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Short Communication

Occupationally Acquired Q Fever in Shepherds and Sheep Milk Cheesemakers

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Abstract: Q fever is a zoonosis caused by *Coxiella burnetii*, and transmission to humans is often associated with contact with ovine and caprine livestock. Those exposed to sheep are particularly at high risk of infection. Recent studies show that Q fever is increasing in sheep farms in Portugal raising alerts on spillover to humans. We detected anti-*C. burnetii* IgG in shepherds and sheep milk cheesemakers (27 [28.1%] in a total of 96; 95% confidence interval [CI] 19.4–38.2%) and in controls (21 [8.1%] in a total of 260; 95% CI 5.1–12.1%), pointing to an increased risk of *C. burnetii* infection ($P = 0.0001$), with an odds ratio for *anti-C. burnetii* of 4.45 (95% CI 2.4–8.4%; $P = 0.0001$), in individuals with occupational contact with sheep in Portugal.

Keywords: *Coxiella burnetii*, Portugal, Occupational exposure

Q fever is a zoonotic disease with an almost worldwide occurrence caused by *Coxiella burnetii*, a nonmotile Gram-negative and obligate intracellular bacterium that has the ability to subsist several weeks under environmental stresses (Maurin and Raoult 1999; Angelakis and Raoult 2010; Mori and Roest 2018). Transmission to humans is often associated with abortive episodes in domestic ruminants, particularly in ovine and caprine livestock, mainly through inhalation of contaminated aerosols but also by the alimentary route and through the bite of ticks (Maurin and Raoult 1999; Angelakis and Raoult 2010; Mori and Roest 2018). Thus, populations that are in particularly high risk often include individuals with occupational contact with animals, such as workers in tanneries, abattoirs, fur and meat industries, on agricultural farms, and in veterinary profession (Fenga et al. 2015; Szymańska-Czerwińska et al. 2015). Q fever in humans may present a vast clinical range, from asymptomatic seroconversion, to acute (mild, flu-like to severe pneumonia) and chronic disease (endocarditis manifestation), also causing death (Angelakis and Raoult 2010). Little is known regarding *C. burnetii* circulation in Portugal, both in animals and in humans. Recent longitudinal studies in

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Portugal have provided data supporting the hypothesis that Q fever is widely present and currently increasing in small domestic ruminants of central Portugal (Cruz et al. 2018a, b). On the other hand, little is known regarding *C. burnetii* circulation in humans in Portugal. A 2012 retrospective study in Portugal describing epidemiological and clinical features of 32 hospitalized patients found that five of them had professions with higher contact with animals (one farmer, three rural workers and one horse handler), suggesting an association between Q fever and occupation health (Palmela et al. 2012).

In the present study, a cross-sectional serological evaluation of *C. burnetii* antibodies in shepherds and sheep milk cheesemakers (SSMCs) of central Portugal and general population controls (matched by age, sex and region) was performed to assess the occupational risk of *C. burnetii* infection in shepherds and cheesemakers in Portugal.

Ethics permission was obtained from a national ethics board (University of Trás-os-Montes e Alto Douro (DOC 15/CE/2017—under the project "*Coxiella burnetii* surveillance in small ruminants of Portugal and the zoonotic impact to humans occupationally exposed"), and all participants were informed about the study goals and procedures. All individuals agreeing to participate have provided informed consent, and study methodologies were conducted following the recommendations described in the Declaration of Helsinki (World Medical Association 2014). Shepherds and sheep milk cheesemakers from the "Serra da Estrela" sheep breed were selected if registered in the local "Serra da Estrela" Sheep Breed Association (ANCOSE). A list of SSMCs was retrieved from ANCOSE (3295 shepherds and 156 cheesemakers), and individuals were contacted and asked to provide an anonymized blood sample. In total, 96 blood samples from SSMCs were collected in a 2-month period, between November and December 2017. From these, 21 samples were from shepherds (18 male and 3 female; mean age = 57.6) and 75 samples were from sheep cheesemakers (44 male and 31 female; mean age = 56.3). Sera from anonymous volunteers ($n = 260$) matched with the SSMCs by sex, region (all from the NUTSII center region, the same as "Serra da Estrela") and age (within 5-year age-group) were used as control group. After collection, sera were separated and stored at − 20°C within 24 h. All sera ($n = 356$; 96 from SSMCs and 260 from controls) were tested for anti-*C. burnetii* IgG using the commercial enzyme immunoassay SERION ELISA classic *Coxiella burnetii* Phase II IgG (Virion/Serion, Würzburg, Germany) according to the manufacturer's instructions. This ELISA delivers quantitative data by using one-point calibration of a standard curve with values < 20 U/mL considered negative and > 30 U/mL positive and has demonstrated specificity and sensitivity of 97% and 89%, respectively (Sanz et al. 2006). Statistical analysis and data processing were done using dedicated statistical software GraphPad Prism (Version 5.04; GraphPad Software Inc., CA, USA). Chi-square test for homogeneity of proportions was applied to examine on possible significant differences between anti-*C. burnetii* IgG seroprevalences. Seropositivities between SSMCs and controls were compared by calculating odds ratio. Only P values < 0.05 were considered statistically significant.

We have detected anti-*C. burnetii* IgG in 27 (28.1%; 95% confidence interval [CI]: 19.4–38.2%) of the 96 SSMCs and in 21 (8.1%; 95% CI: 5.1–12.1%) of the 260 controls. Anti-*C. burnetii* IgG was found to be significantly higher in SSMCs than in population controls X^2 (1, $n = 356$) = 24.16, $P = 0.0001$, with an SSMCs odds ratio for *anti-C. burnetii* of 4.45 (95% CI 2.4–8.4%; $P = 0.0001$) pointing to an increased risk of *C. burnetii* infection in individuals with occupational contact with sheep. Anti-*C. burnetii* IgG antibodies were found in four of the 21 shepherds (19.0%; 95% CI 5.4–41.9%) and in 23 of the 75 sheep milk cheesemakers (30.7%; 95% CI 20.5–42.4%); however, this difference was not found to be statistically significant. The lack of difference in anti-*C. burnetii* seroprevalences of shepherds and cheesemakers could be due to the low sample size. Nonetheless, the high seroprevalences found in both shepherds and cheesemakers point to higher exposures to *C. burnetii*.

This study presents novel seroprevalence data for *C. burnetii* among SSMCs in Portugal and indicates substantial zoonotic transmission in a country where a strong tradition of sheep farming exists. In particular, this study showed that SSMCs are 4.45 more likely to be positive for anti-*C. burnetii* IgG than population controls (odds ratio for *anti-C. burnetii* of 4.45), pointing to an increased risk of *C. burnetii* infection in SSMCs.

Higher anti-*C. burnetii* IgG has been reported in individuals with occupational contact with small ruminants (agricultural workers, farmers, veterinarians) with seroprevalence values ranging from 8.5% to 62.9% among exposed workers (Reid and Malone 2004; Whitney et al. 2009; Fenga et al. 2015; Meadows et al. 2017), linking the frequent and close contact with sheep with *C. burnetii* in humans. Interestingly, *C. burnetii* infection in sheep of Portugal seems to be frequent and has recently been reported to be increasing, presenting a risk for zoonotic spill-over (Cruz et al. 2018a, b), and the proximity to small ruminants has been suggested as a contributing factor to the endemicity of Q fever in Portugal (Alves et al. 2016)

The infection routes to SSMCs are yet to be clarified; however, it is tempting to hypothesize that shepherds have a higher exposure to *C. burnetii* through direct contact with animals, stools, urine, aerosols or abortions caused by *C. burnetii*. On the other hand, cheesemakers are working exclusively with milk; hence, exposure could be related to the handling of sheep milk during the process of cheesemaking or inhalation during processes that produce aerosols. A recent study has detected viable *C. burnetii* in cheese from Italy (Barandika et al. 2019), supporting our results that cheesemakers might have been exposed to *C. burnetii* through contaminated sheep milk. Contaminated dust particles and aerosolization of contaminated body fluids are generally associated with human infection, as well as consumption of unpasteurized dairy products and direct contact with fecally contaminated fomites (Babudieri 1959; Oren et al. 2005). If any of these routes of transmission alone or combined have contributed to the increased anti-*C. burnetii* IgG in SSMCs of central Portugal, it is not known and deserves further exploration.

Noteworthy, sheep farming is widespread in Portugal and sheep meat and milk are an integral part of the Portuguese diet. Hence, given the results of the present study, alerts have to be made to increase surveillance in a One Health approach, not only in small ruminants but also in SSMCs, so as to mitigate the impact of Q fever.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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