for determining the prevalence of Q fever in small ruminants and humans at El Minya Governorate, Egypt. BMC research notes 10(1): 538.

Angelakis E, Mediannikov O, Socolovschi C, Mouffok N, Bassene H, Tall A, Niangalye H, Doumboe O, Znazenf A, Sarihg M, Sokhnaa C, Raoult D, 2014. *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa. International Journal of Infectious Diseases 28: 107-110.

Avbersek J, Pate M, Skibin A, Ocepek M, Krt, B, 2019. Management of a *Coxiella burnetii*-infected sheep flock after an outbreak of Q fever in humans. Turkish Journal of Veterinary and Animal Sciences 43(2): 264-270.

Bogunović D, Stević N, Sidi-Boumedine K, Mišić D, Tomanović S, Kulišić Z, Magaš V, Radojičić S, 2018. Molecular evidence of Q fever agent *Coxiella burnetii* in Ixodid ticks collected from stray dogs in Belgrade (Serbia). Acta veterinaria 68(3): 257-268.

Boschini A, Di Perri G, Legnani D, Fabbri P, Ballarini P, Zucconi R, Boros S, Rezza G, 1999. Consecutive epidemics of Q fever in a residential facility for drug abusers: impact on persons with human immunodeficiency virus infection. Clinical infectious diseases 28(4): 866-872.

Botros B, Soliman A, Salib A, Olson J, Hibbs R, Williams J, Darwish M, El Tigani A, Watts DM, 1995. *Coxiella burnetii* antibody prevalences among human populations in north-east Africa determined by enzyme immunoassay. The Journal of tropical medicine and hygiene 98(3): 173-178.

Cardinale E, Esnault O, Beral M, Naze F, Michault A, 2014. Emergence of *Coxiella burnetii* in ruminants on Reunion Island? Prevalence and risk factors. PLoS neglected tropical diseases 8(8): e3055.

Chang K, Lee NY, Ko WC, Tsai JJ, Lin WR, Chen TC, Lu PL, Chen YH, 2017. Identification of factors for physicians to facilitate early differential diagnosis of scrub typhus, murine typhus, and Q fever from dengue fever in Taiwan. Journal of Microbiology, Immunology and Infection 50(1): 104-111.

Chen NY, Huang PY, Leu HS, Chiang PC, Huang CT, 2008. Clinical prediction of endemic rickettsioses in northern Taiwan—relevance of peripheral blood atypical lymphocytes. J Microbiol Immunol Infect 41(5): 362-368.

Cikman A, Aydin M, Gulhan B, Karakecili F, Ozcicek A, Kesik OA, Parlak M5, Ozcelik F, Gültepe B, 2017. The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical features. Journal of vector borne diseases 54(2): 157-163.

CDC, 2002. Q fever--California, Georgia, Pennsylvania, and Tennessee, 2000-2001. *MMWR.* Morbidity and mortality weekly report 51(41): 924.

Dabaja MF, Greco G, Villari S, Bayan A, Vesco G, Gargano V, Arnone M, Hneino M, Lelli R, Ezzedine M, Berry A, Mortada H, Tempesta M, Mortada M, 2018. The first serological study of Q fever in humans in Lebanon. Vector-Borne and Zoonotic Diseases 18(3): 138-143.

Dhaka P, Malik SS, Yadav JP, Kumar M, Baranwal A, Barbuddhe SB, Rawool DB, 2019. Seroprevalence and molecular detection of coxiellosis among cattle and their human contacts in an organized dairy farm. Journal of infection and public health 12(2): 190-194.

Dorko E, Pilipcinec E, Rimárová K, Kostovcikova J, 2010. Serological study of Q fever in sheep in the territory of Eastern Slovakia. Annals of Agricultural and Environmental Medicine 17(2): 323-325.

Duron O, Noël V, Mccoy KD, Bonazzi M, Sidi-Boumedine K, Morel O, Vavre F, Zenner L, Jourdain E, Durand P, Arnathau C, Renaud F, Trape JF, Biguezoton AS, Cremaschi J, Dietrich M, Léger E, Appelgren A, Dupraz M, Gómez-Díaz E, Diatta G, Dayo GK, Adakal H, Zoungrana S, Vial L, Chevillon C, 2015. The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. PLoS pathogens 11(5): e1004892.

Edalati-Shokat H, Abbasi-Doulatshahi E, Hajian-Bidar H, Gharekhani J, Rezaei A, 2015. Q fever in domestic ruminants: A Seroepidemiological survey in Hamedan, Iran. Int J Curr Microbiol App Sci 4: 589-596.

Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, Raoult D, 2017. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clinical microbiology reviews 30(1): 115-190.

El-Mahallawy HS, Abou-Eisha A, Fadel H, 2012.

Coxiella burnetii infections among small ruminants in Ismailia Governorate. SCVMJ 17: 39-50.

El-Mahallawy HS, Kelly P, Zhang J, Yang Y, Wei L, Tian L, Fan W, Zhang Z, Wang C, 2016. Serological and molecular evidence of *Coxiella burnetii* in samples from humans and animals in China. Annals of Agricultural and Environmental Medicine 23(1):87-91

Esmaeili S, Golzar F, Ayubi E, Naghili B, Mostafavi E, 2017. Acute Q fever in febrile patients in northwestern of Iran. PLoS neglected tropical diseases 11(4): e0005535.

Ezatkhah M, Alimolaei M, Khalili M, Sharifi H, 2015. Seroepidemiological study of Q fever in small ruminants from Southeast Iran. Journal of infection and public health 8(2): 170-176.

Fard SN, Ghashghaei OO, Khalili M, Sharifi H, 2016. Tick diversity and detection of *Coxiella burnetii* in tick of small ruminants using nested Trans PCR in southeast Iran. Tropical Biomedicine 33(3): 506-511.

Fard SN, Khalili M, 2011. PCR-detection of *Coxiella burnetii* in ticks collected from sheep and goats in southeast Iran. Iranian journal of arthropod-borne diseases 5(1): 1-6.

Fenga C, Gangemi S, De Luca A, Calimeri S, Giudice DL, Pugliese M, Licitra F, Alibrandi A, Costa C, 2015. Seroprevalence and occupational risk survey for *Coxiella burnetii* among exposed workers in Sicily, Southern Italy. International journal of occupational medicine and environmental health 28(5): 901-907.

Filioussis G, Theodoridis A, Papadopoulos D, Gelasakis AI, Vouraki S, Bramis G, Arsenos G, 2017. Serological prevalence of *Coxiella burnetii* in dairy goats and ewes diagnosed with adverse pregnancy outcomes in Greece. Ann Agric Environ Med 24(4): 702-705.

Gangoliya SR, Kumar S, Alam SI, Sharma HK, Singh M, Kotwal SK, Berri M, Kamboj DV, 2019. First molecular and serological evidence of Coxiella burnetti infection among sheep and goats of Jammu province of India. Microbial pathogenesis 130: 100-103.

Ghashghaei O, Fard SR, Khalili M, Sharifi H, 2017. A survey of ixodid ticks feeding on cattle and molecular detection of *Coxiella burnetii* from ticks in Southeast Iran. Turkish Journal of Veterinary and Animal Sciences 41(1): 46-50.

González-Quijada S, Salazar-Thieroldt E, Mora-Simón MJ, 2015. Persistent Q fever and ischaemic stroke in elderly patients. Clinical Microbiology and Infection 21(4): 362-367.

Hazlett MJ, McDowall R, DeLay J, Stalker M, McEwen B, van Dreumel T, Spinato M, Binnington B, Slavic D, Carman S, Cai HY, 2013. A prospective study of sheep and goat abortion using real-time polymerase chain reaction and cut point estimation shows *Coxiella burnetii* and *Chlamydophila abortus* infection concurrently with other major pathogens. Journal of veterinary diagnostic investigation 25(3): 359-368.

Hellenbrand W, Breuer T, Petersen L, 2001. Changing epidemiology of Q fever in Germany, 1947-1999. Emerging infectious diseases 7(5): 789.

Jarelnabi AA, Alshaikh MA, Bakhiet AO, Omer SA, Aljumaah RS, Harkiss GD, Mohammed OB, Hussein MF, 2018. Seroprevalence of Q fever in farm animals in Saudi Arabia. Biomed Res 29: 895-900.

Johnson SA, Kaneene JB, Asare-Dompreh K, Tasiame W, Mensah IG, Afakye K, Simpson SV, Addo K, 2019. Seroprevalence of Q fever in cattle, sheep and goats in the Volta region of Ghana. Veterinary medicine and science 5(3):402-411.

Karki S, Gidding HF, Newall AT, McIntyre PB, Liu BC, 2015. Risk factors and burden of acute Q fever in older adults in New South Wales: a prospective cohort study. *Medical* Journal of Australia 203(11): 438-438.

Khalifa NO, Elhofy FI, Fahmy H, Sobhy MM, Agag M, 2016. Seropervelance and molecular detection of *Coxiella burnetii* infection in sheep, goats and human in Egypt. ISOI J Microbiol Biotechnol Food Sci 2: 1-7.

Khalili M, Rezaei M, Akhtardanesh B, Abiri Z, Shahheidaripour S, 2018. Detection of *Coxiella burnetii* (Gammaproteobacteria: *Coxiellaceae*) in ticks collected from infested dogs in Kerman, Southeast of Iran. Persian Journal of Acarology 7(1) 93-100.

Khoo JJ, Lim FS, Chen F, Phoon WH, Khor CS, Pike BL, Chang LY, AbuBakar S, 2016. *Coxiella* detection in ticks from wildlife and livestock in Malaysia. Vector-Borne and Zoonotic Diseases 16(12): 744-751.

Kilic S, Yilmaz GR, Komiya T, Kurtoglu Y, Karakoc EA, 2008. Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolia, Turkey. New Microbiol 31(4): 527-534.

Koulla-Shiro S, Kuaban C, Bélec L, 1997. Microbial etiology of acute community-

acquired pneumonia in adult hospitalized patients in Yaounde-Cameroon. Clinical Microbiology and Infection 3(2): 180-186.

Lai CH, Chin C, Chung HC, Huang CK, Chen WF, Yang YT, Chen W, Lin HH, 2007. Acute Q fever hepatitis in patients with and without underlying hepatitis B or C virus infection. Clinical infectious diseases 45(5): e52-e59.

Lai CH, Huang CK, Chen YH, Chang LL, Weng HC, Lin JN, Chung HC, Liang SH, Lin HH, 2009. Epidemiology of acute Q fever, scrub typhus, and murine typhus, and identification of their clinical characteristics compared to patients with acute febrile illness in southern Taiwan. Journal of the Formosan Medical Association 108(5): 367-376.

Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy IM, Moriarity JR, Dasch GA, 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. Experimental & applied acarology 40(1): 67.

Lucchese L, Capello K, Barberio A, Zuliani F, Stegeman A, Ceglie L, Guerrini E, Marangon S, Natale A, 2015. IFAT and ELISA phase I/phase II as tools for the identification of Q fever chronic milk shedders in cattle. Veterinary microbiology 179(1-2): 102-108.

Madariaga MG, Pulvirenti J, Sekosan M, Paddock CD, Zaki SR, 2004. Q fever endocarditis in HIV-infected patient. Emerging infectious diseases 10(3): 501.

Mazyad S, Hafez AO, 2007. Q fever (*Coxiella burnetii*) among man and farm animals in North Sinai, Egypt. Journal of the Egyptian Society of Parasitology 37(1): 135-142.

Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez JF, Sokhna C, Trape J, Raoult D, 2010. *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS neglected tropical diseases 4(4): e654.

Melenotte C, Protopopescu C, Million M, Edouard S, Carrieri MP, Eldin C, Angelakis E, Djossou F, Bardin N, Fournier PE, Mège JL, Raoult D, 2018. Clinical features and complications of *Coxiella burnetii* infections from the French National Reference Center for Q fever. JAMA network open 1(4): e181580.

Minnick MF, Raghavan R, 2011. Genetics of *Coxiella burnetii*: on the path of specialization. Future microbiology 6(11): 1297-1314.

Mohammed OB, Jarelnabi AA, Aljumaah RS, Alshaikh MA, Bakhiet AO, Omer SA, Alagaili AN, Hussein MF, 2014. *Coxiella burnetii*, the causative agent of Q fever in Saudi Arabia: molecular detection from camel and other domestic livestock. Asian Pacific Journal of Tropical Medicine 7(9): 715-719.

Mostafavi E, Molaeipoor L, Esmaeili S, Ghasemi A, Kamalizad M, Behzadi MY, Naserifar R, Rohani M, Shahraki AH, 2019. Seroprevalence of Q fever among high-risk occupations in the Ilam province, the west of Iran. PLoS one 14(2): e0211781.

Noh Y, Lee YS, Kim HC, Chong ST, Klein TA, Jiang J, Richards AL, Lee HK, Kim SY, 2017. Molecular detection of Rickettsia species in ticks collected from the southwestern provinces of the Republic of Korea. Parasites & vectors 10(1): 20.

Nurkunasegran M, Kho K, Koh F, Tan P, Nizam Q, Ong B, Panchadcharam C, Mat Amin M, Abdul Majid N, Ramli R, Tay ST, 2017. Molecular Detection of *Coxiella burnetii* from Farm Animals and Ticks in Malaysia. Tropical Biomedicine 34(3): 675-680.

Orr H, Christensen H, Smyth B, Dance D, Carrington D, Paul I, Stuart J, 2006. Case-control study for risk factors for Q Fever in southwest England and Northern Ireland. Euro surveillance: bulletin européen sur les maladies transmissibles= European communicable disease bulletin 11(10): 13-14.

Oskam CL, Gofton AW, Greay TL, Yang R, Doggett S, Ryan UM, Irwin PJ, 2017. Molecular investigation into the presence of a *Coxiella* sp. in *Rhipicephalus sanguineus* ticks in Australia. Veterinary microbiology 201: 141-145.

Oyston P, Davies C, 2011. Q fever: the neglected biothreat agent. Journal of medical microbiology 60(1): 9-21.

Parin U, Kaya O, 2015. Detection of *Coxiella burnetii* prevalence in bovine, ovine and caprine herds. Ankara Universitesi Veteriner Fakultesi Dergisi 62(3): 177-181.

Rahaman MR, Milazzo A, Marshall H, Bi P, 2019. Is a one health approach utilized for Q fever control? A comprehensive literature review. International journal of environmental research and public health 16(5): 730.

Reinthaler F, Mascher F, Sixl W, Arbesser C, 1988. Incidence of Q fever among cattle, sheep and goats in the Upper Nile province in southern Sudan. Veterinary Record 122(6): 137.

Reller ME, Chikeka I, Miles JJ, Dumler JS, Woods CW, Mayorga O, Matute AJ, 2016. First identification and description of rickettsioses

and Q fever as causes of acute febrile illness in Nicaragua. PLoS neglected tropical diseases 10(12): e0005185.

Roest H, Tilburg J, Van der Hoek W, Vellema P, Van Zijderveld F, Klaassen C, Raoult D, 2011. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiology & Infection 139(1): 1-12.

Ruiz-Fons F, Astobiza I, Barandika JF, Hurtado A, Atxaerandio R, Juste RA, García-Pérez AL, 2010. Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. BMC veterinary research 6(1): 3.

Samaha H, Haggag Y, Nossair M, Samar A, 2012. Serological detection of IgG against *Coxiella burnetii* phase II in Behera Province Western Egypt. Alexandria Journal of Veterinary Sciences 37(1): 33-40.

Schets F, De Heer L, de Roda Husman A, 2013. *Coxiella burnetii* in sewage water at sewage water treatment plants in a Q fever epidemic area. International journal of hygiene and environmental health 216(6): 698-702.

Seo MG, Lee SH, Ouh IO, Lee GH, Goo YK, Kim S, Kwon OD, Kwak D, 2016. Molecular detection and genotyping of *Coxiella*-like endosymbionts in ticks that infest horses in South Korea. PLoS one 11(10): e0165784.

Sidi-Boumedine K, Rousset E, Henning K, Ziller M, Niemczuck K, Roest H, Thiéry R, 2010. Development of harmonised schemes for the monitoring and reporting of Q-fever in animals in the European Union. EFSA Supporting Publications 7(5): 48E.

Singh A, 2010. Modern medicine: Towards prevention, cure, well-being and longevity. Revista Latinoamericana de Psicopatologia Fundamental 13(2): 265-282.

Souza EA, Castro EM, Oliveira GM, Azevedo SS, Peixoto RM, Labruna MB, Horta MC, 2018. Serological diagnosis and risk factors for *Coxiella burnetii* in goats and sheep in a semi-arid region of Northeastern Brazil. Revista Brasileira de Parasitologia Veterinária 27(4): 514-520.

Sulyok KM, Hornok S, Abichu G, Erdélyi K, Gyuranecz M, 2014. Identification of novel *Coxiella burnetii* genotypes from Ethiopian ticks. PLoS one 9(11): e113213.

Trinachartvanit W, Maneewong S, Kaenkan W, Usananan P, Baimai V, Ahantarig A, 2018. *Coxiella*-like bacteria in fowl ticks from Thailand. Parasites & vectors 11(1): 670.

van der Hoek W, Morroy G, Renders NH, Wever PC, Hermans MH, Leenders AC, Schneeberger PM, 2012. Epidemic Q fever in humans in the Netherlands. Adv Exp Med Biol 984: 329-64.

Walker, A. R. (2003): Ticks of domestic animals in Africa: a guide to identification of species. (pp 3-210). Edinburgh. Bioscience Reports.

Wardrop NA, Thomas LF, Cook EA, de Glanville WA, Atkinson PM, Wamae CN, Fèvre EM, 2016. The sero-epidemiology of *Coxiella burnetii* in humans and cattle, western Kenya: Evidence from a cross-sectional study. PLoS neglected tropical diseases 10(10): e0005032.

Watanabe M, Nakao R, Amin-Babjee S, Maizatul A, Youn J, Qiu Y, Sugimoto C, Watanabe M, 2015. Molecular screening for Rickettsia, *Anaplasmataceae* and *Coxiella burnetii* in *Rhipicephalus sanguineus* ticks from Malaysia. Trop Biomed 32: 390-398.

Zaki ME, Goda T, 2009. Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study. The Journal of Infection in Developing Countries 3(3): 199-205.

Zhang G, Nguyen SV, To H, Ogawa M, Hotta A, Yamaguchi T, Kim HJ, Fukushi H, Hirai K, 1998. Clinical evaluation of a new PCR assay for detection of *Coxiella burnetii* in human serum samples. Journal of clinical microbiology 36(1): 77-80.