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Seroprevalence of *Bartonella* spp., *Coxiella burnetii*, and *Hantavirus* among people who inject drugs in Rio de Janeiro, Brazil: a retrospective assessment of a biobank

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ABSTRACT

The increasing use of illicit drugs imposes a public health challenge worldwide. People who inject drugs (PWID) are more susceptible to health complications due to immunosuppression associated with drug use and non-hygienic self-administration of substances, contaminants, and liquids. PWID are subjected to increased risk of acquiring and transmitting different pathogens (frequently functioning as sentinel cases for (re)emerging pathogens), including those transmitted by arthropods and vertebrate reservoirs in unhealthy environments. A clear association between injection drug use and HIV, HBV, and HCV infections has been described; however, other infectious viral and bacterial agents have been seldomly assessed. In this study, we investigated the seroprevalence of *Bartonella* spp., *Coxiella burnetii*, and *Hantavirus* among 300 randomly selected PWIDs from Rio de Janeiro, as part of a multi-city cross-sectional study carried out in the 1990s. Point seroprevalences and respective 95% CIs are as follows: 9.3% for *C. burnetii* (95% CI: 6.0%-13.0%), 1.0% for *Bartonella* spp. (95% CI: 0.0%-3.0%), and 4.0% for *Hantavirus* (95% CI: 2.0%-7.0%). In addition to the blood-borne pathogens, the results of this study increase our knowledge on other transmissible infectious agents in PWID. The high seroprevalence of *C. burnetii* and *Hantavirus* found among PWID is intriguing and suggests the need to carry out prospective studies, including molecular analyses, to confirm these findings and allow a better understanding of the putative relevance of these zoonotic infectious agents among PWID.

KEYWORDS: PWID (Persons who inject drugs). Intravenous drug user. Emergent infections. *Bartonella* spp. *Coxiella burnetii.* Hantavirus.

INTRODUCTION

Drug abuse is increasing worldwide, with approximately 87,000 associated deaths reported in 2014[1]. According to the World Drug Report[1], there are more than 12 million People Who Inject Drugs (PWID) in the population aged 15-64 years old (a prevalence of 0.27%; 0.19%-0.48%) worldwide, predominantly in Eastern and Central Europe, as well as in certain areas of Southeast and Central Asia[2]. In these regions, the rate of injection drug use is 4.6 times higher than the world average[1].

The implementation of measures to reduce drug-related harm, such as syringe exchange programs providing sterile syringes to PWIDs, condom distribution, hepatitis B virus (HBV) vaccination and treatment, have significantly decreased new HIV and hepatitis cases among PWID[3] leading to a substantial decline in the number of new AIDS cases in this population between 2002 and 2016 according

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to the Brazilian Ministry of Health[4]. A decrease in the frequency of HBV and hepatitis C virus (HCV) infections in PWID has also been described[5-7]. Altogether, these figures reinforce the success of harm reduction policies and the need to maintain and refocus them, considering the most at-risk subgroups and contexts.

Injection drug abuse usually takes place in scenarios of poor sanitation, with the presence of rodents, arthropods and vertebrate reservoirs for different zoonotic pathogens, in such a way that intravenous/subcutaneous ("skin popping") administration of non-sterile substances and syringe/needle sharing favor the recurring transmission of viral and bacterial infections[8]. Besides the most-frequently assessed infectious diseases in PWID, including HIV and hepatitis, other emerging and re-emerging infectious diseases prevalent among PWID, including tetanus, visceral leishmaniasis, and malaria, should be investigated and properly managed[9-12]. The injection of illicit drugs is important as an ecological niche and route for the worldwide diffusion of emerging blood-borne pathogens. Many PWID travel widely, including internationally. The international diffusion of PWID includes spreading pathogens to geographically different areas, leading to contact with reservoirs for emerging diseases. Infective endocarditis is another important and serious, potentially short-term lethal medical condition among PWID, and can be acquired through direct injection of the bacteria (*Staphylococcus*) or through secondary spread, via skin and soft tissue abscesses, into the bloodstream[13-15].

Studies on *C. burnetii*, *Bartonella*, and Hantavirus infections among PWID are still scarce[8,16-25] and are totally unavailable in Brazilian drug use scenarios.

Coxiella burnetii, the causative agent of Q fever, is transmitted to both humans and animals by the inhalation of aerosols or contaminated excreta such as milk, feces, urine, saliva and conception products (placenta, vaginal secretions, and amniotic fluid samples), derived from infected animals. Ticks are suspected of having a role in the transmission of the bacteria among wild vertebrates[26]. In Brazil, despite the existence of serologic evidence of Q fever since the 1950s, reports of this zoonosis have been rare, considering a timespan of almost seven decades[27-34].

Bartonella spp. are intracellular hemotropic bacteria that grow fastidiously, transmitted by flea, lice and ticks bites in a range of mammalian hosts, including rodents, cats, dogs, humans, and bats[35,36]. These proteobacteria can be transmitted directly from a cat to humans during a scratch and less often by the bite of infected fleas in the case of *Bartonella henselae*. In Brazil, little information is available about the diversity of *Bartonella*. At present, *B. henselae*, *B. quintana*, *B. clarridgeiae*, *B. vinsonii* subsp. *berkhoffii*, and *B. vinsonii* subsp. *arupensis* have been detected in human and animal populations[37-43].

Hantaviruses are spherical, enveloped RNA viruses classified into the *Bunyaviridae* family and are known to cause Hemorrhagic Fever with Renal Syndrome (HFRS) in Eurasia and Hantavirus Pulmonary Syndrome (HPS) in the American continent[44,45]. Human infection occurs by aerosol droplet of infected excrements or contact with contaminated rodent secretions[46]. In Brazil, HPS is an important public health issue and cases have been associated with six different genotypes - Anajatuba, Araraquara, Castelo dos Sonhos, Juquitiba, Laguna Negra, and Rio Mamore. Other hantaviruses - Jabora, Rio Mearim, and Seoul - were also detected in rodents, but have not yet been associated with HPS/HFRS[44,45].

We investigated the prevalence of *C. burnetii, Bartonella,* and *Hantavirus* infections among 300 PWID, previously assessed by a cross-sectional multicity study conducted in Rio de Janeiro and two other sites in Brazil during 1999-2001[6].

This information is valuable to warn policymakers and health professionals about infections that have been neglected by policies and initiatives that aimed to reduce drug-related harms and curb their spread.

MATERIALS AND METHODS

PWID

This is a retrospective assessment of a subsample from a cross-sectional multicity study conducted from 1999-2001. PWID were assessed in "drug scenarios" (public places, nightclubs and bars) of Rio de Janeiro, Brazil. The targeted communities were defined after a comprehensive effort of interviewing key informants and mapping drug scenarios from different communities using a brief ethnographic assessment, in-depth interviews, and focus groups, according to the World Health Organization (WHO) guidelines, after Watters and Biernacki's original contribution on targeted sampling procedures. New sampling methods that have been used in recent studies, such as respondent-driven sampling or time-location sampling, were virtually ignored in Brazil in that period and were introduced much later[47].

The original database belongs to a large multicity study conducted in 13 cities, worldwide, entitled "Multicenter study of WHO-II, Brazil. Epidemiological survey - risk of HIV infection and viral hepatitis among drug users and transitions of cocaine use routes" under the coordination of Dr. Francisco I. Bastos and developed in the 1990s; this database was used in the present study for the analysis of

a core set of original variables. A total of 300 biological samples, out of the existing 608 samples, were randomly selected with the help of the Epidemiological calculator (Epicalc R) version 2.15.1.0, taking into account the constraints secondary to high-cost laboratory inputs, as well as the necessary statistical power to carry out simple statistical tests for contingency tables.

Ethics Statement

The study was approved by the Institutional Review Board (IRB) of the Fundação Oswaldo Cruz (FIOCRUZ) after addition of tests for *Bartonella* spp, *Coxiella burnetii*, and Hantavirus infection (amendment). In accordance with ethical guidelines defined by National Commission for Research Ethics (CONEP), and international norms under the aegis of WHO regulations. Formal written consent forms were obtained from each adult individual (none of the participants were children; > 18 years old – Brazilian national adult legal age), who was interviewed using a standardized questionnaire that included questions about socio-demographic status, pattern of injection drug use and sexual behavior.

Characterization of *Coxiella*, *Bartonella,* and *Hantavirus* infections

The presence of immunoglobulin G antibodies (IgG) against *Coxiella burnetii* and *Bartonella* spp. was determined by the indirect immunofluorescence assay (IFA), using commercial kits from ScimedX® (Dover, NJ, USA) and BION Enterprises® (Des Plaines, IL, USA), respectively[48,49], in accordance with the manufacturer´s instructions. Serum samples were screened at a 1:64 dilution and samples positive at 1:64 were further diluted and tested by IFA to determine the end titers. Negative and positive controls were included for each test run.

For *Hantavirus* investigation, IgG-ELISA was performed using a recombinant nucleocapsid protein from the Araraquara virus as the antigen, according to the protocol described by Figueiredo *et al*.[50]. Serum samples were screened at a 1:400 dilution and the sample was considered positive when the optical density (OD) was higher than 0.3. Negative and positive controls were included for each test run.

Statistical analysis

Statistical analysis was conducted with the help of Microsoft Office Professional Plus 2010 - Excel. Contingency table analysis (e.g., chi-square or Fisher's exact test and Student's *t*-test for means) were employed. Results were considered significant when $P<0.05$.

Associations between the results of the serological tests and demographic (sex, ethnicity, education and place of residence) parameters as well as drug use variables (date of the first drug use, first injection, use of a previously used syringes and location where drug use took place) were analyzed in bivariate analyses.

RESULTS

The socio-demographic and drug use patterns of the 300 PWID included in the study are shown in Table 1. The mean age was 31.7 ± 10 years (range 16-68 years).

Table 1 - Socio-demographic and drug use patterns in the population of people who inject drug (PWID), Rio de Janeiro, Brazil (1999-2001)

Variables		Study population (300) n (%)
Gender	Male	280 (93.3)
	Female	20 (6.7)
Age	<20	27 (9.0)
	20-29	119 (39.7)
	30-39	83 (27.7)
	40-49	58 (19.3)
	>49	13 (4.3)
Marital status	Single	187 (62.3)
	Living as married	50 (16.7)
	Legally married	34 (11.3)
	Legally separated	19 (6.3)
	Divorced	6 (2.0)
	Widowed	4 (1.3)
Formal education (years)	0	7 (2.3)
	1-8	130 (43.3)
	9-12	108 (36.0)
	>12	55 (18.3)
How many times injected in life	Once	10 (3.3)
	2-9 times	59 (19.7)
	10-99 times	115 (38.3)
	100-999 times	108 (36.0)
	1000 or more times	8 (2.7)
How long without injecting (months)	0	69 (23.0)
	1-11	92 (30.7)
	12-24	53 (17.7)
	>24	86 (28.7)

The average number of years of formal education was 9.4 ± 4.2 years (range 0-29 years): 2.3% had no schooling at all, 52% had not completed the 1st grade, 18% had completed the 1st grade, 10.3% had not completed high school, 11% had completed high school and 6.3% had college degree.

Regarding the level of education of the head of the family: 23% were illiterate or studied until the the 5th grade; 36.7% studied beyond the 5th grade, but did not complete the elementary school; 19.7% concluded the elementary school; 16.7% had completed high school; 2.7% had completed higher education and in 1.3% there was no information about the education level of the head of the family.

Most subjects (66.0%) did not report any treatment for chemical dependency. Among those who had been previously treated for drug abuse (34.0%), 39 were submitted to more than one treatment schedule. The most frequently reported approaches were admission to drug treatment clinics (49.7%), support from mutual aid groups (18.6%) and support from religious institutions (8.6%). Overall, 44.7% (n=134) of the sampled PWID had been imprisoned at some point; among those, incarceration occurred once among 56% of the interviewees, twice among 20.9%, three times among 17.9%, and more than three times among 5.2%. Among those arrested, 23.1% injected drugs inside jails/prisons, and 71% injected drugs with a previously used needle/syringe.

About half of the sampled PWID (53.7%) started consuming drugs before they were 17 years old and the majority (78.0%) reported using marijuana or hashish in the six months before the interview.

At the time of the study admission, the main route of drug use was: (i) non-injectable by 87.0% of the interviewees, (ii) injectable by 5.7%, (iii) both routes by 4.3%, and (iv) 3.0% refused to answer. A total of 23.0% had injected drugs in the month before the survey, 30.7% did not inject drugs for more than a month and less than a year before the survey and 46.3% did not inject drugs for more than one year.

PWID and anti-*Coxiella burnetii* antibodies

Point seroprevalence for *C. burnetii* was 9.3% (28/300) (95% CI: 6.0%-13.0%). There were no statistically significant associations between the demographic or behavioral data and this biological outcome.

PWID characteristics and anti-*Bartonella* spp. antibodies

Point seroprevalence for anti-*Bartonella* spp. was 1.0% (3/300) (95% CI: 0%-3.0%). All individuals were male, with incomplete elementary educationand did not report treatment for chemical dependency. At the time they were interviewed, two individuals reported previous non-injectable use of the same drug and to have used non-sterile injection equipment in their first episode of drug use.

Besides seropositivity for *Bartonella*, antibodies to *Hantavirus* and *C. burnetii* were also detected in a 29-year-old and a 43-year-old PWID, respectively. These very low figures preclude any further assessment of putative associations between socio-demographic and behavioral variables and these outcomes.

PWID characteristics and anti-*Hantavirus* antibodies

Anti-hantavirus point seroprevalence was 4.0% (12/300) (95% CI: 2.0%-7.0%). All individuals were male. The first illicit drug consumed was cocaine by another non-injectable route, with the exception of a 42-year-old individual, who had first injected amphetamine. The other subjects used cocaine as the first intravenous drug and 50% used previously used syringes.

DISCUSSION

This was the first Brazilian seroprevalence study on *C. burnetii, Bartonella,* and *Hantavirus* exposure among PWID.

The demographic profile of the PWID population, composed mainly of young male adults with a low degree of formal education,was similar to those observed in previous reports[8,16,18,20,22,24,51,52].

We found a relatively high seroprevalence of *C. burnetii* in this population (9.3%; 95% CI: 6.0%-13.0%), almost twice the frequency found among non-PWID populations. Lamas *et al*.[53] found a seroprevalence of 3.2% among HIV-positive patients from Rio de Janeiro, similar to the percentage (3.9%) found in the general population of Minas Gerais[37]. The greater susceptibility of PWID to infectious agents due to non-hygienic injection practices, immunosuppression and sharing of injection paraphernalia, may explain these findings. It is likely that the lack of a detailed assessment of specific risk factors for these infections (the overall study targeted HIV and viral hepatitis) did not help in the effort to find statistically significant associations between socio-demographic and behavioral variables and *C. burnetii* infection.

In a Spanish study conducted in 590 PWID, a *C. burnetii* seroprevalence of 21.0% was reported[21]. This high frequency could be partially attributed to a lower definition of the IFA cutoff titer (1:16). If a cutoff point of 1:64 was used instead, as adopted in the present study, the reported seroprevalence would decrease to 14.6%, in agreement with our study since the confidence intervals overlap. A second study was carried out in Italy, where Boschini *et al*.[16] detected a seroprevalence of 33% (using the complement fixation test) after two outbreaks that

occurred among residents in an agricultural community for the rehabilitation of drug users. It is pertinent to consider that, in this case, both methodological and epidemiological differences compromise comparative analyses.

There was no significant association between *C. burnetti* infection and socio-demographic variables. The seroprevalence of *Bartonella* infection was 1.0%, far below that observed in other Brazilian seroepidemiological studies, as reported by Lamas *et al.*[53] in blood banks from Rio de Janeiro (24.0%) or among HIV-positive patients (38.4%).

Although there are no Brazilian studies on *Bartonella* seroprevalence among PWIDs, six international studies show a high prevalence of anti-*Bartonella* antibodies in this population. In European studies, the prevalence is significantly higher in this population, compared to people who do not inject drugs. In Sweden, a prevalence of 39.0% was found among 59 PWID, in contrast to the 21.0% identified in the control group[20]; in Slovenia, these percentages were 43.0% vs. 25.0%, respectively[25]. In two Spanish studies, the seroreactivities for *Bartonella* among PWID were 13.7%[24]; and 24.2%[22], whereas in other two American studies, the seroprevalences were 47.5% and 37%, respectively[8,18]. The discrepancies in *Bartonella* seroprevalence in PWID found in our study and in other international studies could be partially explained by the global differences in the geographical distribution of *Bartonella* species. The highest reported seropositivities were observed against *B. elizabethae*, followed by *B. quintana*, both considered non-existent and/or rarely detected in Brazil. In our study, the *B. henselae* antigen was used. Therefore, it is reasonable to speculate that if other *Bartonella* species were included in our assay, a different seroprevalence could be observed. Unfortunately, commercial kits that include other species than *B. henselae* are not available. Moreover, our sample included a low frequency of homeless PWID and about half of the subjects did not report to have injected drugs in the last year, in contrast with other studies. This could be explained by the route transition hypothesis[54].

Hantavirus seroprevalence was 4.0%, consistent with values found in other Brazilian studies, including 314 blood donors of Santa Catarina, a Southern State (4.4%)[55], and 1,063 individuals from the South and Southeast regions, including residents of rural areas, harbor employees and leptospirosis patients (3.0%)[56]. These samples are likely to contain the hantavirus genotype of Seoul, identified in the 1980s in the North of Brazil, for which urban rats (*Rattus norvegicus*) are reservoirs[57].

Although reference serological tests for the detection of Q fever (97.7% sensitivity and 100% specificity), bartonellosis (97.4% sensitivity and 100% specificity), and HPS/HCPS (97,2% sensitivity and 100% specificity) were used in the present study, we cannot rule out the possibility of cross-reactivity with other infectious agents; however, our results were based on IgG detection, which is less prone to low specificity than IgM detection.

Additional studies on bartonellosis, including detection of a range of different bacterial species, would be valuable to assess comparable seroprevalences in different geographical regions. The high seroprevalence of *C. burnetii* among PWID must be highlighted and reinforces the need for further prospective studies, including serological and molecular analyses, to confirm these first findings and to allow a better understanding of the importance of Q fever among this population. This paper highlights the findings of a targeted surveillance of a highly vulnerable population. This study has been carried outwith the double purpose of identifying "sentinel populations", as well as the effort to discern the putative bridging between vulnerable populations with other populations or the general population at large.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or disclosures concerning this work.

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