

## Seroprevalence of *Coxiella burnetii* antibodies in human immunodeficiency virus-positive patients in Jacarepaguá, Rio de Janeiro, Brazil

C. C. Lamas<sup>1</sup>, T. Rozental<sup>1</sup>, M. N. Bóia<sup>2</sup>, A. R. M. Favacho<sup>1</sup>, A. H. Kirsten<sup>1</sup>, A. P. M. da Silva<sup>3</sup> and E. R. S. de Lemos<sup>1</sup>

<sup>1</sup>Laboratório de Hantavírus e Rickettsioses, Instituto Oswaldo Cruz, FIOCRUZ, <sup>2</sup>Laboratório de Doenças Parasitárias, Instituto Oswaldo Cruz, FIOCRUZ and <sup>3</sup>Hospital Municipal Raphael de Paula Souza, Rio de Janeiro, Brazil

### INTRODUCTION

*Coxiella burnetii* infection is a worldwide zoonosis; the sporulation capacity and high infectivity of *Coxiella* explain its ubiquity. Human infection is mainly related to exposure to farm animals; urban outbreaks have implicated cats, dogs and rabbits. There is an association between the chronic form of the infection and immunosuppression, including human immunodeficiency virus (HIV) seropositivity, although the prevalence and severity of illness in this group remains controversial [1]. Recent publications have reinforced the role of pregnancy in perpetuating infection [2,3].

The aim of this study was to describe the risk factors for acquisition of infection of *C. burnetii*, to determine the seroprevalence, and to identify the presence of DNA in blood samples of HIV-positive patients in a semi-rural area in the city of Rio de Janeiro.

### METHODS

This was a prospective study of HIV-positive individuals followed up in an AIDS clinic in HMRPS, Jacarepaguá, Rio de Janeiro. Patients were interviewed and had peripheral blood collected after informed consent on their routine consultation day. Active disease and viral loads above 100 000 copies/mL (NASBA) were exclusion criteria. IFI assays using PANBIO slides were performed for detection of IgG antibodies; titres  $\geq 1$ :64 were considered to be reactive. DNA extraction from blood clots was performed using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's

instructions. The extract was screened for the presence of *Coxiella* DNA by PCR using one set of primers, QBT-1/QBT-2 (QBT-1, 5'-TATGTATCCACCGTAGCCAGTC-3'; and QBT-2, 5'-CCCAACAACACCTCCTTATTC-3') (hspAB, 687 bp)[4]. PCR conditions consisted of an initial DNA denaturation and hot start at 95°C for 5 min, followed by 40 consecutive cycles of 30 s of denaturation at 95°C, primer annealing at 60°C for 30 s, extension at 72°C for 1 min, and a 5-min extension at 72°C. Control reactions were always the last to be set up in PCR strips and were the last to be loaded onto gels. Each PCR reaction contained 0.3  $\mu$ L of Platinum Taq DNA Polymerase (5 units) (Invitrogen, Carlsbad, CA, USA), 10.5  $\mu$ L of nuclease-free water, 1.25  $\mu$ L of each primer (1 pmol), and 8  $\mu$ L of DNA extract in water. PCR products were separated by electrophoresis on 1% agarose gels, and visualized under ultraviolet light with ethidium bromide.

### RESULTS

One hundred and twenty-five patients were included, aged  $37.1 \pm 10.1$  years; 64 were females. None used intravenous drugs; 20% inhaled cocaine. Ninety-four of 125 (75.2%) were on antiretroviral therapy. Mean most recent CD4 count was 351 to 500  $\text{mm}^3$ . Contact with cats and dogs were reported by 60/125 (48.0%) and 98/125 (78.4%). The frequency of watching and/or helping with animal birth was similar between men and women. Two of 64 women (3.1%) and 10/60 (16.7%) men milked animals, and there was a tendency towards statistical significance in this exposure by the Fisher's correction of chi-square ( $p$  0.06). Exposure to animal hide and wool was infrequent in both sexes. *C. burnetii* phase I antibodies were found in four of 125 (3.2%) samples, and in four of 64 (6.3%) females. Patient 3 had attended a dog birth 3–5 years previously. No DNA amplification was obtained from these patients' clots (Table 1).

Obstetric history showed that patient 1 had four recent pregnancies (childbirths 5, 4 and 3 years previously, and one miscarriage),

Corresponding author and reprint requests: C. Lamas, Laboratório de Hantavírus e Rickettsioses, Pavilhão Hélio e Peggy Pereira, 1º andar, sala B115, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, Mangueiras, Rio de Janeiro, RJ-Brazil 21045-900.

E-mail: [cristianelamas@gmail.com](mailto:cristianelamas@gmail.com)

No conflicts of interest declared.

**Table 1.** Characteristics of patients with *Coxiella burnetii* antibodies

Age	Sex	<i>C. burnetii</i> antibody dilution titres	Cat exposure	Dog exposure	Potential exposure to <i>C. burnetii</i> <sup>a</sup>
25	F	1 : 128	0	0	0
28	F	1 : 64	0	1	0
40	F	1 : 64	0	1	1
53	F	1 : 64	1	0	0

0 = no; 1 = yes.

<sup>a</sup>Exposure to wool, hides, animal birth and milking.

patient 2 had one childbirth 7 years previously (miscarriages were not reported), and patient 3 had seven pregnancies (one childbirth 4 years before, six miscarriages, three of which were spontaneous). Patient 4 did not reliably provide her obstetric history, and had no live children.

## CONCLUSIONS

Although serological evidence must be analysed carefully, the seroprevalence to *C. burnetii* in Jacarepaguá, Rio de Janeiro of 3.2% suggests its circulation between HIV-positive individuals. This rate is higher than that found in asymptomatic adults (0.9%) in Minas Gerais State, a rural area in south-east Brazil [5]. In this same study, none of 269 ill AIDS patients presented antibodies to *C. burnetii*. In our study, the absence of DNA

amplification suggests inactivity of the disease, despite HIV serostatus. This is not surprising, as stable clinical condition was an inclusion criterion. All seroreactive patients were female, and pregnancy may have played a role in perpetuating *Coxiella* antibodies, as the obstetric history was active for most patients. Diagnosis of Q-fever should be considered in cases of acute pneumonia and prolonged fever, especially when there is associated immunosuppression, valvulopathy or pregnancy, and a history of potential exposure to the organism.

## REFERENCES

1. Raoult D, Levy PY, Dupont HT *et al.* Q fever and HIV infection. *AIDS* 1993; **7**: 81–86.
2. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. *Clin Infect Dis* 2007; **44**: 232–237.
3. Raoult D, Tissot-Dupont H, Foucault C *et al.* Q fever 1985–1998: clinical and epidemiologic feature of 1,383 infections. *Medicine* 2000; **79**: 109–123.
4. Hoover M, Vodkin H, Williams JC. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. *J Bacteriol* 1992; **174**: 5540–5548.
5. da Costa PS, Brigatte ME, Greco DB. Antibodies to *Rickettsia rickettsii*, *Rickettsia typhi*, *Coxiella burnetii*, *Bartonella henselae*, *Bartonella quintana*, and *Ehrlichia chaffeensis* among healthy population in Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz* 2005; **100**: 853–859.