Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolia, Turkey

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SUMMARY

The aims of this study were to determine the prevalence of *Coxiella burnetii* antibodies among blood donors and to examine the epidemiological characteristics of *C. burnetii* infection in Ankara, Turkey. A total of 601 serum samples were collected from blood donors aged 18-61 years. Donor samples were stratified by age, sex, and residence (rural or urban). IgG and IgM antibodies to the *C. burnetii* phase II antigen were determined using a commercial ELISA. Blood samples reactive in the ELISA were also analysed using a commercial indirect immunofluorescence assay (IFA). The prevalence of anti-phase II IgG was 32.3%, and 17 (2.8%) were IgM positive. Seropositivity in men was higher than in women (33.2% vs. 21.7%, OR:1.88;95% CI: 0.88-4.14) and the difference in seroprevalence rates between genders was not related to occupational exposure to domestic animals. 87.6% of seropositive donors reported no contact with farm animals. Our results revealed that *C. burnetii* infection is highly endemic in Ankara and that the majority of seropositive cases are not linked to specific occupational exposure in this area. In conclusion, the high rate of *C. burnetii* phase II antibodies among blood donors is a reflection of the high prevalence of Q fever in this area of Turkey and indicates the need for further studies, not only to determine the risk of transfusion-transmitted Q fever, but also to elucidate the epidemiology of Q fever in Turkey. These studies should be conducted through improved collaboration between the veterinary and medical services.

KEY WORDS: Coxiella burnetii, Q fever, Blood donors, Seroprevalence, ELISA, IFA

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INTRODUCTION

Coxiella burnetii, the causative agent of Q fever, is an obligate intracellular Gram-negative bacterium formerly classified in the Rickettsiaceae family (Maurin and Raoult, 1999).
The disease is endemic worldwide, occurring in all geographic regions and climatic zones, with New Zealand remaining an exception (Hilbink *et al.,* 1993).
Cattle, sheep and goats are considered to be the main reservoirs for infection of humans (Arricau-Bouvery and Rodolakis, 2005). Humans are primarily infected through inhalation of air-borne contaminated dust, through direct contact with infected animals or by ingestion of unpasteurized milk or fresh dairy products. There is little evidence for direct human to human transmission through blood transfusion, during autopsy, delivery, via transplacental transmission or via sexual contact (Milazzo *et al.,* 2001; Raoult *et al.,* 2002; Maurin and Raoult, 1999; Weber and Rutala, 2001).
In humans, the clinical presentation of Q fever varies widely, depending on a number of factors,

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including the initial health of the host (Kazar, 2005). Primary infection in humans is believed to be asymptomatic in approximately 60% of those infected (Peter *et al.*, 1987). *C. burnetii* infection may be acute (self-limited febrile or flu-like illness, pneumonia, granulomatous hepatitis) or chronic (mainly endocarditis, hepatitis, chronic fatigue syndrome, graft infection) (Raoult *et al.*, 2000). The acute form is often under-diagnosed because of the non-specific clinical picture, and thus serology is extremely important diagnosis of the disease (Arricau-Bouvery and Rodolakis, 2005; Fournier *et al.,* 1998).

C. burnetii is considered to be a risk of blood transfusion because asymptomatic and subclinical infections are believed to be common in acute Q fever and the pathogen resides and replicates in host monocytes and macrophages. However, there is only one report of blood transfusion associated transmission of Q fever in the literature in the past 30 years (Anonymous, 1977).

Little is known about the incidence and geographical distribution of Q fever in humans and animals in Turkey, although *C. burnetii* has been known to occur here since 1947. To date, no clinical case of Q fever has been documented in Ankara. We therefore aimed to assess the prevalence of *C. burnetii* antibodies in blood donors and examine the epidemiological characteristics of *C. burnetii* infection in Ankara, Central Anatolia, Turkey.

MATERIAL AND METHODS

Blood samples obtained from the local blood bank at Ankara Hospital came from donors living in Ankara and its neighbouring areas during a 4-month period from April and July 2007. Sera and EDTA blood were stored at -20°C on the day of blood donation. 601 blood donations were screened over a period of 1 month (July to August 2007).

A standard blood bank questionnaire was used to obtain informed consent from all blood donors participating in this investigation, and the approval of local ethics committees was obtained prior to conducting the study. Information on age, sex, place of residence, contact with farm animals, contact with wild animals and occupation was collected from each study subject.

Serological assays

The presence of IgM and IgG antibodies to *C. burnetii* phase II was investigated using a commercially avaliable ELISA (Vircell SL® Granada, Spain). The assays were conducted according to the manufacturer's instructions. Blood samples reactive in the ELISA were also analysed using a commercial indirect immunofluorescence assay (IFA) (Vircell SL® Granada, Spain) to confirm the ELISA results.

Statistical analyses

Data were analyzed using SPSS software. The Student t test was used to compare quantitative variables. Univariate group comparisons were performed using x^2 and the Fisher exact test. A $P<0.05$ was considered significant. The statistical analyses odds Ratio (OR), with its interval of confidence of 95% (CI 95%), with and without stratifying by the main variables: sex, rural or urban origin, contact with animals and greater or smaller age of 40 years, were calculated using EpiInfo 2000 software.

RESULTS

The group of blood donors investigated included 555 (92.3%) males aged from 18 to 61 years, and 46 (7.7%) females aged from 18 to 55 years. The ratio of men to women was 12.1:1 with a median age of 32.2 years.

Serology results

Of the 601 samples studied, 194 (32.3%) were positive for anti-phase II Coxiella IgG and 17 (2.8 %) were positive for Coxiella IgM antibodies. All IgM positive cases were also positive for IgG. In males (n=555), IgM and IgG antibodies were found in 33.1% and 2.9%, respectively. In females (n=46), IgM and IgG antibodies were found in 21.7% and 2.2%, respectively (Table 1).

The median age of the 194 IgG positive donors was 31 years (range, 18-58 years), with a male predominance of 94.8% (184 males and 10 females). No statistically significant difference in IgG seroprevalence was observed between male and female blood donors (33.2 % vs 21.7%, P= 0.142).

The prevalence of seropositivity varied among the different age groups (Table 1). IgG seropositivity

TABLE 1 - *Prevalence of C.burnetii determined by ELISA according to sex and age groups.*

Sex and age (Years)	*No. of tested*	*C. burnetii phase II ELISA*	
		IgG	*IgM*
Males			
18-29	232	64 (27.6%)	10 (4.3%)
30-39	188	66 (35.1%)	3 (1.6%)
40-49	117	47(40.2%)	3 (2.6%)
50-59	17	7 (41.2%)	-
>60	1	-	-
Total	555 (92.3%)	184 (33.2%)	16 (2.9%)
Females			
18-29	19 (41.3%)	3 (15.8%)	-
30-39	19 (41.3%)	5 (26.3%)	-
40-49	6 (8.7%)	2 (33.3%)	1 (16.7%)
50-59	2 (4.3%)	-	-
Total	46 (7.7%)	10 (21.7 %)	1 (2.2 %)
Total	601 (100 %)	194 (32.3%)	17 (2.8%)

increased with age, but the difference was not statistically significant (P=0.336).

Study subjects resided in a total of seven towns and nine villages; 537 (89.4%) in urban areas and 64 (10.6%) in rural areas. *C. burnetii* IgG seroprevalence was 31.7% in urban residents and 37.5% in rural residents (Table 2), a difference that was not significant (P=0.631).

There were no significant differences in the prevalence of antibodies to *C. burnetii* related to reported contact with any kind of animal or to occupation.

No statistically significant differences were found between IgM positivity and sex, age and place of residence. All samples positive by ELISA were tested by IFA for *C. burnetii* Phase II IgM and IgG

TABLE 2 - *C. burnetii ELISA IgG prevalences according to considered variables.*

Variable	*IgG Positivity (%)*	*OR (CI 95%)*	*P*
Sex			
Male	184/555 (33.2)	1.88 (0.88-4.14)	0.11
Female	10/46 (21.7)		
Age			
18-39	138/458 (30.1)	0.67 (0.45-1.01)	0.056
40-61	56/143 (39.2)		
Residence			
Urban	170/537 (31.7)	0.77 (0.44-1.37)	0.42
Rural	24/64 (37.5)		
Occupation			
Livestock/crop breeder	14/41(34.1)	1.09 (0.53-2.23)	0.92
Other	180/560 (32.1)		
Contact with animals			
Yes	24/73(32.9)	1.03 (0.59-1.79)	
No	170/528 (32.2)	0.99	

OR: odd ratio; CI 95%: interval of confidence of 95%.

antibodies. From 17 ELISA IgM positive samples, 15 (88.2%) were also positive for *C. burnetii* phase II IgM by IFA at titres ranging from 1:24 to 1:96. From 194 ELISA IgG positive cases, IgG antibodies were found in 191 (98.5%) donors by IFA at titres ranging from 1:64 to 1:512.

DISCUSSION

Seroepidemiological studies suggest that *C. burnetii* infection is common worldwide (Hellenbrand *et al.,* 2001; Tissot dupont *et al.,* 1992; Suarez-Estrada *et al.,* 1996; Houwers and Ricardus, 1987; Pascual-Velasco *et al.,* 1998), but a lack of clinical data and diagnosis makes it impossible to estimate the true incidence of human Q fever. The disease is probably underdiagnosed because it is not a notifiable disease in most countries, the non-specific nature of its clinical signs, the requirement for a laboratory test to establish a diagnosis, and the lack of awareness of this disease in medical and veterinary communities (Kazar, 2005; Kovakova and Kazar, 2002).

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).

The seroprevalence of *C. burnetii* in blood donors determined by different serological techniques has been reported to be 1 to 18.3% in Morocco (Meskini *et al.,* 1995), 10% to 20 % in Northeast Africa (Botros *et al.,* 1995), 26% in Tunisia (Omezzine-Letaief *et al.,* 1995), 4.2% to 15.9% in Canada (Marrie *et al.,* 1983; Marrie, 1988), 3,6% in Japan (Abe *et al.,* 2001), 1.1% in Czechoslavakia (Literak, 1993), 4.03 to 5 % in France (Tissot-Dupont *et al.,* 1992; Raoult *et al.,* 1987), 15% to 22% in Germany (Hellenbrand *et al.,* 2001), 23.1% in Spain (Bortolome *et al.,* 2007), 22,6% to 28.6% in Switzerland (Peter *et al.,* 1987) and 45% in The Netherlands (Houwers and Ricardus, 1987).

In individual patients, recent infection is most often identified by IgM specific antibodies. Only two seroprevalence studies have been carried out in blood donors using IgM antibody assays reported in the literature so far. The prevalence of *C. burnetii* IgM antibodies was reported to be 0.3% in blood donors in Spain and in Japan (Bortolome *et al.,* 2007; Abe *et al.,* 2001).

In our study, the prevalence of IgG and IgM antibodies to *C. burnetii* phase II antigen was 32.3% and 2.8%, respectively. These seroprevalence rates suggest that *C. burnetii* infection is endemic at a high level in the area around Ankara.

The higher IgM prevalence rate found in this study may be related to the time of collection of samples during late spring and early summer seasons. Since sheep parturition takes place in the spring in Turkey as in Europe, the peak of infection has been attributed to spring lambing and sharing and subsequent environmental contamination with *C. burnetii* (Hellenbrand *et al.,* 2001, Tissot-Dupont *et al.,* 1992).

Q fever was first recognized in Turkey in 1947, when a small outbreak occurred in a rural community. It was suspected that infectious dust produced by shearing of sheep whose wool was contaminated with the bacteria was the source of transmission (Payzin, 1948). Following the first reported outbreak, several epidemiological studies in Turkey in the late 1940s and 1950s suggested that Q fever was widespread in both humans and animals. Seroprevalence was seen to differ from one region to another, probably because of different epidemiologic and climatic environments (Payzin, 1953). The highest seroprevalence rates were found in Central Anatolia (Ankara [28.0%] and in eastern Turkey [40.3%], probably because of more extensive sheep- and goat-raising activities (Payzin and Akan, 1964). In contrast, Q fever seems to be less prevalent in the north of Turkey (Samsun [1.8%] and Trabzon [11.2%] and South of Turkey (13.2%) (Payzin and Akan 1964, Berberoglu *et al.,* 2004). In a 1975 a serosurvey in Izmir, western Turkey, 4.5% of humans tested were positive (Karakartal, 1975). Conversely, recent serosurveys in the same area found a 39.3% seropositivity rate among blood donors (Sertpolat and Karakartal, 2005).

In the present study, the seroprevalence of *C. burnetii* IgG was 32.3%, indicating endemic infection by *C. burnetii* in this predominantly urban area. This result agrees well with those obtained in the previously study carried out in Izmir, where the seropositive rate was 39.3%.

An earlier study conducted in Ankara in 1964, of rural residents and patients hospitalized for oth-

er diseases reported an overall prevalence of *C. burnetii* infection of 28% (Payzin and Akan, 1964). This is slightly lower than the level found in the present study, but is in general agreement. Although the nature and socio-demographic properties of the city have been significantly changed from a moderate service sector to important industrial centre, it has been suggested that prevalence of Q fever has not changed dramatically since 1964 (28.0% compared with 32.3% in the current study). In 1960 Ankara was a residential city with a population of 1.3 million and an economy based mainly on agriculture and stockbreeding. During the last two decades of the 20th century the population of Ankara almost quadrupled and the city has become an important industrial centre. From the results of our study, it appears that urbanization of rural areas and industrialisation of the city seems to have had little effect on the prevalence of antibodies to *C. burnetii*.

The prevalence of Q fever has generally been considered higher in rural areas (Payzin and Akan, 1964, Karakartal, 1975; Suarez-Estrada *et al.,* 1996; Pascual-Velasco *et al.,* 1998), because Q fever has typically been regarded as an occupational disease affecting people in contact with livestock and their products (Maurin and Raoult, 1999). In our study, only a small proportion of blood donors reported being involved in stockbreeding, and most of them lived in urban areas. No statistically significant correlation was found between seropositivity and place of residence (p=0.42). In addition, no significant differences were detected between seropositive rates and contact with animals or occupation. However, the small number of people who reported contact with animals may be a limitation.

Recent studies have suggested that there is an increasing number of cases among urban residents (Hellenbrand *et al.,* 2001). Some increased exposure to farm animals caused by travel to the countryside and outside activities, contact with pets, and urbanization of rural areas could contribute to the rising probability among urban residents of being infected (Arricau-Bouvery and Rodolakis, 2005, Tissot-Dupont *et al.,* 2004; Sampere *et al.,* 2003; Hellenbrand *et al.,* 2001; Carrieri *et al.,* 2001; Maurin and Raoult 1999).

Another possible explanation for the higher prevalence of Q fever in urban areas is windborne spread. *C. burnetii* is extremely resistant to hostile environmental conditions, due to the unusual spore-like structures, and remains viable in the environment over long periods of time (Kovakova and Kazar, 2002; Kazar, 2005). Infectious particles can stick to wool and be carried in dust, spreading from farmlands to urban areas by the wind, and infected people sometimes never recall direct contact with animals (Tissot-Dupont *et al.,* 2004). This may help to explain the high seroprevalence figure found in this study.

Ingestion of unpasteurized milk or consumption of fresh dairy products may also contribute to humans being exposed to infection. Traditional eating habits, including the consumption of unpasteurized milk and dairy products (the production of fresh cheese and butter from milk immediately after obtaining it from the animal is particularly common in this region) may be a possible source of infection. Although in earlier studies it had been assumed that consumption of raw milk and dairy products can lead to seroconversion without developing clinical disease, human infection through unpasteurized milk and goat dairy products has been reported (Fishbein and Raoult,1992; Tissot-Dupont *et al.,*1992). Moreover, in a recent outbreak in Newfoundland, Canada, consumption of cheese made from pasteurized milk emerged as a risk factor for infection for the first time (Hatchette *et al.,* 2001). Further studies are necessary to determine the risk factors in our predominantly urban region.

The prevalence of *C. burnetii* infection is reported to be more frequent in men in areas where the most common risk factor is exposure to infected domestic ruminants. This has been reported from Spain, France, USA, and Australia (Maurin and Raoult 1999; Raoult *et al.,* 2000; Pascual-Velasco *et al.,* 1998; Suarez-Estrada *et al.,* 1996). This may be partly attributable to the larger number of men in occupations in close contact with farm animals and increased recreational exposure. In contrast, there was no statistically significant difference in prevalence between male and female donors in this study, which is in accordance other studies conducted in Turkey (Kalkan *et al.,* 1999, Berberoglu *et al.,* 2004, Sertpolat and Karakartal, 2005).

The seroprevalence of *C. burnetii* infection demonstrates a trend for increasing with age, due to longer exposure time in industrial and farming

activities and persistence of IgG antibodies (Dupuis *et al.,* 1985; Marrie and Pollack, 1995; Pascual-Velasco *et al.,* 1998; Suarez-Estrada *et al.,* 1996; Karakartal, 1975; Ozyer *et al.,*1989; Sertpolat and Karakartal, 2005). Although we found a higher rate among older blood donors (over 40 years of age), no statistically significant difference was observed (p>0.05). This is in agreement with other studies (Kalkan *et al.,* 1999; Berberoglu *et al.,* 2004).

The diagnosis of Q fever is mainly based on serological analysis because isolation of the causative agent is time-consuming, extremely hazardous and available only in reference laboratories. The ELISA and IFA remains the most common method used to detect antibodies against *C. burnetii*. IFA is the current reference method for the serodiagnosis of Q fever (Fournier *et al.,* 1998; Maurin and Rauolt, 1999; Kovacova and Kazar, 2000). The ELISA is purported to be more sensitive than IFA and CF for the diagnosis of Q fever. *C. burnetii* ELISA demonstrated a sensitivity, specificity and agreement of 96.8-100%, 96-99%, and 71-100%, respectively, for IgG, and 95.65-99%, 88-98.9%, and 92-96.8%, respectively, for IgM, when tested against the immunofluorescence reference method (Peter *et al*., 1988; Cowley *et al*., 1992; Uhaa *et al*., 1994; D'harcourt *et al.* 1996; Fournier *et al*., 1998; Field *et al*., 2000; Field *et al*., 2002; Frangoulidis *et al.*, 2006).

Although ELISA and IFA are well-established routine techniques, the high proportion of positive results due to cross-reactions with other microorganisms raised questions concerning the specificity of the test used. Cross-reactivity has been described to occur between *C. burnetii* and *Legionella* (*L. pneumophila* and *L. micdadei*) or *Bartonella* (*B. henselae* and B*artonella quintana* (Maurin and Raoult, 1999; Fournier *et al*., 1998; Kovacova and Kazar, 2000; Field *et al*., 2000). Specificity of the ELISA can be increased with confirmation by IFAT (Cowley *et al*., 1992; Hillbrink *et al.*, 1993; Field *et al.,*2000; Kovacova and Kazar, 2000). To confirm the specificity of both tests, confirmation of the presence of *C.burnetii* in seropositive samples is highly desirable, e.g. by PCR. (Fournier *et al.,* 1998; Kazar, 2005; Kovacova and Kazar, 2000). Although PCR appears to be a very sensitive method for the laboratory diagnosis of *C. burnetii* infection, the presence of inhibitors in serum and the usually small amount of bacterial DNA present in serum samples limit the efficiency of PCR (Maurin and Raoult, 1999). It has recently been emphasized that in acute Q fever, PCR was not particularly sensitive and should be performed together with serology (Fournier and Raoult, 2003).

In conclusion, 32.3% of a sample of blood donors from Ankara province and its surrounding areas were positive for antibodies against *C. burnetii,* confirming the endemic nature of this infection and indicating that the disease is more widely spread in Turkey than was previously assumed. Q fever should be taken into consideration in the differential diagnosis of a febrile diseases, pneumoniae, hepatitis and culture-negative endocarditis.

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