Frequency of seropositivity for *Coxiella burnetii* immunoglobulins in livestock and abattoir workers in Trinidad

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SUMMARY

Coxiella burnetii is a zoonotic, rickettsial pathogen which causes mild and severe diseases often referred to as Q-fever in humans, particularly those occupationally exposed. This study determined the seropositivity for *Coxiella burnetii* IgM immunoglobulins using the enzyme immunoassay (EIA) in livestock and abattoir workers in Trinidad and related to selected personal characteristics to seroprevalence. Overall, of the 455 humans whose serum samples were tested, 20 (4.4%) were seropositive for *C. burnetii* IgM immunoglobulin, comprising 13 (4.6%) out of 283 livestock workers, 4 (4.7%) out of 85 abattoir workers and 3 (3.4%) out of 87 office workers ($P>0.05$; χ^2). The age, sex and race of workers were not significantly associated with the occurrence of acute Q-fever ($P>0.05$; χ^2). This is considered the first documentation of Q-fever in the human population in Trinidad. It is difficult to assess the impact of the disease in the country since the disease is not routinely tested for in the local hospitals or diagnostic laboratories.

KEY WORDS: Seropositivity for *Coxiella burnetii*, Immunoglobulins, Livestock workers, Abattoir workers, Trinidad and Tobago

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INTRODUCTION

Q-fever caused by *Coxiella burnetii*, a member of the family Rickettsiaceae, is considered a zoonotic and public health disease as well as an emerging or re-emerging disease (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005). The pathogen has worldwide distribution and is usually transmitted through animals including cattle, sheep, goats, rodents and cats (Arricau-Bouvery and Rodolakis, 2005).

The disease has several variable clinical manifestations ranging from acute to fatal chronic infections. The acute form of the disease is primarily a flu-like disease or atypical pneumonia or hepatitis (Maurin and Raoult, 1999), other clinical signs and symptoms include menningoencephalitis, severe headache, pericarditis, pancreatitis and abortion (Kagawa *et al.,* 2003; Madariaga *et al.,* 2003). The majority of infections by *C. burnetii* in humans are however asymptomatic seroconversions (Arricau-Bouvery and Rodolakis, 2005). Q-fever is also considered an occupational disease associated with individuals in contact with animals (Dolcé *et al*. 2003; Mazyad and Hefez, 2007; Zhang *et al.,* 2008; Whitney *et al.,* 2009) or consumption of animal products such as raw milk or improperly heat-treated milk (Dorko *et al.,* 2008).

Q-fever outbreaks have been documented in several countries including Spain (De los rios-Martin *et al*, 2006), Poland (Tylewska-Wierzbanowska *et al.,* 1996), The Netherlands (Karagiannis *et al.,* 2009), Japan (Kawamoto *et al*., 2002) and Canada (Langley *et al*., 2003). Similarly, reported asymptomatic seroprevalence of Q-fever have ranged

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from 0% to 37% in various countries in different groups of individuals, particularly livestock farmers and their family members, abattoir workers, hunters, veterinarians and animal health assistants (Kelly *et al.,* 1993; Thibon *et al*., 1996; Deutz *et al*., 2003; Zhang *et al.,* 2008; Whitney *et al*., 2009).

Diagnosis of Q-fever in humans could be made using a combination of clinical manifestations (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005), isolation of the microorganism which is long, tedious and hazardous to perform (Fournier *et al*., 1998), polymerase chain reaction (PCR) for direct diagnosis (Stein *et al*., 1992; Arricau-Bouvery and Rodolakis, 2005) and serological tests (Fournier *et al*., 1998; Kovacoa and Kazar, 2002; Santoro *et al.,* 2004). In view of the fact that most chronic Q-fever or asymptomatic infections are primarily diagnosed by the use of serological tests which detect antibody response to antigens of Phase I, Phase II or both. Serological tests include the immunoflourescence test (IFT) which remains the most common method used to detect antibodies against *C. burnetii* because it is considered simple, accurate and able to distinguish between acute and chronic Q-fever using phase I and phase II antigens (Fournier *et al.,* 1998), complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) which have been used extensively for serological surveys or diagnosis of clinical cases where sensitivities of 99% and specificity of 88% have been documented (Abe *et al*., 2001; Field *et al.,* 1983, 2000). The possibility of false-positive test results therefore exists with the use of serological tests.

There is a dearth of information on infection by *Coxiella burnetti* in humans in Trinidad and Tobago and the Caribbean region at large since the infection or disease is not routinely assayed for. Infection by *C. burnetii* was first documented in livestock (cattle, sheep, goats, pigs, chickens, and water buffalo) in Trinidad in 1996 where an overall prevalence of 4.8% for *C. burnetii* IgG was reported (Adesiyun and Cazabon, 1996) using the capillary agglutination test, but to date, no publication exists on infection in humans. The primary objective of this study was therefore to determine the seroprevalence of IgM immunogolobulins for acute or current infections in high risk members of the population (farm and abattoir workers and veterinarians) and the secondary objective was to relate selected demographic risk factors (age, gender, race and duties performed) to infection by *C. burnetii*.

MATERIALS AND METHODS

Sources of samples

For this study, owners of large, privately-owned livestock farms and institutional farms as well as personnel of local abattoirs were informed by the Veterinary Public Health Unit of the study, its voluntary nature and their participation was solicited. The study design was to collect blood samples from consenting individuals. On the livestock farms, samples were collected from individuals/farmers (stockmen, labourers, animal health assistants, veterinarians) at 12 large farms/institutional farms and from workers at 10 abattoirs across the country (Table 1). In addition, at the farms and abattoirs, samples were collected from workers with minimal animal contact such as secretaries, office assistants, laboratory technicians, cleaners, security guards and drivers, amongst others.

Overall, a total of 283 livestock and 85 abattoir workers and 87 other workers were sampled.

During visits to the facilities, the workers were again informed of the voluntary nature of their participation in the study and all consenting individuals were requested to complete consent forms. Also, a questionnaire was administered to each person who agreed to participate in the study to elicit information on age, gender, race and the type of functions they performed.

Collection of samples

Approximately five milliliters of blood were collected from the median cubital vein of each individual into vacutainer tubes without anticoagulant and transported ice-cooled to the laboratory. This volume of blood was collected since this is considered a large study screening for exposure experience of livestock farmers, veterinarians and abattoir workers to a number of zoonotic pathogens including leptospirosis, brucellosis, toxoplasmosis, hanta virus and Q-fever. After overnight storage at 4^{o}C, the sera were harvested by centrifugation and stored -20^{o}C until tested.

Detection of IgM immunoglobulins in serum samples

To detect *Coxiella burnetii* IgM immunoglobulins, enzyme-linked immunosorbent assay (ELISA) test kits (Panbio Diagnostics, Australia) were used with appropriate controls provided by the manufacturer.

Statistical analysis

The frequency of detection of *C. burnetii* IgM immunoglobulins in the three groups of workers were compared as well as relating seroprevalence to the age, gender and race of workers using the Statistical Package for Social Sciences (SPSS), version 10. All statistical tests were two-sided and interpreted at the 5% level of significance using the Chi-square (χ^2) test.

Ethics committee approval

The Ethics Committees of the Faculty of Medical Sciences, University of the West Indies, St. Augustine Campus and the Ministry of Health approved the research proposal.

RESULTS

Table 1 shows the overall frequency of detecting *C. burnetii* IgM immunoglobulins was 4.4% (20 of 455). For the three groups of workers, the seropositivity rates were as follows: 13 (4.6%) of 283 amongst livestock workers, 4 (4.7%) of 85 abattoir workers and 3 (3.4%) of 87 office workers associated with livestock farms and abattoirs. The differences were not statistically significant ($P>0.05$; χ^2). On the 12 livestock farms tested, the range of seropositive humans was from 0.0% on 5 farms to 16.7% on 2 farms while the range for the 10 abattoirs was from 0.0% in 7 abattoirs to 12.5% in 1 abattoir. The frequency of *C. burnetii* IgM immunoglobulin by age, sex and race is shown in Table 2. Overall, the differences in frequency of seropositive humans were not statistically significant ($P>0.05$; χ^2).

DISCUSSION

It was significant that although all the individuals sampled in the current study were apparently healthy, 4.4% had significant titres of *C. burnetii* IgM immunoglobulin, the first demonstration in humans of any type immunoglobulins to *C. burnetii* in the country. Demonstration of *C. burnetii* IgM has been reported to be useful in arriving at a diagnosis clinical Q-fever (Murphy and Hunt, 1981; Field *et al*., 2000), however the farm and abattoir workers in the current study were apparently healthy. The findings may be explained, in part, by the possibility that the seropositive individuals had mild cases of Q-fever, may have just recovered from the undiagnosed clinical form of the disease since *C. burnetii* IgM immunoglobulin titres have been known to remain in infected cases for as long as 4 to 6 months after the onset of clinical disease (Field *et al*., 1983; Guigno *et al*., 1992).

TABLE 1 - *Frequency of Coxiella burnetii IgM immunoglobulins in farm and abattoir workers.*

Type of workers	*No. of workers tested*	*No. (%) positivea for C. burnetii IgM immunoglobulin*
Abattoir	85	4 (4.7)
Farm/Livestock	283	13 (4.6)
Office/Othersa	87	3 (3.4)
Total	455	20 (4.4)

[a]At abattoirs and livestock farms.

The overall seropositivity of 4.4% for *C. burnetii* immunoglobulins detected in this study is in agreement with the seroprevalence rates of 2.8% to 13.5% for Q-fever by others who similarly assayed for *C. burnetii* IgM reported for high risk groups or blood donors elsewhere (Abe *et al.* 2001; Kilic *et al.*, 2008). In a study of apparently healthy blood donors in Turkey, Kilic *et al.* (2008) using a commercial ELISA kit for *C. burnetii* antibodies reported that 32.3% and 2.8% were seropositive for IgG and IgM antibodies respectively. Bartolom *et al*. (2007) also detected IgM *C. burnetii* antibodies in 0.3% apparently healthy blood donors in Albacete.

The detection of *C. burnetii* immunoglobulin in livestock workers (4.6%), abattoir workers (4.7%) and office workers associated with the livestock farmers and abattoirs (3.4%) who had minimal contact with animals were not statistically significantly different is an indication that other fac-

TABLE 2 - *Frequency of C. burnetti IgM immunoglobulin by age, gender and race.*

Type of workers	*Category*	*Frequency of C. burnetii IgM immunoglobulin:*											
				Gender				*Race*					
				Female		*Male*		*African*		*Indian*		*Others*	
	Age (y)	*No. tested*	*No. (%) positive*	*No. tested*	*No. (%) positive*	*No. tested*	*No. (%) positive*	*No. tested*	*No. (%) positive*	*No. tested*	*No. (%) positive*	*No. tested*	*No. (%) positive*
Abattoir	≤30	19	1 (5.3)	3	0 (0.0)	16	1 (6.3)	8	0 (0.0)	10	1 (10.0)	1	0 (0.0)
	31-60	53	3 (5.7)	0	0 (0.0)	44	3 (6.8)	23	2 (8.7)	27	1 (3.7)	3	0 (0.0)
	>60	13	0 (0.0)	0	0 (0.0)	13	0 (0.0)	8	0 (0.0)	5	0 (0.0)	0	0 (0.0)
	Subtotal	85	4 (4.7)	3	0 (0.0)	82	4 (4.9)	39	2 (5.1)	42	2 (4.8)	4	0 (0.0)
Farm/Livestock	≤30	55	9 (16.4)	13	4 (30.8)	42	5 (11.9)	26	6 (23.1)	25	3 (12.0)	4	0 (0.0)
	31-60	216	3 (1.4)	22	0 (0.0)	194	3 (1.5)	52	1 (1.9)	149	2 (1.3)	15	0 (0.0)
	>60	9	0 (0.0)	1	0 (0.0)	8	0 (0.0)	4	0 (0.0)	5	0 (0.0)	0	0 (0.0)
	NA	3	1 (33.3)	0	0 (0.0)	3	1 (33.3)	1	1 (100.0)	2	0 (0.0)	0	0 (0.0)
	Subtotal	283	13 (4.6)	36	4 (11.1)	247	9 (3.6)	83	8 (9.6)	181	5 (2.8)	19	0 (0.0)
Office workers	≤30	11	1 (9.1)	10	1 (10.0)	1	0 (0.0)	7	1 (14.3)	4	0 (0.0)	0	0 (0.0)
	31-60	28	1 (3.6)	22	1 (4.5)	6	0 (0.0)	8	0 (0.0)	20	1 (10.0)	0	0 (0.0)
	>60	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
	NA	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
	Subtotal	39	2 (5.1)	32	2 (6.3)	7	0 (0.0)	15	1 (6.7)	24	1 (4.2)	0	0 (0.0)
Others[a]	≤0	3	0 (0.0)	2	0 (0.0)	1	0 (0.0)	2	0 (0.0)	0	0 (0.0)	1	0 (0.0)
	31-60	31	1 (3.2)	12	1 (8.3)	29	0 (0.0)	18	1 (5.6)	21	0 (0.0)	2	0 (0.0)
	>60	4	0 (0.0)	0	0 (0.0)	4	0 (0.0)	1	0 (0.0)	3	0 (0.0)	0	0 (0.0)
	Subtotal	48	1 (2.1)	14	1 (7.1)	34	0 (0.0)	21	1 (4.8)	24	0 (0.0)	3	0 (0.0)

[a]People perfoming the following function-- Laboratory Technicians, Security personnel, Drivers, Cleaners, Vendors, Veterinarians, Tradesmen, Customers. NA = Not available

tors may have been more important for exposure of the people sampled to *C. burnetii*. It may also merely demonstrate seropositivity for C. burnetii immunoglobulins rather than infection. It has been documented by others that association with farm animals, working in slaughter houses or abattoirs are risk factors for *C. burmetii* infection or clinical Q-fever (Dolcé *et al*., 2003; Zhang *et al*., 2008; Whitney *et al*., 2009). However, it has been reported that in some studies the seroprevalence of *C. burnetii* antibodies was not significantly associated with occupation or exposure to animals (Carde osa *et al*., 2006; Kilic *et al*., 2008).

It was also of interest to find that infection by *C. burnetii* in the population studied was not significantly affected by the age, sex, race or the types of duties performed on the livestock farms, abattoir or in the offices by the workers, again suggesting seropositivity rather than current infection. It is also possible the factors investigated in the current study may be more important in the epidemiology of Q-fever in the country. In agreement with the findings of the current study, others (Kelly *et al.,* 1993; Carde osa *et al.,* 2006) reported that age- or sex-related differences were not detected in a study on human Q-fever. However, there are reports of association of age and sex with C. burnetii infection by others (Cisak *et al*., 2003, Carde osa *et al*., 2006; Whitney *et al.,* 2009; Munoz-Sanz *et al*., 2007; Kilic *et al*., 2008). This study is the first to demonstrate seropositivity for *C. burnetii* immunoglobulins in the human population in Trinidad. It is imperative that local physicians be aware of the possibility of the pathogen in human infection and clinical disease and should be suspected in patients with clinical signs and symptoms associated with Q-fever, a practice which does not occur at present.

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REFERENCES

ABE T., YAMAKA K., HAYAKAWA T., FUKUDA H., ITO Y., KUME H., KOMIYA T., ISHIHARA K., HIRAI K. (2001). A seroepidemiological study of the risks of Q-fever in Japanese veterinarians. *Eur. J. Epidemiol*. **17,** 1029-1032.

ADESIYUN A.A., CAZABON E.P.I. (1996). Seroprevalence of brucellosis, Q-fever and toxoplasmosis in slaughter livestock in Trinidad. *Rev. d'Elev. et de Med. Vet. Pays Trop*. **49**, 28-30.

ARRICAU-BOUVERY N., RODOLAKIS A. (2005). Is Q-fever emerging or re- emerging zoonosis? *Vet. Res*. **36**, 327-349.

BARTOLOM J., RIQUELME E., HERN NDEZ-P REZ N., GARCIA-RUIZ S., LUJ N R., LORENTE S., MEDRANO-CALLEJAS R., CRESPO M.D. (2007). Seroepidemiology of *Coxiella burnetii* infection among blood donors in Albacete. *Enferm Infecc Microbiol Clin.* **25**, 382-386.

CARDE OSA N., SANFELIU I., FONT B., MU OZ T., NOGUERAS M., SEGURA, F. (2006). Short report: seroprevalence of human infection by *Coxiella burnetii* in Barcelona (northern Spain). *Am. J. Trop. Med. Hyg.* **75**, 33-35.

CISAK E., CHMIELEWSKA J., MACKIEWICZ B., DUTKIEWICZ J. (2003). Prevalence of antibodies to *Coxiella burnetii* among farming populations in Eastern Poland. *Ann. Agric. Environ. Med*. **10,** 265-267.

DE LOS RIOS-MARTIN R., SANZ-MORENO J.C., MARTIN-MARTINEZ F., T BAR-BETEG N M.A., CORT S-GARCIA M., ESCUDERO-NIETO R. (2006). Q-fever outbreak in an urban area following a school farm visit. *Med. Clin. (Barc)* **126**, 573-575.

DEUTZ A., FUCHS K., NOWOTNY N., AUER H., SCHULLER W., ST NZNER D., ASP CK H., KERBI U., K FER J. (2003). Seroepidemiological studies of zoonotic infections in hunters - comparative analysis with veterinarians, farmers, and abattoir workers. *Wien Klin. Wochenschr.* **115** (Suppl. 3), 61-67.

DOLCÉ P., BLANGER M.J., TUMSNOWICZ K., GAUTHIER C.P., JUTRAS P., MASS R., MONTPETIT C., BERNATCHEZ H., MCCOLL D., ARTSOB H. (2003). *Coxiella burnetii* seroprevalence of shepherds and their flocks in the lower Saint-Lawrence River region of Quebec, Canada. *Can. J. Infect. Dis*. **14**, 97-102.

DORKO E., KALINOV Z., WISSOVA T., PILICINEC E. (2008). Seroprevalence of antibodies of *Coxiella burnetii* among employees of the Veterinary University in Kosice, eastern Slovakia. *Ann Agric. Environ. Med*. **15**, 119-124.

FIELD P.R., HUNT J.G., MURPHY A.M. (1983). Detection and persistence of specific IgM antibody to *Coxiella burnetii* by enzyme-linked immunosorbent assay: a comparison with immunofluorescence and complement fixation tests. *J. Infect. Dis*. **148**, 477-487.

FIELD P.R., MITCHELL J.L., SANTIAGO A., DICKESON D.J., CHAN S.W., HO D.W., MURPHY A.M., CUZZUBBO A.J., DEVINE P.L. (2000). Comparison of a commercial enzyme-linked immunosorbent assay with immunofluorescence and complement fixation tests for detection of *Coxiella burnetii* (Q-fever) immunglobulin M. *J. Clin. Microbiol.* **38**, 1645-1847.

FOURNIER P.E., MARRIE T.J., RAOULT D. (1998). Diagnosis of Q-fever. *J. Clin. Microbiol.* **36**, 1823-1834.

GUIGNO D., COUPLAND B., SMITH E.G., FARRELL I.D., DESSELBERGER U. CAUL E.O. (1992). Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q-fever. *J. Clin. Microbiol*. **30**, 1958-1967.

KAGAWA F.T., WEHNER J.H., MOHINDRA V. (2003). Q-fever as a biological weapon. *Semin. Respir. Infect.* **18**, 183-195.

KILIC S., YILMAZ G.R., KOMIYA T., KURTOGLU Y., KARAKOC E.A. (2008). Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolis, Turkey. *New Microbiol.* **31**, 527-534.

KARAGIANNIS I., SCHIMMER B., VAN LIER A., TIMEN A., SCHNEEBERGER, P., VAN B., ROTTERDAM B., DE BRUIN A., WIJKMANS C., RIETVELD A. AND VAN DUYNHOVEN Y. (2009). Investigation of a Q-fever outbreak in a rural area of The Netherlands. *Epidemiol. Infect.* **23**, 1-12.

KAWAMOTO T., OGAWA,M., KISHIMOTO T., UCHIDA Y., KATO K. (2002). Three imported cases of acute Q fever after an inspection tour of Australia and New Zealand. *Kansenshogaku Zasshi.* **76**, 1030-1034.

KAVACOVA E., KAZAR J. (2002). Q-fever-still a query and underestimated infectious disease. *Acta Virol.* **46**, 193-210.

KELLY P.J. MATTHEWMAN L.A., MASON P.R., RAOULT D. (1993). Q-fever in Zimbabwe. A review of the disease and the results of a serosurvey of humans, cattle, goats and dogs. *South Afr. Med. J.* **83**, 21-25.

LANGLEY J.M., MARRIE T.J., LEBLANC J.C., ALMUDEVAR A., RESCH L., RAOULT D. (2003). *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. *Am. J. Obstet. Gynecol.* **189**, 228-232.

MADARIAGA M.G., REZAI K., TRENHOLME G.M., WEINSTEIN R.A. (2003). Q-fever: a biological weapon in your backyard. *Lancet Infect. Dis.* **3**, 709-721.

Maurin M., Raoult D. (1999). Q-fever. *Clin. Microbiol*. **12**, 518-553.

Mazyad S.A., Hafez A.O. (2007). Q-fever (*Coxiella burnetii*) among man and farm animals in North Sinai, Egypt. *J. Egypt Soc. Parasitol.* **37**, 135-142.

Mu oz-Sanz A., Vera A., Rodriguez Vidigal F.F. (2007). Q-fever in Extramadura: an emerging infection. *Enferm Infecc Microbiol. Clin*. **25**, 230-234.

Murphy A.M., Hunt J.G. (1981). Retrospective diagnosis of Q-fever of Q fever in a country abattoir by the use of specific IgM globulin estimations. *Med. J. Austr*. **3**, 326-327.

Stein A., Raoult D. (1992). Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. *J. Clin. Microbiol*. **30**: 2462-2466.

Santoro D., Giura R., Colombo M.C., Antonelli P., Gramegna M., Gandola O., Gridavilla G. (2004). Q-fever in Como, northern Italy. *Emerg. Infect. Dis.* **10**, 159-160.

Thibon, M., Villiers, V., Souque, P., Dautry-Varsat, A., Duquesnel, R., Ojcius, D.M. (1996). High incidence of *Coxiella burnetii* markers in a rural population in France. *Eur. J. Epidemiol.* **12**, 509-513.

Tylewska-Wierzbanowska S., Kuszewska D., Chmielewski T. (1996). Epidemics of Q-fever in Poland in 1992-1994. *Rocz. Akad. Med. Blalymst*. **41**, 123-128.

Whitney E.A., Massung R.F., Candee A.J., Ailes E.C., Myers L.M., Patterson N.E., Berkelman R.L. (2009). Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. *Clin. Infect. Dis.* **48**, 558-559.

Zhang L., Shan A., Matthew B., Yin J., Fu X., Zhang J., Lu J., Xu J., Dumier J.S. (2008). Rickettsial seroepidemiology among farm workers, Tianjin, People's Republic of China. *Emerg. Infect. Dis.* **14**, 938-940.