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BRIEF REPORT

Seroprevalence of *Coxiella burnetii* (Q fever) Exposure in Humans on Reunion Island

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After the documentation of sporadic cases of Q fever endocarditis, we conducted a serosurvey to assess *Coxiella burnetii* exposure on Reunion Island. Two hundred forty-one stored frozen human sera were analyzed using an immunofluorescence assay. The weighted seroprevalence of Q fever was of 6.81% (95% confidence interval, 4.02%–9.59%). Despite the absence of infection in youths <20 years of age, exposure was not driven by age or by gender. There was a spatial disparity in exposure across the island, with higher prevalence being reported in regions where ruminant farms are present. The seroprevalence pattern suggests that Q fever is endemic on Reunion Island.

Keywords.: *Coxiella burnetii*; general population; immunofluorescence; prevalence; Q fever; serology; seroepidemiologic study; serosurvey; stored frozen serum; zoonosis.

Q fever is a widespread zoonosis of public health importance caused by *Coxiella burnetii*, an obligate gram-negative intracellular bacterium [1]. *Coxiella burnetii* has a wide host range, including mammals, birds, and probably arthropods (mainly ticks). Ruminants (cattle, goats, and sheep) are the primary sources of human infection. Humans are mainly infected either through aerosolization or manipulation of infected birth products from farm ruminants, or by direct contact with bodily fluids of infected animals. With the exception of French Guiana [2], a hyperendemic area where transmission has not been linked with ruminants, the burden of Q fever is far higher in rearing countries, especially in regions of traditional pastures [1].

The first identifications of human clinical cases in the Indian Ocean date back to the late 1950s [3]. On Reunion Island, the circulation of *C. burnetii* has been confirmed for about 30 years, with several epizootic events in cattle during the nineties, and is the subject of an intensive veterinary surveillance. In 2011–2012, the seropositivity rate was 11.8% in cattle, 1.4% in sheep, and 13.4% in goats, whereas their vaginal shedding of *C. burnetii* was 0.8%, 4.4%, and 20.1%, respectively [4].

After the documentation of sporadic cases of Q fever endocarditis and *C. burnetii*–related interstitial lung disease in the South Western Indian Ocean [5, 6], we conducted a serosurvey using stored human sera frozen in 2009 to assess the magnitude of *Coxiella burnetii* exposure on Reunion Island.

METHODS

Setting and Population

La Réunion is a small tropical island (2512 km^2) located in the southwestern Indian Ocean, 700 km east of Madagascar. Its volcanic landscape gives rise to contrasted climates with a mountainous center separating a humid windward east coast from a dry leeward west coast. The ruminant population comprises roughly 40 000 cattle, 30 000 goats, and 2000 sheep, mainly based in the West and South microregions [4]. In 2009, most of the 816 000 inhabitants lived in the coastal area where the cities lie. The structure by age, gender, and microregion (ie, 4 administrative regional subdivisions) of the general population is presented in Supplementary Table 1.

The study population was a subset of the CoPanFlu-RUN cohort, dedicated to the 2009 H1N1 pandemic flu [7]. Age, gender, and place of residence were retrieved for each participant and compared with the general population (Supplementary Table 1).

Ethical Considerations

CoPanFlu-RUN was funded by the French National Institute of Health and Medical Research (INSERM). The study was conducted in accordance with the Declaration of Helsinki and the French law for biomedical research (Nu ID RCB AFSSAPS: 2009-A00689-48). It was approved by the Ethics Committee of

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the University of Bordeaux, which allowed the reuse of serum samples for investigation of other infectious diseases. Written consent was obtained from all adult participants or from the legal representatives of all underage participants.

Serology

Two hundred forty-one sera were tested using an indirect immunofluorescence assay with commercially available *C. burnetii* antigens (I+II IFA IgG/IgM/IgA, Vircell, Grenade, Spain). Seropositivity for *C. burnetii* antibodies was defined by a phase 2 IgG titer ≥1:64. Further dilutions (<1:128 and ≥1:256) were chosen as conservative thresholds to fulfill the National Reference Centre requirements and minimize the number of false positives in order to provide public health stakeholders with more stringent definitions of exposure [8]. Phase 2 IgM and phase 1 IgG antibodies were not considered discriminant for Q fever diagnosis in this sample. Sera were also checked for typhus group (TGR) and spotted fever group *Rickettsiae* (SGFR).

Statistical Analysis

Statistical analysis was performed using Stata 14.2 (StataCorp, College Station, TX). The seroprevalence rates were weighted to account for the discrepancy between the CoPanFlu-RUN subset and the general population. Associations between *C. burnetii*–seropositive specimens and age, gender, and geographic subdivision were determined using weighted chi-square tests. Survey-adjusted log-binomial regression models were used to estimate prevalence proportion ratios (PPRs) and 95% confidence intervals (95% CIs). A P value < .05 was considered significant.

RESULTS

The seropositivity rate of Q fever, as defined by a phase 2 IgG titer ≥1:64, was 8.71% (21/241), and the weighted seroprevalence was 6.81% (95% CI, 4.02%–9.59%). These figures were, respectively, 7.47% (18/241) and 5.38% (95% CI, 2.88%–7.87%) using the more stringent titer ≥1:128, and 4.56% (11/241) and 3.94% (95% CI, 1.79%–6.09%) using the more conservative titer ≥1:256. In detail, participants with high IgG2 titers (≥1:256) accounted for 52% (11/21) of all seropositive subjects, participants with intermediate titers (≥1:128) represented at least a third (7/21), and those with low titers (≥1:64) represented 14.2% (3/21), respectively.

Seropositivity for Q fever was not observed in youths (<20 years of age), although prevalence did not progress with age through adulthood (Table 1; Supplementary Table 2). Participants living in the dry "leeward" west and the south microregions were more likely to show exposure to *C. burnetii* than those living in the humid "windward" east microregion (adjusted PPR, 4.70 and 5.82, respectively). They also presented higher rates of intermediate to high IgG2 titers (adjusted PPR, 16.24 and 20.10, respectively).

At the cutoff value of 1:64, of the 68 sera reactive with *Rickettsiaceae*, 2 sera reacted to both *C. burnetii* and TGR, 3 to both *C. burnetii* and SFGR, and 2 to *C. burnetii*, TGR, and SFGR. Cross-reactions represented 33.3% (7/21) of the sera reactive with *C. burnetii*. Of the 7 participants with cross-reactive sera, 5 lived in the western microregion. After exclusion of cross-reactions, the seropositivity rate was 5.81% (14/241) and the weighted seroprevalence was 4.73% (95% CI, 4.17%–5.29%).

Table 1. Factors Independently Associated With Q Fever at a Cutoff of 1:64 or 1:128 in Multivariate Analysis, Reunion Island, 2009 (n = 241)

	Coxiella burnetii IgG ≥64				*Coxiella burnetii* IgG ≥128			
Variables	No.	%	Adjusted PPR	95% CI	No.	%	Adjusted PPR	95% CI
Age, y								
<20	0/24	0.00	NA	-	0/24	0.00	NA	
20–39	6/77	7.98	1.37	0.41–4.55	5/77	6.05	1.01	0.28–3.54
40–59	10/102	8.33	1.48	0.47–4.65	8102	6.48	1.11	0.34–3.59
≥60	5/38	6.10	1	-	5/38	6.10	1	-
Gender								
Female	10/136	6.41	1	-	7/136	4.66	1	-
Male	11/105	8.55	1.06	0.45–2.45	11/105	8.55	1.42	0.56–3.59
Microregion**								
North	0/17	0.00	NA	-	0/17	0.00	NA	-
South	8/80	7.39	4.70	0.83–26.66	7/80	6.11	16.24*	1.89–139.24
West	11/78	13.59	5.82*	1.06–31.78	10/78	11.21	20.10**	2.43–165.86
East	2/66	2.14	1		1/66	0.05	1	-

Data are numbers, weighted seropositive rates (%), adjusted prevalence proportion ratios, and 95% confidence intervals. P values linked to variable names are given for overall design-based Pearson chi^2 tests. P values linked to PPRs are given for within-each-category Wald tests.

Abbreviations: CI, confidence interval; NA, not assessed; PPR, prevalence proportion ratio.

*P < .05; **P < .01.

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DISCUSSION

We provide herein the first serological evidence of a low to moderate exposure to *Coxiella burnetii* of the Reunion Island human population.

Q fever has been rarely reported for the 3 last decades on the island, albeit *C. burnetii* circulation has been recently documented in farm ruminants [4]. The occurrence of persistent Q fever of autochthonous origin [5, 6] and the low to moderate seroprevalence reported herein suggest that Q fever is weakly endemic on the island, as compared with other endemic countries such as Northern Ireland or Turkey, which exhibit higher seroprevalence averaging 13% [9, 10]. Interestingly, the weighted seroprevalence observed in Reunion Island falls within the range reported in affected areas of the Netherlands postepidemic [11]. In the absence of clinical data mentioning a history of Q fever in CoPanFlu-RUN questionnaires, and with none of the Q fever cases diagnosed at the hospital enrolled in the cohort, the relative importance of highly positive IgG2 titers found in our study might suggest that a significant proportion of Q fever cases have been asymptomatic or paucisymptomatic or have escaped medical attention in the primary care setting [1, 12]. This raises questions about the virulence of *C. burnetii* in our setting and highlights the importance of genotyping the circulating strains on Reunion Island to assess how they may drive the antibody response and influence seroprevalence.

It is worth noting that the seropositive participants and infected patients predominantly lived in the west and south of the island, where the farms are [4]. In the absence of significant associations with age (beyond the relative protection of children) and gender that might suggest an occupational exposure, this unique risk factor is more compatible with an airborne (windborne) transmission from ruminant farms [13]. Though our data have not been spatialized, they are nevertheless in accordance with our current knowledge about this transmission pathway. In the *Bouches-du-Rhone* department of France, for example, a seasonality and a strong correlation between sheep densities, wind speed, and Q fever cases have been identified [14]. In the Netherlands, during epidemics, a remarkable spatial dose–response relationship was found in relatively small livestock-dense areas, the seroprevalence increasing with decreasing distances to the closest goat farm that was infected [11].

This study has potential limitations. First, despite weighting our data to limit resampling bias, a bias might still occur, as the sera were originally sampled for another purpose. Second, a third (7/21) of the sera reactive with *C. burnetii* also reacted with SFGR or TGR or both, and those cross-reactions may result in Q fever seroprevalence overestimation. However, 71% of the participants showing cross-reactions lived in the western dryer part of the island, which is also the habitat of *Xenopsylla cheopis*, the rat flea, vector of murine typhus [15, 16]. This suggests that cross-reactions might signal previous exposure to multiple pathogens rather than false positives, which is more in keeping with seroprevalence figures.

Finally, our findings support the need for a larger-scale seroepidemiologic study on Reunion Island aimed at assessing the prevalence of Q fever with more accuracy and at better understanding the transmission pathways of *C. burnetii*. The collection of detailed clinical data will be needed to identify symptomatic vs asymptomatic infections. In the meantime, special attention should be paid to occupations at risk, unprotected ritual slaughtering practices that are popular on the island, and possible high exposure of people living near ruminant farms.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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