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SEROPREVALENCE OF *Coxiella Burnetii* AMONG MEAT HANDLERS IN DUHOK CITY, IRAQ.

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ABSTRACT

Background: Q fever is a zoonotic disease caused by *Coxiella burnetii*, most commonly infect people who work in close contact with animals and their carcasses like butchers and abattoir workers.

Objective: This study was carried out for the first time in the area to show the prevalence of Q fever among butchers and abattoir workers in Duhok city, as one of the main risk groups.

Methods: A total of 86 serum samples were collected from butchers (54) and abattoir workers (32) working in Duhok city from June to August, 2018. ELISA kit (Vircell SL, Spain) was used to detect IgG and IgM antibodies against *C. burnetii* phase II antigen.

Results: Out of 86 serum samples with mean age 31.8 ±11.8, 23 (26 %) were positive for IgG in which 12 (22.2%) were butchers and 11 (34.4%) were abattoir workers, while 5 (5.8%) were positive for IgM and only recorded among butchers. The percentages of IgM positive cases were decreased with increasing of age in opposite of IgG cases in which the highest percentage of IgG was 30.2% in age group 16-30 years while the lowest percentage was 16.7% in age group more than 45 years. The same picture was found with years of occupation in which the highest percentage of IgG was 31.8% in those worked from 0.5 to 10 years, while the lowest percentage was 11.8% in those who have more than 20 years of working.

Conclusion: Based on the results of this study, it can be concluded that *C. burnetii* infection is widespread among butchers and abattoir workers.

KEYWORDS: Coxiella burnetii, seroprevalence, abattoir workers, butchers.

INTRODUCTION

Q fever was named by Derrick in 1935 after an outbreak of febrile illness among abattoir workers in Brisbane, Queensland, Australia. The letter Q refers to query because of the unknown cause of the infection (Derrick, 1937). In 1937, Burnet and Freeman isolated a fastidious, Gram negative, rickettsia like, obligate intracellular bacterium from guinea pigs that had been injected with blood and urine from Derrick's patients and named it *Rickettsia burnetii* (Maurin and Raoult, 1999). At the same time, Herald Cox and Gordon Davis isolated an agent responsible for human infections from ticks in Rocky Mountain laboratory in Hamilton, Montana, USA (Davis and Cox, 1938). They named it the nine-mile fever agent and later found that this new agent is immunologically similar to Q fever (Burnet, 1967). Due to its specific cultural and biochemical characteristics, Philip reclassified *R. burnetii* in a genus that he named Coxiella, after Herald R. Cox, the scientist that was first able to isolate it in America. Hence, the causative agent for Q fever got its final name, *C. burnetii*. (Angelakis and Raoult, 2010; Hopper *et al*., 2016)

Epidemiological studies point out that Q fever should be considered a public health problem in France, Italy, Germany, Canada, the United Kingdom, Spain, and other nations including Iraq where Q fever is prevalent but poorly diagnosed. (Maurin and Raoult, 1999). Q fever might become latent in women and re-emerge during pregnancy causing spontaneous abortion.

Acute Q fever progresses to chronic Q fever in a small proportion of infected patients (0.2-1.4%). Chronic Q fever is more severe and complications last for many years. Chronic Q fever is namely represented as endocarditis or other cardiovascular infections which is lethal if neglected. (lockheart, 2010)

The inhalation of polluted aerosols with *C. burnetii* is the main mode of transmission to human beings. Direct contact with infected reservoirs, or indirectly through contact with their urine, feces and birth products is another method, also, cases of infection through ingestion of contaminated milk products, blood transfusion and sexual intercourse have been reported. (Cikman *et al*., 2017)

Since inhalation is considered the main mode of transmission, outbreaks of Q fever have generally been associated with slaughterhouses, farmhouses, and foundations concerned with sheep research programs. Large outbreaks have been linked to wind dispersion from locations where infected animals were diagnosed, (Hadush *et al*., 2016). The ID_{50} is one via inhalation, meaning, inhalation of one bacteria causes infection in 50% of people. This extremely low infectious dose (only 1-10), making it one of the most infectious organisms identified (Tigrett & Beneson, 1961).

Q fever is problematic to be clinically or radiologically diagnosed (Berktaş *et al*., 2011). A dependable diagnostic method of this infection is the isolation of this pathogen, namely, in cell tissue culture or embryonated chicken eggs, conversely, it is time consuming and hazardous as the bacteria is highly infectious and airbourne, requiring biosafety level 3. Therefore, PCR or serological diagnosis is preferred. (Vaidya *et al*., 2008). The most commonly used serological test is ELISA technique which detect IgG and IgM antibodies against phase I and II of *Coxiella burnetii*. Serologically the antiphase II antibodies are detected first in acute infection even though this phase is a virulent. High levels of anti-phase I antibodies usually suggest a chronic Q fever infection. Whereas specific anti-phase II antibodies are associated with acute Q fever infections. This is due to the phase II antigens being more immunogenic than the phase I surface components (Williams *et al*. 1984; Dupius *et al*., 1985). Phase I antibody may be found after initial infection but rarely as much as phase II (Dupius *et al*., 1985).

Iraq is one of the countries of middle east which are considered endemic for Q fever disease according to many cases reported among US soldiers who served in Iraq. (Bamberg *et al*., 2007; Banazis *et al*., 2009; Beare *et al*., 2006)

Data concerning prevalence of this zoonotic disease are scarce or absent in Iraq especially in Kurdistan region, north part of Iraq and it is even a neglected disease and out of the differential diagnosis of physicians, therefore this study is aimed to shed the light on the prevalence of Q fever among meat handlers in the city of Duhok as one of the important risk groups for contracting this infection from direct contact with animal and animal products.

MATERIALS AND METHODS

Sample collection

This study was focused on butchers and abattoir workers who working in Duhok city as an important risk group for contracting Q fever and clinically asymptomatic. Consent was obtained from all contributors and a special questionnaire was designed with specific questions to be answered by the participants. A total of 86 blood samples were collected from butchers and abattoir workers. The samples were collected from June to August 2018. From each participant, 5 ml of blood was collected aseptically into sterile test tubes and transferred to the laboratory of College of Health sciences, University of Duhok. Serum samples were separated from clotted blood by centrifugation and kept in freezer (-20 ℃) for ELISA technique.

Ethical approval

The study was approved by the ethics committee of Duhok Directorate General Health No.26062018-5.

Serological test

ELISA kit (Vircell SL, Spain) was used to detect IgG and IgM antibodies against *C. burnetii* phase II antigen. The test was carried out according to the leaflet instructions supplied with the kit by the manufacture. The mean OD for cut-off serum and antibody index (AI) was calculated using an ELISA reader (BioTek Instruments, Inc., Winooski, VT, USA) at 450/620 nm wavelengths. Antibody Index (AI) was calculated according to the following formulas:

AI= (Sample OD/ Cut-off serum mean OD X 10. Samples with indexes < 9.0 were considered as negative, samples with indexes 9.0-11.0 were equivocal and retested for confirmation. Samples

with indexes > 11.0 were considered positive for IgG and IgM antibodies against *C. burnetii*.

Statistical analysis

Statistical analysis was applied using SPSS version 24, origin USA, IBM 2017. The differences by age groups, occupation, years of occupation, work region, clinical signs and symptoms to Q fever infection were analyzed using Fisher's exact test which is a better fit test due to the low number of samples. The significant level was considered to be $p< 0.05$.

RESULTS

A total of 86 serum samples were collected in which 54 (62.8%) were butchers and 32 (37.2 %) were slaughter house workers. Their ages ranged from 16 to 75 years old with mean age: 31.8 ±11.8, in which 50% were in age group 16-30, 36% in age group 31-45 and 14% in age group 45 years and above. The years of working in this job were varied from 5 months to 40 years. Concerning clinical signs and symptoms, 11 (12.8%) of the participants complained from fever, chest infection symptoms, chills and/or sweats, while 13 (15.1%) of them suffered from respiratory distress, cough and breathlessness and 2 (2.3%) complained from cardiovascular problems as shown in table 1. All participants enrolled in the study were male. No significant association was found between studied risk characteristics and IgM positive (5 out 86 samples) based on the Fisher's exact test although the percentages of positivity were increased with increasing of age, in which the highest percentage of positive IgM (16.7%) was found in age group more than 45 years and the lowest percentage was 2.3% in age group 16-30 years. The same results were found with years of occupation with positive IgM in which the highest percentage was 11.8% in butchers working more than 20 years compared to 2.3% in those with less than 10 years of working as shown in table 2.

Out of 86 serum samples, 23 (26 %) were positive for IgG in which 12 (22.2%) were butchers and 11 (34.4%) were abattoir workers. No significant correlation was found between the studied risk factors and IgG positive cases, although the percentages of IgG cases were more among abattoir workers than butchers. The percentages of IgG positive cases were decreased with increasing of age in opposite of IgM cases, in which the highest percentage of IgG was 30.2% in age group 16-30 years while the lowest percentage was 16.7% in age group more than 45 years. The same picture was found with years of occupation in which the highest percentage of IgG was 31.8% in those worked from 0.5 to 10 years, while the lowest percentage was 11.8% in those who have more than 20 years of working as shown in table 3.

Table (1): Distribution of characteristics of all men in the study sample (n = 86)

Characteristic/manifestation		No.	%
Age (years)	16 - 30	43	50.0
	31 - 45	31	36.0
	> 45	12	14.0
Occupation	Butcher	54	62.8
	Abattoir worker	32	37.2
Years in occupation	0.5 - 10	45	52.3
	11 - 20	25	29.1
	> 20	16	18.6
Work region	Duhok	53	61.6
	Khanke slaughter h.	32	37.2
	Shingar and Duhok	1	1.2
Fever, chills and/or sweats		11	12.8
Respiratory distress, cough, breathlessness		13	15.1
Cardiovascular problems		2	2.3
Malaise		Nil	
Endocrine pathology		Nil	

Autoimmune disease		Nil	
Other manifestations	Impaired immunity	1	1.2
	Allergy	1	1.2
	Congenital malform.	1	1.2

Table (2): Comparison of men with positive and negative *C. burnetii* specific IgM

Characteristic/manifestation		IgM				*P*-value*
		Positive		Negative		
		No.	%	No.	%	
Age (years)	16 – 30	1	2.3	42	97.7	0.131
	31 – 45	2	6.5	29	93.5	
	> 45	2	16.7	10	83.3	
Occupation	Butcher	5	9.3	49	90.7	0.152
	Abattoir worker	0	0	32	100.0	
Years in occupation	0.5 – 10	1	2.2	44	97.8	0.156
	11 – 20	2	8.0	23	92.0	
	> 20	2	12.5	14	87.5	
Work region	Duhok	5	9.4	48	90.6	0.201
	Khanke slaughter h.	0	0	32	100.0	
	Shingar and Duhok	0	0	1	100.0	
Fever, chills and/or sweats	Yes	1	9.1	10	90.9	0.504
	None	4	5.3	71	94.7	
Respiratory distress, cough, breathlessness	Yes	1	7.7	12	92.3	0.569
	None	4	5.5	69	94.5	
Cardiovascular problems	Yes	0	0	2	100.0	1.000
	None	5	6.0	79	94.0	
Other manifestations	Impaired immunity	0	0	1	100.0	1.000
	Allergy	0	0	1	100.0	
	Congenital malform.	0	0	1	100.0	
	None	5	6.0	78	94.0	
Total		5	5.8	81	94.2	

* Based on Fisher's exact test.

Table (3): Comparison of men with positive and negative *C.burnetii* specific IgG

Characteristic/manifestation		IgG				*P*-value*
		Positive		Negative		
		No.	%	No.	%	
Age (years)	16 - 30	13	30.2	30	69.8	0.672
	31 - 45	8	25.8	23	74.2	
	> 45	2	16.7	10	83.3	
Occupation	Butcher	12	22.2	42	77.8	0.313
	Abattoir worker	11	34.4	21	65.6	
Years in occupation	0.5 - 10	15	33.3	30	66.7	0.096
	11 - 20	7	28.0	18	72.0	
	> 20	1	6.3	15	93.7	
Work region	Duhok	12	22.6	41	77.4	0.498
	Khanke slaughter h.	11	34.4	21	65.6	
	Shingar and Duhok	0	0	1	100.0	
Fever, chills and/or sweats	Yes	4	36.4	7	63.6	0.475
	None	19	25.3	56	74.7	
Respiratory distress, cough,	Yes	4	30.8	9	69.2	0.740

breathlessness	None	19	26.0	54	74.0	
Cardiovascular problems	Yes	1	50.0	1	50.0	0.466
	None	22	26.2	62	73.8	
Other manifestation	Impaired immunity	0	0	1	100.0	1.000
	Allergy	0	0	1	100.0	
	Congenital malform.	0	0	1	100.0	
	None	23	27.7	60	72.3	
Total		23	26.7	63	73.3	

* Based on Fisher's exact test.

DISCUSSION

Coxsiella burnetii causes Q fever which is a zoonotic disease that is spread throughout the world except New Zealand (Hilbink, 1993; Vogel, 2004). The Middle East, including Iraq is considered endemic for Q fever as shown through a case series recorded in American troops who served in Iraq (Gleeson *et al*., 2007). There is no data concerning the seroprevalence of *Coxiella burnetii* in both human and animals in our area, while few studies are carried out in other parts of Iraq. Moreover, this important public health disease is out of the list of the differential diagnosis followed by physician in the area. Therefore, this study was done primarily to investigate the seroprevalence of *C. burnetii* among the butchers and slaughterhouse workers who come into direct contact with animals and constitute one of the most important risk groups for this infection.

In this study, the prevalence of IgG antibodies against *C. burnetii* phase II was detected in 23 (26.7%) out of 86 serum samples from both butchers and abattoir workers, while IgM antibodies were found in 5 (5.8%) serum samples from butchers only indicating to both chronic and acute stage of *C.burnetii* infection since IgG appears in the secondary immune response and last for years, while IgM appears during the primary immune response and lasts for few weeks.. The percentage of IgG antibodies was 34.4% among abattoir worker and 29.6% in butchers. The seroprevalence of *C. burnetii* significantly differ according to region and occupation even in the same country. No data are available concerning seroprevalence of *C. burnetii* among butchers and abattoir workers in Iraq and this is the first study to be carried out in Iraq. Our results were lower than those found by other researchers in Turkey and Iran. Esmaeili *et al* (2016) found that 68% of butchers and abattoir workers in Kerman Province, Iran were positive for Phase II IgG. Ismaeili *et al*. (2014) found 38% of these risk groups were positive for phase II IgG in Kurdistan Province, Iran. In Turkey, the seroprevalnce was 65.9% and 42.9% among abattoir workers and butchers respectively (Berktas *et al*., 2011). In another side, the results were higher than those found in Turkey and Iran in which 18.1% and 14.4% were positive among butchers and abattoir workers respectively from Sistan Ve Baluchestan of Iran (Esmaeili., 2016) and in Turkey, 1.8%, 11.2% and 13.2% in Samsung, Trabzon and Antalya respectively (Cetinkaya *at el*., 2000). In most of the studies, the percentage of positivity was higher among abattoir workers than butchers because abattoir workers are more prone to blood splashing to their faces, hands and clothes during slaughtering process and all of them were without personnel protective equipment, while butchers receive clean washed carcasses with minimum amounts of blood.

The prevalence of Phase II IgG was decreased with increasing of age, while the picture was opposite in case of IgM in which the percentages were increased with increasing of age. These results were in contrast with other studies which found that the percentage of positivity (IgG) was increased with increasing of age and these discrepancies can be attributed to the low number of samples covered by this study because a lot of butchers and abattoir workers were not willing to give blood samples for personal reasons. In case of IgM which increased with increasing of ages which may be due to the weakened immunity or reactivation of chronic infection into acute infection. In this study no significant correlation was found between studied risk factors and positive cases which may be due to the low number of samples.

Based on the results of this preliminary study, it can be concluded that *C. burnetii* infection is widespread among meat handlers like butchers and abattoir workers in this region who come in contact with animals which are the main source of human infection. These results showed the importance of Q fever as an occupational risk factor for abattoir workers and butchers. Moreover, more investigations are required on other risk groups and animals in the area in order to make epidemiological features of this disease in the area and to increase the awareness among meat handlers for prevention purposes as well as its contribution to the abortion among pregnant women.

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Conflict of interest

We declare that we have no conflict of interest.

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