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SEROPREVALENCE AND OCCUPATIONAL RISK SURVEY FOR *COXIELLA BURNETII* AMONG EXPOSED WORKERS IN SICILY, SOUTHERN ITALY

CONCETTINA FENGA[1], SILVIA GANGEMI[1], ANNAMARIA DE LUCA[1], SEBASTIANO CALIMERI[2], DANIELA LO GIUDICE[2], MICHELA PUGLIESE[3], FRANCESCA LICITRA[4], ANGELA ALIBRANDI[5], and CHIARA COSTA[1]

[1] University of Messina, Messina, Italy
Occupational Medicine Section – Department of the Environment, Security, Territory, Food and Health Sciences
[2] University of Messina, Messina, Italy
Department of Biomedical Sciences and Morphological and Functional Images
[3] University of Messina, Messina, Italy
Department of Veterinary Medical Sciences
[4] Veterinary Doctor, Ragusa, Italy
[5] University of Messina, Messina, Italy
Department of Economical, Business and Environmental Sciences and Quantitative Methods

Abstract
Objectives: The aim of this survey was to assess the seroprevalence of antibodies against *Coxiella burnetii* (*C. burnetii*) in subjects at risk of exposure in Sicily, Southern Italy. **Material and Methods:** Prevalence of IgG antibodies to *C. burnetii* phase II antigens was evaluated by ELISA in a group of 140 workers at risk of exposure (38 veterinarians, 38 slaughterhouse workers, 44 livestock handlers, 20 laboratory and technical personnel) included in a medical surveillance program and in 42 control subjects. Positive samples were classified as suggestive of prior exposure to *C. burnetii*. **Results:** Antibodies against *C. burnetii* were detected in 88 out of 140 (62.9%) exposed workers and in 6 out of 42 (14.3%) subjects of the control group. The variables evaluated did not seem to have a significant effect on seropositivity to *Coxiella* with the exception of symptoms in the last 6 months preceding the survey. **Conclusions:** Our study demonstrated a high seroprevalence of *C. burnetii* in the group of exposed workers in comparison to non-exposed subjects of the control group. Clinical illness appears to be rare; nevertheless, physicians should consider Q fever in patients with compatible symptoms and occupational exposure to animals and their products. As aerosols represent the main route of infection in animals and humans, these workers are strongly advised to wear respiratory masks. In addition, occupational physicians should consider routine serologic evaluation and vaccination of occupationally exposed workers.

Key words:
Seroprevalence, *Coxiella burnetii*, Occupational hazard, Anthropozoonosis, Health surveillance, Prevention

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Corresponding author: S. Gangemi, University of Messina, Occupational Medicine Section, Department of the Environment, Security, Territory, Food and Health Sciences, Policlinico Universitario "G. Martino," Via Consolare Valeria 1, Messina 98125, Italy (e-mail: silviagangemi@hotmail.com).

INTRODUCTION

Q fever is caused by the obligate intracellular bacterium *Coxiella burnetii*. This zoonotic disease is endemic throughout the world, occurring in diverse geographic regions and climate zones.

Infected domestic animals, particularly sheep, goats, cattle and cats (but also dogs, horses, rabbits and other animals) represent the main source of infection for humans, but free-living mammals and birds are also important reservoirs [1–4].

In humans, infection is usually acquired from aerosols generated from infected placenta, body fluids or contaminated dust, after desiccation of the primary source. People can also acquire infection by ingestion of unpasteurized dairy products [5] and direct contact with material contaminated with animal excreta [6]. The clinical signs of Q fever in humans are often described as "flu-like," but the illness may vary from self-limiting non-specific fever to atypical pneumonia, endocarditis, hepatitis and neurological manifestation [7].

Q fever is also recognized as an occupational risk for people who work with animals or animal products, including veterinarians, sheep and dairy workers, meat processing plant workers, laboratory workers, hide handlers, wool spinners, taxidermists and butchers [8]. Few occupational health studies have been conducted to examine occupational exposure and rates of infection among exposed workers [9].

In Italy, a seroprevalence of Q fever in sheep, goats, cows and buffaloes has been reported, and the presence of DNA of *C. burnetii* in dogs was 31.5% in Sicilian area and 7% in Southern Italy [10,11]. Despite these findings, few data are reported in Italy on the seroprevalence of *C. burnetii* in humans [12,13]; but there are no data in occupationally exposed subjects in Sicily.

The aim of this survey was to assess the seroprevalence of antibodies against *C. burnetii* in a group of exposed workers and to identify possible risk factors.

MATERIAL AND METHODS

A group of 140 workers at risk for exposure to *C. burnetii* (38 veterinarians, 38 slaughterhouse workers, 44 livestock handlers, 20 laboratory and technical personnel) were recruited as study subjects.

All participants were Caucasians, age: 25–75 years (mean ± standard deviation: 48.45±10.81), length of employment: 28.45±9.75 years. All workers were included in a medical surveillance program for the prevention of occupational diseases and gave their informed consent before inclusion into the study.

In order to obtain sociodemographic, occupational and clinical data, all participants included in the study were interviewed by a well-trained occupational physician to fill out a questionnaire providing the following information: sex, age, residence (rural or urban); profession and occasional temporary work during which the individual came into contact with hay, soil, manure, animal skins and furs, wool, milk, meat and similar, or work in a dusty environment; use of respiratory and skin protection devices; sporting activities associated with animals; stay abroad with potential contact with animals and their products; consumption of raw meat, non-pasteurized milk and dairy products; pet ownership; contact with farm animals or pregnant dog or cat; tick bite.

Clinical history interview included questions on fever of unknown origin, flu-like symptoms during the last 6 months, rheumatic disease, diseases involving heart, liver, respiratory tract (atypical pneumonia), chronic fatigue syndrome and in females also spontaneous abortion.

After the completion of the survey, a single blood sample was collected in a 10 ml serum separator from each participant and stored at –20°C until analysis.

The control group consisted of 42 samples collected from healthy blood donors, comparable for age and sex to the study subjects, employed in public offices and in whom questionnaire indicated no known risk factor for exposure to *C. burnetii*.

Sera from workers and controls were tested by a Q fever phase II IgG ELISA kit (PanBio, Brisbane, Australia) according to the manufacturer's instructions. Microwell plates were finally read in a microtiter plate reader at a wavelength of 450 nm. Positive control, negative control and triplicate wells with calibrator control sera were used on each plate.

Data were analyzed according to the protocol provided by manufacturer. Briefly, sample absorbance was divided by the average absorbance of calibrator control wells, then multiplied by 10 to obtain "PanBio units." Samples with calculated PanBio units < 9 were considered negative, samples with PanBio units 9–11 were equivocal and with > 11 PanBio units were considered positive results. Positive samples were classified as suggestive of prior exposure to *C. burnetii*.

Logistic Regression Models [14] were evaluated to verify the possible dependence of the results (positive or negative) on some potential explicative variables, such as age, sex, work place, smoking status, intake of crude food etc. Only for subjects with symptoms we investigated the association between flu-like and not flu-like symptoms and the results, using the Log-likelihood Ratio test. Statistical analyses were performed using SPSS 11.0 for Windows package. P value below 0.05 was considered to be statistically significant.

RESULTS

Data provided by questionnaires regarding sociodemographic characteristics, occupational and clinical history are shown in Table 1. A total of 88/140 (62.9%) subjects met the criteria for seropositivity to *C. burnetii*. Six out of 42 (14.3%) subjects of the control group were positive for IgG to *C. burnetii*. Table 1 also reports the seroprevalence of phase II IgG for *C. burnetii* in the different subpopulations individuated by questionnaire submission.

Application of logistic regression models on the variables evaluated did not seem to have a significant effect on seropositivity to *Coxiella*, with the exception of symptoms in the last 6 months preceding the survey (Table 2).

Table 1. Sociodemographic and clinical characteristics and seropositivity for *C. burnetii* in study group

Variable	Respondents [n (%)]	
	total (N = 140)	with IgG for *C. burnetii*
Presence of IgG for *C. burnetii*		
negative	52 (37.1)	
positive	88 (62.9)	
Sex		
male	136 (97.1)	86 (63.2)
female	4 (2.9)	2 (50.0)
Residence		
urban	112 (80)	70 (62.5)
rural	28 (20)	18 (64.3)
Job type		
veterinary	38 (27.1)	28 (73.7)
slaughterer	38 (27.1)	28 (73.7)
animal farmer	44 (31.4)	24 (54.5)
other	20 (14.3)	8 (40.0)
occasional exposure	0 (0)	–
Workplace		
slaughterhouse	46 (32.9)	36 (78.3)
laboratory	12 (8.6)	6 (50.0)
animal farm	82 (58.6)	46 (56.1)
Smoking		
no	96 (68.6)	60 (62.5)
yes	44 (31.4)	28 (63.6)
Drug intake		
no	108 (77.1)	60 (55.5)
yes	32 (22.9)	28 (87.5)
Raw food consumption		
no	108 (77.1)	64 (59.3)
yes	32 (22.0)	24 (75.0)
Sporting activity involving animals		
no	134 (95.7)	82 (61.2)
yes	6 (4.3)	6 (100)
Exposure abroad		
no	140 (100)	88 (62.9)
yes	0 (0)	–

Table 1. Sociodemographic and clinical characteristics and seropositivity for *C. burnetii* in study group – cont.

Variable	Respondents [n (%)]	
	total (N = 140)	with IgG for *C. burnetii*
Pets		
no	103 (73.6)	61 (59.2)
yes	37 (26.4)	27 (73.0)
Non-occupational contact with animals		
no	134 (95.7)	85 (63.4)
yes	6 (4.3)	3 (50.0)
Use of respiratory protection devices		
no	140 (100)	88 (62.9)
yes	0 (0)	–
Use of gloves		
no	29 (20.7)	25 (86.2)
yes	111 (79.3)	63 (56.8)
Symptoms in the last 6 months		
no	96 (68.6)	64 (66.7)
yes	44 (31.4)	24 (54.5)

Table 2. Association between prior exposure to *C. burnetii* and presence of symptoms in the last 6 months in study group

Symptom	Respondents with IgG for *C. burnetii* [n]	
	negative	positive
Not flu-like	0	6
Flu-like	20	18
Likelihood ratio	p = 0.045	

DISCUSSION AND CONCLUSIONS

In this cross-sectional study, an overall *C. burnetii* seroprevalence of 62.9% among occupationally exposed workers was found.

Few data are reported in Italy on the seroprevalence of *C. burnetii* in humans [15–18] and little is known about occupational risk for the above mentioned zoonosis. In the current study, the 1st that demonstrated a seropositivity for *C. burnetii* in occupationally exposed workers in Sicily, there was a seroprevalence higher than that reported in a previous study conducted in Northern Italy (50%) in agricultural workers [12].

Our results are also similar to those reported in France (71%), in Netherlands (83.8%) [19] and in Slovakia (63%) [20]. International prevalence rates vary greatly. For example, published prevalence rates for cattle workers in Sweden, Austria, Bavaria and Spain vary from 10% to 30% [21–25]. Such variation may reflect either geographical differences and/or variable sensitivity of the available testing techniques (e.g., complement fixation test – CFT, ELISA, immunofluorescence assay, skin prick testing) [8].

The results of the present study also showed a positive correlation between subjects positive for IgG to *C. burnetii* and those reporting either flu-like or not flu-like symptoms in the last 6 months.

In general, clinical illness appears to be rare; nevertheless, physicians should consider Q fever in patients with compatible symptoms and occupational exposure to animals and their products [24]. The infection by *C. burnetii* in the population studied was not significantly affected by drinking unpasteurized milk, age and sex. The role of unpasteurized milk in *C. burnetii* infection is controversial. In agreement with the findings of the current study, other authors reported that the age- or sex-related differences were not detected in human Q fever. However, there are reports of associations of age and sex [26].

In our study, 70 subjects lived in rural, and 18 in urban area. Residence in rural dwellings was not a statistically significant risk factor for seropositivity; nevertheless, various authors demonstrated that subjects living in rural and sub-urban area were significantly more often seropositive than subjects living in an urban area [20,27].

The results also showed a seroprevalence rate of 78.3% among veterinarians and slaughterhouse workers and

a rate of 54.5% in animal farmers. The frequency of antibody is constant, irrespective of type of occupation. This suggests that the organism is widely dispersed in such environments and that all employees in such areas are at risk of exposure. Furthermore the questionnaire indicated that, with the exception of 8 subjects (laboratory and technical personnel), all employees with a high seroprevalence for *C. burnetii* reported a frequent contact with hay, manure, soil and straw and animal products, which is natural regarding the respective profession. Hay on the floor of animal housings is contaminated by *C. burnetii* in faeces, urine and birth products. Removing the bedding would generate aerosols containing *C. burnetii* [20]. These findings are consistent with what is known of the biology of Q fever. *C. burnetii* differs from others rickettsia crucially in its resistance to physical conditions – such as extremes of temperature and desiccation. As a consequence, infection in an animal reservoir may result in wide dissemination of the organism in the environment [28,29].

Among exposed workers who reported using various personal protective equipment, all subjects reported never wearing a mask. This could explain the high seropositivity to *C. burnetii* observed in our sample. In fact, the contamination by aerosols represents the main route of infection in animals and humans. As a result, a suitable prevention must be guaranteed and people who work in contact with animals should wear appropriate personal protective equipment, including a lab coat or equivalent and a mask, when treating animals possibly infected with *C. burnetii* and especially when assisting birth, or during the handling of birth products of animals known to be carriers of *C. burnetii*.

Public health policy (e.g. pasteurization of milk and milk products, and the provision of adequate quarantine facilities for imported animals) and farm hygienic measures (such as manure sterilization/composting and management, disinfection of the paths and ways to the pastures, air filter systems in housing and movement controls) are effective in reducing infection risk [19].

Measures that can be applied to reduce environmental transmission of *C. burnetii* from infected animals to humans also include vaccination. There is evidence that vaccine for goat and sheep can reduce the number of infections and abortions, as well as decrease the environmental transmission of the pathogen [30,31]. A vaccine against *C. burnetii* has been developed for use in humans and it is available in Italy and throughout the whole of the European Union. Preexposure vaccination of those in high-risk occupations is routinely carried out in some countries, and has been shown to be both safe and 100% effective for at least 5 years [8].

In conclusion, exposed workers should consider undergoing routine serologic follow-up as well as obey basic safety rules in order to better define the risk and if necessary take appropriate measures to prevent zoonotic diseases.

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