

SEROPREVALENCE OF Q FEVER, BRUCELLOSIS AND LEPTOSPIROSIS IN FARMERS AND AGRICULTURAL WORKERS IN BARI, SOUTHERN ITALY

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Abstract: To establish the seroprevalence of antibodies to *C. burnetii*, *Leptospira* and *Brucella* in subjects at risk of exposure, 128 workers exposed to farm animals and 280 healthy blood donors were studied. Antibodies to *C. burnetii*, *Leptospira* and *Brucella* were determined by indirect immunofluorescence assay, by microagglutination test (MAT) and by standard tube agglutination test, respectively. Of subjects exposed to farm animals 73.4% were positive for anti *C. burnetii* IgG (titer ≥ 20) compared to 13.6% of control subjects ($p < 0.0001$). The seroprevalence was found mainly among the veterinarians (100%) and the animal breeding workers (84%). The trend of percentage of positivity to antibodies to *C. burnetii* is significant only in the group of workers ≥ 70 years, when adjusted for sex and occupation. None of the subjects examined had antibodies to *Leptospira* and *Brucella*. Further improvements in the occupational hygiene of the work environment is advisable.

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INTRODUCTION

Zoonosis refers to diseases that can be passed from animals to humans by contact with infected animals or their products. Several factors exist that promote zoonotic infection in an agricultural setting such as frequent contact with domestic animals or poor animal sanitation. 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 cows with clinical signs of abortion and neonatal mortality was demonstrated [4, 5, 16]. Despite these findings, few cases of human Q fever have been reported in Italy [14]. In Italy, leptospirosis is a disease reported both in animals and humans. In fact, a study conducted in Northern and Central Italy from 1995–2001 demonstrated that 6.81% of animals

and 5.6% of humans sera scored positive to the presence of antibodies to *Leptospira* [6].

Apulia, Southern Italy, is one of the Mediterranean regions in which brucellosis is present. During the year 2005, 39 cases of human brucellosis from this region and 681 from other Italian regions were notified to the Italian Ministry of Health [17]. In the rural area of Apulia, there are several farms that produce milk for the production of typical Apulian cheeses, such as fresh soft cheese (mozzarella, ricotta).

Because in Italy studies of occupational risks for the above-mentioned diseases are scarce, we carried out a serological investigation to estimate the presence of antibodies against of *C. burnetii*, *Leptospira* and *Brucella*, in subjects at risk of exposure to these microorganisms.



Table 1. Occupational, demographic profile and seroprevalence of *C. burnetii* of study population.

Subjects	Number	Mean age		Male/Female ratio	<i>C. burnetii</i> seropositivity (titre \geq 20)	
		Male	Female		N	%
Animal breeding workers	50	46	55	36/14	42	84.0*
Agricultural/animal breeding workers	66	49	54.7	36/30	40	60.6*
Veterinarians	12	37		12/0	12	100.0*
Controls Healthy blood donors	280	49.8	52.3	142/138	38	13.6

*Significantly greater compared to blood donors ($p < 0.0001$)

POPULATION AND METHODS

Study population. The study was conducted between June 2002–July 2004. Eligible subjects were workers and veterinarians working in rural areas near Bari, Southern Italy. The control group consisted of 280 healthy blood donors (students, medical and technical staff, nurses, teachers: 142 males aged 18–73, mean age 49.8, and 138 females aged 28–71, mean age 52.3). Workers were approached one by one and asked to volunteer for the study and all were informed of its purpose. A standard questionnaire was completed by direct interview performed by trained interviewers to obtain individual socio-demographic data regarding age, gender, residence, economic activity of the workers exposed to animals.

These subjects include 84 males aged 20–70 (mean age 46.3 years) and 44 females, aged 33–76 (mean age 53.8 years). Blood was taken, kept on ice during transport to the laboratory, and centrifuged within 6 hours. Sera were stored at -20°C in aliquots until tested.

Serology. IgG and IgM antibodies to *C. burnetii* were determined by the indirect immunofluorescence assay (*Coxiella burnetii*-Spot IF BioMerièux, Milan, Italy). The assay was performed according to manufacturer's instructions. Sera were initially diluted 1 : 20 in PBS, pH 7.4 for antibody screening. Where the screening test was found to be positive, further serial twofold dilutions to 1 : 320 were tested. Specimens were considered reactive if typical fluorescence was present at a dilution of 1 : 20 or more. Sera positive for *C. burnetii* were also tested for antibodies against *B. henselae* (*Bartonella* IgG IFA, Focus Diagnostics, USA) and *Chlamydomphila pneumoniae* (*Chlamydia* MIF IgG, Focus Diagnostics, USA) according to manufacturer's instructions.

Leptospiral antibodies were tested by the microscopic agglutination test (MAT). Suspensions of 20 strains (belonging to 19 serovars of *Leptospira interrogans sensu lato*, including all the serovars known to circulate in Italy, were used as antigens). The strains were provided by the National Centre for Leptospirosis, Istituto Superiore di Sanità, Rome, Italy. Each sample of serum was tested with each of the 20 serovars at a 1 : 50 dilution according to

procedure previously reported [7]. The sera were considered positive if 50% or more of leptospire were agglutinated.

The standard tube agglutination test using standardized commercial *Brucella abortus* antigen (Tube Test *Brucella*, Sclavo, Siena, Italy) was used to detect antibodies to *Brucella*. The test was performed following the manufacturer's specifications, using a screening serum dilution of 1 : 50.

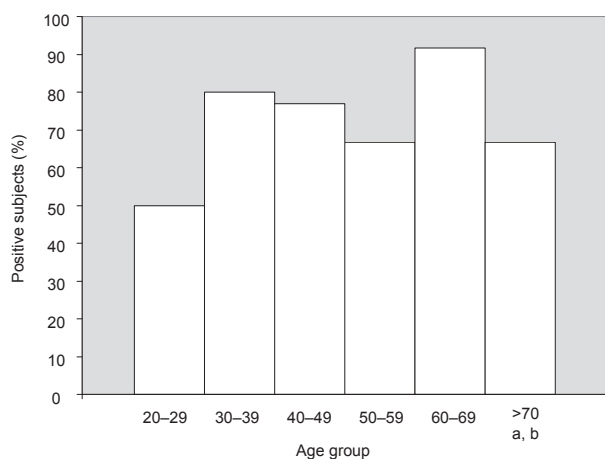
Statistical analysis. Categorical variables (sex, occupational exposure and age class) are described as count and percentage. The Fisher Exact Test was used to compare the independent groups. Trend in antibody titer was evaluated by means of the Cochran-Armitage test for trend when appropriate.

A 2-sided p value < 0.05 was considered statistically significant. To account for multiple comparisons performed in the analysis, p values were adjusted using permutational tests. All data were analysed using the statistical package SAS 9.1, PC version.

RESULTS

Demographic, occupational data and the seroprevalence of *C. burnetii* in the 408 subjects studied are shown in Table 1.

In the group of workers at risk, the male/female ratio was 84/44 because in Apulia animal breeding and mixed agriculture/animal breeding are mainly a male occupation. Serological testing revealed that 73.4% (94/128) of subjects exposed to farm animals (cattle and sheep) were positive for anti *C. burnetii* IgG (titer \geq 20) compared to 13.6% (38/280) of control subjects ($p < 0.0001$). In particular, the IgG seroprevalence for *C. burnetii* was 84.0% (42/50) in the group of animal breeding workers, 60.6% (40/66) in that of agriculture/animal breeding, and 100% in the group of veterinarians. Subjects in direct contact with animals are more at risk for exposure to *C. burnetii* compared to controls and those in contact with several kinds of animals, such as veterinarians who are even more exposed in comparison to animal breeding workers, and both of them are more exposed to those subjects dedicated to agricultural and farming. Of the 94 workers who scored



- a. The trend of percentage of positivity is significant when adjusted for sex ($p = 0.0005$) and for type of job ($p = 0.04$)
 b. Workers of ≥ 70 years of age have higher antibody titer (1 : 160) in comparison to all other age groups $p = 0.0145$

Figure 1. *Coxiella burnetii* IgG seroprevalence in workers at risk depending on age.

positive for IgG to *C. burnetii*, 64 (68.1%) were males and 30 (31.9%) were female, and a high antibody titer was observed in males compared to females for antibody titer of 80 ($p = 0.048$) and for antibody titer of 160 ($p = 0.009$). The trend of percentage of subjects scoring positive at high antibody titers is statistically significant both in males and females when adjusted for age ($p < 0.001$) and for type of job ($p = 0.091$). In particular, an antibody titer of 160 was found in 8 subjects (all males), an antibody titer of 80 in 4 men and 4 women, 28 were reactive at titer of 40, and 48 scored positive at titer of 20. All 38 sera of the control group scored positive at titer of 20. Both groups of subjects exposed to animals and control subjects scored negative for IgM to *C. burnetii* and for antibodies to *Brucella* and *Leptospira*. The 128 serum samples of the risk group were divided into 6 age groups each containing from 14–28 samples. The prevalence of IgG antibodies to *C. burnetii* at titer ≥ 20 in each age group of the risk group was as follows: 20–29 years old – 50%; 30–39 years old – 80%; 40–49 years old – 76.9%; 50–59 years old – 66.7%; 60–69 years old – 91.7%; ≥ 70 years old – 66.7%.

The trend of percentage of positivity to antibodies to *C. burnetii* (antibody titer ≥ 20 and ≤ 160) is significant only in the group of workers in the age group ≥ 70 when adjusted for sex ($p = 0.0005$) and/or for type of job ($p = 0.04$). In addition, the risk group of ≥ 70 years of age have a statistically higher antibody titer (160) when compared to all the remaining groups ($p = 0.0145$). All these data suggest that prolonged contact with animals represents a real risk factor for *C. burnetii* infection (Fig. 1). Control subjects have a significantly low seroprevalence for *C. burnetii* also with aging, and no trend in the percentage of *C. burnetii* seropositive subjects was observed with respect to aging also when adjusted for age and sex ($p = 0.25$). Because the cross reaction with other bacteria are described in literature [12, 24]

all sera scoring positive for *C. burnetii* were tested for the presence of antibodies to *B. henselae* and *C. pneumoniae* by IF. Only 2 of the risk group scored positive for *C. pneumoniae* (2 subjects in the ≥ 70 years age group) at titer of 64; none scored positive for *B. henselae*. In the group of controls, 14 subjects scored positive for *C. pneumoniae* at titer between 32 and 64.

DISCUSSION

People working with animals or their products may contract several infections. In a study conducted in the United Kingdom, 105 out of 385 farm workers were found seropositive to *C. burnetii*: no association between seroprevalence and age was found, and the time of contact with animals seemed more important than exposure to any specific animal [21]. In a study conducted in Eastern Poland, antibodies to *C. burnetii* phase II antigen were found in about 18% of farmers, but not in any blood donors living in an urban environment [9]. In the Slovak republic, *C. burnetii* phase I and phase II antibodies were detected in 38% and 63% respectively of employees (of the Veterinary University) [11]. In Sweden, a seroprevalence of 24–30% in sheep farmers and of 12% in veterinarians has been found in comparison to 5–17% of non-risk groups [13].

In Italy, a survey carried out throughout the Campania area has shown a seroprevalence of Q fever of 11.8% within sheep, 6.3% within goats, 14% in cattle and 7% in dogs. In addition, 77 out of 305 animals were found positive for *C. burnetii* by PCR [5]. A seroprevalence of about 8% was found in cattle from an Apenninic area of the Emilia-Romagna Region [15].

Recently in Sardinia, Italy, specific IgG antibodies have been detected in 38% and 47% of sheep and goat herds, and *C. burnetii* was found by PCR in 10% and 6% of ovine and caprine foetuses, respectively [16]. In Northern Italy, 44.9% out of cattle which experienced abortion were seropositive for *C. burnetii* [2]. In a serological survey in the province of Bologna, Northern Italy, 0.87% of dogs were found to have antibodies to *C. burnetii* and 35% of dog owners were also found seropositive [1]. The seroprevalence for *C. burnetii* in dogs was 31.5% in Sicilian areas [22] and 7% in Southern Italy [5]. Despite the presence of *C. burnetii* among animals, few data are reported in Italy on the incidence of *C. burnetii* pneumonia and on the seroprevalence of *C. burnetii* in humans. There was a Q fever outbreak in Northern Italy involving 58 subjects after 3 flocks of sheep had passed through the outbreak area, and later these sheep were shown to be infected [14]. Interestingly, a seroprevalence of 2.8% was found in forestry rangers in North-Eastern Italian regions [6] and ticks are also now considered a reservoir of *C. burnetii* and responsible for the spread of infection to wild and domestic animals [18].

All these data suggest that *C. burnetii* is widespread in several areas of Italy, both in domestic animals and in subjects exposed to animals or ticks.

Q fever is present also in our area, Apulia, as demonstrated by PCR positivity for *C. burnetii* in 18.9% of aborted fetuses from domestic ruminants [19]. Nevertheless, cases of human Q fever in our area have not been reported. Our laboratory serves patients admitted to the Policlinico of Bari, a large hospital in Apulia (1.200 beds). During a period of 6 years we examined for *C. burnetii* infection 398 patients with lower respiratory infections, and a current infection has never been determined.

It may be possible that Q fever in our area is underestimated for the lack of knowledge of diffusion of this infection among animals in our farms, because the detection of antibodies against *C. burnetii* is not included in the panel of indirect diagnosis of respiratory pathogens in many laboratories, and finally because of the fact that many subjects may have flu-like or no symptoms. Our study has demonstrated a high seroprevalence of *C. burnetii* in the group of workers exposed to animal in comparison to non-exposed subjects of the control group.

On the contrary, none of the workers exposed to animals and none of the control group had antibodies to *Leptospira*. In animals living in Northern and Central Italy, seropositivity to *Leptospira* has been reported in ovine, horses, swine, dogs and cattle, and 5.60% of human sera examined scored positive [6]. In a seroepidemiological survey on leptospirosis carried out in 1996 on 75 workers on swine farms in province of Mantua, a seroprevalence of 32% for serovars (i.e. *pomona*, *australis*, *tarassovi*), widespread in pigs, was found [25]. In the 3-year period (1994–1996), 222 reports of human cases of leptospirosis from 16 regions of Italy were received by the Italian Ministry of Health [17]. Of these cases, 18.2% were by direct contact with animals swine, in particular, while in our area pig raising is not popular. In a previous study on 2,534 (apparently in good health) Italian subjects, 305 were found to be MAT reactive to *Leptospira* serovars. Residence in rural dwellings and contact with animals were statistically significant risk factors for seropositive subjects. In the Bari area, no seroprevalence for leptospirosis has been previously reported [3], but there are no data on the seroprevalence of *Leptospira* in animals. In a period of 6 years we diagnosed leptospirosis in 3 patients with fever, jaundice and renal failure. One patient had drunk river water, the other 2 were due to work exposure (in agriculture). Few data are available on the seroprevalence of brucellosis in Italy, although an overall prevalence of antibodies of 3.1% was recorded in the general population in 2 regions of Southern Italy, but was not significantly associated with occupation [23]. In our area, Apulia, 166 cases of brucellosis were notified to the Osservatorio Epidemiologico Regionale in the years 2001–2005. The incidence of human brucellosis in Italy during 1990–2002, decreasing from 2.7 cases/100,000 inhabitants in 1990 to 1.4 cases/100,000 inhabitants in 2002. Occupational exposure may not be the primary route of infection and in the main could be related to exposure through food [10]. In conclusion, further improvements in

the occupational hygiene of the work environment to prevent the exposure to dusts containing *C. burnetii*, and an increased awareness of the presence of this microorganism in cattle and sheep is advisable. All laboratories in our area must include serology for *C. burnetii* in the panel of diagnostic microbiology of lower respiratory tract infections.

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