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Serological Detection of IgG Against *C. Burnetti* Phase II In Behera Province Western Egypt

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ABSTRACT

Little information is available about Q. fever in Egypt, a zoonotic disease caused by *Coxiellaburnetii*, transmitted from domestic ruminants. This study aimed to investigate the prevalence of IgG antibodies against *C. burnetii* phase II antigen in human serum of two different groups (healthy human population of a rural area and a population of an urban area suffering from respiratory complains), in other words determine the percentage of both symptomatic (urban) and asymptomatic (rural) Q. fever infections and also investigate about different risk factors. A total of 92 human serum samples (42 serum samples from patients with respiratory complains and 50 serum samples randomly collected from people free from respiratory complains) were collected. The detection of antibodies against *C. burnetii* was carried out by ELISA method. The obtained results in the current study revealed that 66 out of 92 (72%)humanserum samples were found to be ELISA positive for *C. burnetii* phase II IgG. 31serum samples were found seropositive in patients with respiratory complains with a percentage of (74%) of urban Q. fever, and 35 serum samples were found seropositive in people free from respiratory complains with a percentage of (70%)ofrural Q. fever**.**Statistical analysis of the available data (symptomatic or asymptomatic, urbanism, sex, age, job, history, and pregnancy**)** was carried out using Chi-square test that revealed no statistically significant differences and the values were (0.16, 0.175, 1.62, 2.14, 8.53 and 0.88), respectively. These results prove that Q. fever is present in Egypt and is not restricted to a certain risk group. The public health significance of Q. fever was also discussed.

INTRODUCTION

Zoonoses or diseases transmitted from animals to man, have been recognized as important public health issues for centuries and much of the early history of veterinary science was focused on the control of diseases such as bovine tuberculosis. Ungulates, in particular, are known to carry at least 315 zoonotic pathogens and many emerging and re-emerging infectious disease problems globally are zoonotic **(Cleaveland et al., 2001; Taylor et al., 2001).** Q. fever is a highly contagious zoonotic disease caused by the intracellular pathogen Coxiella burnetii (C. burnetii). It is highly infectious disease, requiring only 1–10 organisms to initiate an infection in an exposed host **(Tigertt et al., 1961)**. This infectious dose is lower than other developed biological weapons **(Bellamy and Freedman, 2001).**

Multiple hosts can serve as a reservoir of infection however; cattle, sheep and goats are the major reservoirs **(Maurin and Raoult, 1999)**.

Infected female animals shed a high concentration of the organism into birth

products and smaller concentrations in urine, feces and milk. This shedding may continue over several months, particularly in vaginal mucus, feces and milk, even in those females with normal parturition **(Masala et al., (2009).** All domesticated ruminants are susceptible but, with the exception of reproductive failures such as abortions, stillbirths, infertility and weak offspring, animals are usually asymptomatic and can remain chronically infected **(Bildfell et al., 2000).** The most common source of human outbreaks of Q. fever due to C. burnetii seems to be domestic ruminants **(EFSA, 2010).** ELISA tests are widely used **(Field et al., 2000)** to detect antibodies in milk but cannot identify shedders **(Berri et al., 2001).** The natural cycle of this bacterium is not reported to include humans, who are considered incidental hosts. The true reservoir is wide and includes mammals, birds and arthropods, mainly ticks. Cattle, sheep and goats are most commonly identified as sources of human infection and the disease is prevalent in mostly rural areas worldwide. Other animals, however, including common pets such as cats, rabbits, pigeons and dogs may also serve as sources **(Tissot-Dupont and Raoult, 2008).** Q. fever is usually transmitted by inhalation of aerosol **(Marrie, 2007).**

Q. fever is an occupational hazard for veterinarians, abattoir workers, dairy farmers, and anyone with regular contact with livestock or their products. After primary infection, about 60% of humans are asymptomatic while 40 % manifest clinical signs consisting of isolated fever, hepatitis and pneumonia. Endocarditis is the major clinical presentation of chronic Q. fever **(Maurin and Raoult, 1999)**. **Abe et al. (2002)** performed a serological survey using serum samples from 267 veterinarians. They measured the antibody titers of the serum samples by indirect immunofluorescence assay (IFA) using phase II *C. burnetii* Nine Mile strain as the antigen. They found that the positive rate of IgG antibody was 13.5% in the veterinarians, which was higher than the blood donors (3.6%) and medical workers (5.1%).In addition, they concluded that Japanese veterinarians have a higher risk of infection by *C. burnetii* than other members of the Japanese population. Also, they found that positive rates of IgG and IgM antibodies were higher in younger individuals and the IgM antibody positive rate was the highest in females under 30 years old. **Dorko et al., (2008)** determined the titers of immunoglobulin IgG against phases I and II of *C. burnetii* in 241 veterinary students by ELISA method and evaluated some risk factors as rural and urban life, consumption of milk, contact with animals and gender. They detected phase I antibodies in 59 serum samples (24.4 %) found Phase II Abs in 179 persons (74.2 %). Also, they found no significant difference in the prevalence of Abs either between the students living in rural and urban environment (78.8 and 73.2 %, respectively) or between males and females (74.0 and 74.7 %, respectively). But they detected Abs more frequently in raw milk consumers (68.1 %) and in students who kept some animals (73.7 %). **Vranakis et al., (2012)** estimated the prevalence of C. *burnetii* phase II antibodies in two different groups (high and low risk) of healthy human population and investigated the epidemiological characteristics of the infection in the island of Crete (southern Greece). They tested 493 sample sera for IgG and IgM antibodies against C. *burnetii* phase II antigen by IFA and found that the prevalence of IgG antibodies of 48.7%. Therefore, the main aim of this work is to provide a recent paper about the presence of C. burnetii among human population in Egypt and find the suitable measures in

order to face this highly infectious disease.

MATERIALS AND METHODS

- **Collection of samples:**

92 human serum samples were collected from two different places in relation to the risk factor. 42 human serum samples were collected from the laboratory of chest hospital of Damanhur, Behera Province, Egypt. In addition, 50 human serum samples were collected from a private laboratory located in a rural area in the same province during the period extended from March 2011 to February 2012. Serum samples were labeled and transferred with the minimum of delay to the laboratory of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University and kept at -20°C till examined by ELISA kits.

- **Serological examination of collected samples:**

Serum samples were tested for presence of antibodies against C. burnetti using **a commercial indirect ELISA kit** (**Vircell SL® Granada, Spain. G1001**, **96 tests)** which was an indirect immune enzyme assay to detect IgG antibodies against C. burnetii phase II in human serum. The test was carried out according to the protocol recommended by the manufacturer.

RESULTS

Table (1): Frequency of detection of IgG antibodies against **C. burnetii** in examined human serum samples by ELISA.

Human serum samples	No.	+ve	%	-ve	%	Chi-square value
Patients with respiratory complains (urban)	42	31	74	11	26	**0.16 NS**
Randomly collected samples (rural)	50	35	70	15	30	
Total	**90**	**66**	**72**	**26**	**28**	

NS = Non-significant

Table (2): Frequency of detection of IgG antibodies against **C. burnetii** in examined serum samples of patients with respiratory complains in relation to **gender** by ELISA.

Gender	No.	+ve	%	-ve	%	Chi-square value
Males	25	19	76	6	24	**0.15 NS**
Females	17	12	70	5	30	**0.15 NS**
Total	**42**	**31**	**74**	**11**	**26**	

NS = Non-significant

Table (3): Frequency of detection of IgG antibodies against **C. burnetii** in randomly collected human serum samples in relation to **gender** by ELISA

Gender	No.	+ve	%	-ve	%	Chi-square value
Males	16	11	69	5	31	**0.2 NS**
Females	34	24	70	10	30	**0.2 NS**
Total	**50**	**35**	**70**	**15**	**30**	

NS = Non-significant

Table (4): Frequency of detection of IgG antibodies against **C. burnetii** in randomly collected human serum samples in relation to **age** by ELISA

Age groups (yeas)	No.	+ve	%	-ve	%	Chi-square value
< 30	13	9	69	4	31	**1.62 NS**
30 - < 45	16	13	81	3	19	
45 - >60	21	13	62	8	38	**1.62 NS**
Total	50	35	70	15	30	

NS = Non-significant

Table (5): Frequency of detection of IgG antibodies against **C. burnetii** in randomly collected human serum samples in relation to **occupational groups** by ELISA

Occupational groups	No.	+ve	%	-ve	%	Chi-square value
Farmers	4	2	50	2	50	**2.14 NS**
House wives	30	21	70	9	30	
Employees	8	5	62	3	38	
Others	8	7	88	1	12	
Total	**50**	**35**	**70**	**15**	**30**	

NS = Non-significant

Table (6): Frequency of detection of IgG antibodies against C. burnetii in randomly collected human serum samples in relation to **medical history** by ELISA

Medical history	No.	+ve	%	-ve	%	Chi-square value
Liver diseases	9	5	55.6	4	44.4	**8.53 NS**
Diabetes mellitus	19	14	74	5	26	**8.53 NS**
Anemia	7	6	86	1	14	**8.53 NS**
Others	15	10	67	5	33	
Total	**50**	**35**	**70**	**15**	**30**	

NS = Non-significant

Table (7): Frequency of detection of IgG antibodies against C. burnetii in randomly collected human serum samples in relation to **pregnancy** by ELISA

Pregnancy status	No.	+ve	%	-ve	%	Chi-square value
Pregnant	6	5	83	1	17	**0.88 NS**
Non pregnant	28	19	68	9	32	**0.88 NS**
Total	**34**	**24**	**70**	**10**	**30**	

NS = Non-significant

DISCUSSION

Q. fever has been described worldwide except in New Zealand. From 1999 to 2004, there were 18 reported outbreaks of Q. fever from 12 different countries involving two to 289 people. Six outbreaks involved sheep; three involved goats; one resulted from exposure to goat manure; one from exposure to ovine manure; one involved exposure to wild animals; one involved exposure to cats and dogs; and in two outbreaks the source was unknown **(Arricau-Bouvery and Rodolakis, 2005).** Data demonstrated in **Table (1)** revealed that the percentage of seropositive subjects for IgG to C. burnetii phase II was 72 %. The

obtained was in harmony with **Mak et al., (2003)** who recorded a detection rate of 66% in Kimberly Western Australia, **Wagner-Wiening et al., (2006)** who recorded a detection rate of 65 % in Germany and **Dorko et al., (2008)** who recoded a detection rate of 74.2 % in Slovakia. On the other hand, this result was higher than those reported by **Loukaides et al. (2006)** (44.4%) in Cyprus**, Kilic et al., (2008**) (32.3%) in Ankara Turkey**, McCaughey et al., (2008)** (13%) in Ireland**, Anderson et al., (2009)** (3%) in USA, **Gozalan et al., (2010)** (13.5%) in Northern Turkey, **Tozer et al., (2011)** (5.2 %) in Australia **and Vranakis et al., (2012)** (48.7%) in Crete. Chi square statistical analysis of the recorded results in **Table (1)** showed no significant difference between the percentage of Q fever antibodies in urban area (74%) and the percentage Q. fever antibodies in rural area (70%). This result agreed with those reported by **Dorko et al., (2008)** who recorded detection rates of 73.2 and 78.8 % of urban and rural areas, respectively with no statistically significant difference, **Tozer et al., (2011)** who recorded detection rates of 5.0 and 5.3% of urban and rural areas, respectively with no statistically significant difference and **Vranakis et al., (2012)** who recorded detection rates of 48.2, 50% of urban and rural areas, respectively with no significant difference between them. However, this result disagreed with that reported by **Pascual-Velasco et al., (1998)** who recorded detection rates of 32.8 and 54 % of urban and rural areas, respectively with a significance difference that prevalence of IgG to c. burnetii phase II was lower among subjects living in the urban zone than in those living in the rural zone.

The prevalence of Q. fever in both rural and urban zones had become significant since it was not necessary to come in direct contact with the infected farm animals to get infected. Other sources of infections may be involved such as improperly heat treated milk and milk products or contact with infected pet animals or infected birds. So, further work needed to improve Q. fever awareness in unexpected and emerging groups and investigate about the exact source of infection. Since it was proven that Q. fever is present in Egypt, further preventive and control measures must be carried out such as investigating all reported abortions, sampling tank milk, and serological screening in various animals. Carrying out a vaccination program in endemic zones and prevention of infection by application of hygienic measures in dealing with various animals and proper heat treatment of milk and milk products must be carried out.

The recorded data in **Table (2) and Table (3)** showed that the detection rate of IgG against C. burnetii phase II in relation to gender was 76% in males, 70% in females among patients with respiratory complains and 69% in males, 70% in females among healthy people. There were no significant differences between males and females in both two groups. These results agreed with those of **Vranakis et al., (2012)** 50.2% in males and 46.3% in females with no statistically significant difference, **Kilic et al., (2008)** 33.2% in males and 21.7 in females with no statistically significant difference, **DORKO et al., (2008)** reported that no significant difference in the prevalence of Abs was detected between males and females (74.0 % and 74.7 %, respectively), and **Gozalan et al., (2010)** 17.3% in males, 10.9% in females and also there was no statistically significant difference between males and females seropositivity. On the other hand, these results disagreed with those of **Abe et al., (2001)** reported that the IgG and IgM antibody positive rates in female subjects were higher than those in male

subjects and **Tozer et al., (2011)** who recorded that the detection rate of Q. fever was higher in males (6.5%) than females (3.9%) This presumably is due to the fact that occupational exposure is the primary cause of infection and occurs predominantly in the male workforce.

Both males and females can be infected with Q. fever equally and that may be due to the exposure to the source of infection equally by the nature of life. Both males and females deal with a variety of animals and exposed to a variety of sources of infection. This may be explained by an increasing need and interest from women to be involved in animal handling jobs that may have previously been performed by men and hence increasing their risk of disease. So, there was no difference between males and females in the capability to contract infection with Q. fever. These differences in results may be due to the different methods of diagnosis and population densities in various countries.

The effect of age groups on the frequency of detection af IgG antibodies against Q. fever was illustrated **Table (4).** It was found that there was no statistically significant difference of detection rate of Q. fever between different age groups in rural area [<30 (69%), 30-<45 (81%), 45- >60 (62%)]. This result agreed with those reported by **Vranakis et al., (2012)** 41–50 (55.3%), 51–60 (47.7%) and no statistical difference was calculated, and **Kilic et al., (2008)** [18-39 (30.1%), 40-61 (39.2%)] with no statistical difference. On the other hand, this result disagreed with those of **Abe et al., (2001)** who recorded a higher prevalence of Q. fever in young people[<29 (5.4%), 30–39 (2.0%), 40–49 (2.5%), >50 (3.3%)]. **Loukaides et al. (2006)** reported that the mean age of seropositive participants was 44.7 years compared with 33.5 for seronegative and the difference was statistically significant. **Tozer et al., (2011)** found that there was an increase in seropositivity with an increase in age [0–14 (1.3%), 15–39 (4.6%), 40–64 (9.9%), 65+ (9.5%)]. **Gozalan et al., (2010)** concluded that seropositivity for C. burnetii was 3.2 times higher in the over 30 years of age group than those below 30 years of age (p=0.001). These data may suggest more frequent exposure to C. burnetii, such as infectious aerosols. The reason that the detection of acute Q fever in over 60-year-olds could be explained by decreased immunity. The differences in results about age groups may be due to the physiological differences of different ages and differences in the ability of acquiring the disease.

Data presented in **Table (5)** showed that there was no statistically significant difference of seroprevalence of C. burnetii between different occupational groups in rural area; farmers (50%), house wives (70%), employees (62%), others (88%). This result agreed with that of **Abe et al., (2001)** who found that there was no significant difference between medical workers and blood donors (p= 0.19) **and Kilic et al., (2008)** reported no statistically significant difference between occupational groups [Livestock/crop breeder (34.1%), Other (32.1%)]. On contrary, this result disagreed with that of **Abe et al., (2001)** who found that IgG antibody against C. burnetii was significantly higher in veterinarians (13.5%) than in either medical workers (5.1%) or healthy blood donors (3.6%), **Dorko et al., (2008)** detected that the percentage of positivity of IgG to c. burnetii phase II among students of faculty of medicine as (74.2 %) and reported that 175 students mentioned work in a dusty environment (field, garden, grain-loft, game preserve, building construction) or in contact with hay, straw, soil, manure and animal products; and assumed that this group was most probably exposed more to *C. burnetii*, and **Gozalan et al., (2010)**

reported prevalence of c. burnetii in hunters, and slaughters higher than the others (p=0.034, and p=0.006, respectively).

Analysis of the data in **Table (6)** showed that there was no statistically significant difference of seroprevalence of C. burnetii between different medical history groups in rural area [liver diseases (55.6%), Diabetes mellitus (74%), Anemia (86%), and Others (67%)], this result agreed with that of **Dorko et al., (2008)** who found that 23 out of 55 students reported liver disease, 18 fevers of unknown etiology, 13 respiratory disease, 6 heart disease and 3 rheumatism and fatigue. In this group phase I Abs were found only in 12 students suffering from various diseases. Phase II Abs was positive in 38 cases. Examination for the presence of phase I and II Abs in additional 17 subjects (who suffered in the past from various diseases) provided negative results. Because of that they could not determine whether the diseases experienced in the past and the Abs detected were really related to infection caused by *C. burnetii*. While, **Wagner-Wiening et al., (2006)** reported the great significance of Q. fever infection for patients with valvular heart defects in rural areas.

Data demonstrated in **Table (7)** showed that there was no statistically significant difference of seroprevalence of C. burnetii between pregnant women (83%), and non pregnant women (68%). In contrast, **Wagner-Wiening et al., (2006)** found a great significance of Q. fever infection for pregnant women. **Carcopino et al., (2009)** mentioned the risk of developing chronic Q. fever infection is especially high for pregnant women.

Finally, the prevalence of *C. burnetii* infection appears to vary considerably in different geographic areas, seasons and populations studied. Reported prevalence may also depend on the techniques used for antibody detection and criteria used to define positive results **(Fournier et al. 1998; Kovacova and Kazar, 2000; Kovacova and Kazar, 2002).**

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