Infectious agents, *Leptospira* spp. and *Bartonella* spp., in blood donors from Cajamarca, Peru

Maria J. Pons[1], Numan Urteaga[2], Carlos Alva-Urcia[1], Pedro Lovato[3], Jaquelyne Silva[3], Joaquim Ruiz[4], Juana del Valle-Mendoza[1]

[1]Research Centre of the Faculty of Health Sciences, Peruvian University of Applied Sciences-UPC, Lima, Peru; [2]Faculty of Human Medicine, Cajamarca National University, Cajamarca, Peru; [3]Cajamarca Regional Hospital, Cajamarca, Peru; [3]ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clinic - University of Barcelona, Barcelona, Spain

Introduction

The implementation of molecular and serological tests has led a great decline in transfusion-transmitted infections. Unfortunately, however, this has only occurred in high-income countries, whereas the scenario is different in low-income countries and in rural areas of middle-income countries, in which access to serological tests is sometimes not feasible or limited by economic factors[1,2]. Furthermore, in middle and low-income countries, the burden of infectious diseases tends to be higher and subjects are often weakened by various factors, including malnutrition. These factors result in a population that is more vulnerable and at increased risk of infections. Although the search for relevant pathogens that can be transmitted by blood transfusion is implemented worldwide, various pathogens that can be present in blood bank donations remain under studied, as in the case of some bacteria such as *Leptospira* spp. and *Bartonella* spp. *Bartonella* species are re-emerging blood-borne organisms, capable of causing prolonged infections in animals and humans[3], while leptospirosis is recognised as an emerging public health problem worldwide[4]. Both infections are considered to be neglected tropical diseases.

In humans, *Bartonella* spp. cause infections of varying severity, ranging from asymptomatic bacteraemia to severe cases with chronic manifestations responsible for persistent disease and death[3]. So far, various members of the *Bartonella* genus have been associated with human disease, the most relevant being *Bartonella henselae*, causing cat scratch disease, *Bartonella quintana*, causing trench fever, and *Bartonella bacilliformis*, the causal agent of Carrion's disease[2]. This last micro-organism is endemic in Latin America, and specifically in the Andean valleys. *B. bacilliformis* infects red blood cells and endothelial cells and, as mentioned above, is the aetiological agent of Carrion's disease, a biphasic disease with two different clinical presentations: an initial febrile phase with haemolytic anaemia, known as Oroya fever, which has a mortality rate ranging from 44-88% in untreated patients, and a second phase characterised by the development of dermal eruptions, known as "Peruvian wart"[5]. It is important to highlight the presence of asymptomatic people, who account for about 45% of the population in some endemic areas[6]; consequently, the risk of transfusion transmission is of note, especially because of the ability of *B. bacilliformis* to survive in blood stored at 4 ºC for up to 1 year[7].

Leptospirosis is one of the most common bacterial zoonotic diseases worldwide. Various mammals such as rodents, livestock and domestic pets can act as reservoir hosts[8]. Traditionally, infection in humans is due to contact with *Leptospira*-contaminated sites in different environments: at work (veterinarians, farmers, sewer cleaners), in alleys and slums with poor drainage, and in recent years, during international travel and recreational activities[9]. Infection by *Leptospira* may be asymptomatic or may cause a mild influenza-like illness that can resolve spontaneously. Symptomatic infection may present as non-specific symptoms such as fever, chills, headache, myalgia and jaundice[8]. As mentioned above, the presence of asymptomatic carriers may favour the risk of transfusion-related transmission. The distribution of Carrion's disease is limited to the Andean valleys, while leptospirosis occurs worldwide, being more common in tropical and subtropical regions due to the wet weather. Rainfall, flooding and climatic phenomena, such as "El Niño", increase the risk of these bacterial infections[4].

There is no information about the prevalence of these bacteria in blood donors in Peru. Moreover, analyses to detect the presence of *Leptospira* spp. are not routine in this country and the presence of *B. bacilliformis* is only tested for in blood banks in endemic areas, but not in the remaining ones, irrespectively of whether there are inhabitants arriving from or travelling to endemic areas[10,11]. This represents a health problem, since subjects receiving a blood transfusion are usually immunocompromised and more vulnerable to the development of severe infectious diseases from potentially transfusion-transmitted micro-organisms.

Blood Transfus 2016; 14: 504-8 DOI 10.2450/2015.0081-15

The aim of the present study was to examine the prevalence of *Bartonella* spp. and *Leptospira* spp. in blood donors in Cajamarca, in northern Peru, and describe the associated epidemiological factors.

Material and methods

Patients and sampling

The study was conducted from March to May of 2014 under a collaborative agreement between the *Universidad Peruana de Ciencias Aplicadas* (UPC) - Institute of Nutritional Studies (UPC-IIN, Lima, Peru) and the Blood Bank Service of the regional hospital of Cajamarca. This hospital is located in the capital of Cajamarca Department, in northern Peru, and serves an area greater than 35,000 km^2 and a population of approximately 1,500,000 people. The hospital's catchment area has great climatic diversity: humid subtropical regions (eastern slopes), dry subtropical and tropical regions (western slopes) and, in the centre, a high mountain climate.

Healthy blood donors were recruited at the time of their volunteer blood donation at the Blood Bank of the regional hospital of Cajamarca. These blood donors were between 18-55 years of age, had no signs or symptoms of unspecified illness (no fever, chills, jaundice or myalgia in the preceding 4 weeks) and signed informed consent to participate in the study. All blood donors included in the study met at least one of the following inclusion criteria: exposure to water sources, waterlogging or other potentially contaminated water collections, such as irrigation canals, ditches, pools, ponds, lakes, and rivers; exposure to drains, latrines or management of wastewater contaminated with urine of rodents and other animals; people at occupational risk, such as farmers, ranchers, garbage collectors, recyclers, ditch cleaners, water and sewer workers, plumbers, veterinarians, agricultural technicians who treat animals and slaughterhouse workers; recreational and adventure sports that are related to potentially contaminated water sources (rivers, lakes, ditches, ponds and other) and living in rural and marginal urban housing with overcrowding or poor or absent sanitation.

Samples of 3 mL of venous blood from blood donors were collected into tubes containing EDTA and citrate, stored at 4 ºC and sent in on a weekly basis to the UPC-IIN (Lima, Peru) facilities where they were stored at 4 °C until processing. The project was approved by the Ethics Committees of the regional hospital of Cajamarca (Peru) and the Hospital Clinic of Barcelona (Spain).

DNA extraction

DNA was extracted from 200 µL of blood samples using a commercial extraction kit (High Pure Kit Preparation template, Roche Applied Science, Mannheim, Germany). Bacterial DNA obtained after extraction was eluted in 100 µL of nuclease free water and then processed or stored at − 20 °C until use.

Amplification of a *Bartonella* spp.-specific 16S rRNA gene fragment

A 438 bp fragment of the *16S rRNA* gene was amplified in blood samples as previously described[12] using the primers: p24Emod 5'-CCTTCAGTTMGGCTGGATC-3' and 16S-R 5'- GCCYCCTTGCGGTTAGCACA-3'. Five microlitres of DNA extracted from blood were used in each polymerase chain reaction (PCR). The 438 bp amplified products were gel recovered, purified (SpinPrepTM Gel DNA Kit, San Diego, USA) and sent to be sequenced (Macrogen, Seoul, Korea).

Detection of *Leptospira* spp.

Specific multiplex PCR able to detect all pathogenic *Leptospira* species described (*L. interrogans*, *L. borgpetersenii, L. weilii, L. noguchii, L. santarosai, L. meyeri* and *L. kirschneri*) was carried out using the primers G1 (5'-CTGAATCGCTGTATAAAAGT) and G2 (5'-GGAAAACAAATGGTCGGAAG), and primers B64-I (5'- CTGAATTCTCCATCTCAACTC) and B64-II (5'-GCAGAAATCAGATGGACGAT) as described previously[13]. In addition, 5 µL of DNA extracted from blood were used in each PCR. A positive sample, used as a control, was provided by the Regional Health Direction - DIRESA, Cajamarca.

Data analysis

Statistical significance was established using the Fisher's exact test. Differences with a p-value <0.05 were considered statistically significant.

Results

During the study period, a total of 581 blood donors were received at the regional hospital of Cajamarca. Of these, 42 (7.23%) blood donors met at least one of the inclusion criteria and were consequently included in the analysis. Of the samples taken from these 42 blood donors, eight (19.05%) were positive for the presence of *Leptospira* spp. and one (2.38%) for *Bartonella* spp., which was classified as *B. bacilliformis* after sequencing (Figure 1).

According to the demographic and epidemiological characteristics of the study population, 57.2% of the blood donors lived in rural areas and 59.5% reportedly worked as farmers and/or ranchers. Overall, 27 (64.3%) of the 42 blood donors studied were inhabitants of rural areas or farm/ranch workers living in urban areas, indicating continuous exposure to a rural environment. The remaining characteristics studied are shown in Table I. The only *B. bacilliformis* carrier lived in a rural area

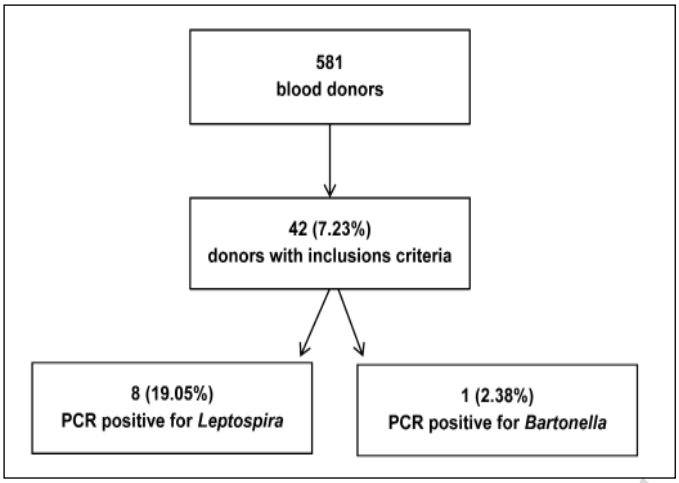

Figure 1 - Flow chart of the presence of both *Lesptospira* and *Bartonella* spp. in blood donors at the regional hospital of Cajamarca.
PCR: polymerase chain reaction.

Table I - Demographic data of the blood donors studied.

		All donors (N=42) N (%)	*Leptospira* positive (n=8) n (%)	*Bartonella* positive (n=1) n (%)
Gender	Male	26 (62)	5 (62.5) (p=1.0)	0
	Female	16 (38)	3 (37.5) (p=1.0)	1
Age	Median (CI)	33.1 (29.8-36.4)	29.6 (22.1-37.1)	28
	18-30	23 (54.8)	6 (75)	1
	31-40	7 (16.6)	1 (12.5)	0
	41-50	8 (19.0)	0	0
	>50	4 (9.5)	1 (12.5)	0
Living area	Urban	18 (42.8)	4 (50) (p=1.0)	0
	Rural	24 (57.2)	4 (50) (p=1.0)	1
Overcrowding[1]		12 (28.6)	4 (50) (p=0.25)	0
Occupation[2]	Farmer/Rancher	25 (59.5)	4 (50) (p=0.71)	1
	Student	6 (14.3)	1 (12.5) (p=1.0)	0
	Health personnel	2 (4.8)	2 (25) (p=0.12)	0
	Others	9 (21.4)	1 (12.5) (p=1.0)	0
Travel[3]		7 (16.6)	3 (37.5) (p=0.33)	0
Animal contact[4]	Cattle	19 (45.2)	3 (37.5) (p=1.0)	1
	Pigs	12 (28.6)	2 (25) (p=1.0)	1
	Sheep	2 (4.8)	0 (p=1.0)	0
	Poultry	9 (21.4)	0 (p=0.32)	0
	Dogs	26 (61.9)	6 (75) (p=0.69)	0
	Cats	19 (45.2)	4 (50) (p=1.0)	1
Other	Backwaters[5]	19 (45.2)	5 (62.5) (p=0.46)	0
	Septic pit latrine[5]	32 (76.2)	8 (100) (p=0.18)	0
	Rodents[5]	22 (52.4)	6 (75) (p=0.44)	1
	Skin wounds[6]	17 (40.5)	5 (62.5) (p=0.28)	0

[1]More than three people per bedroom; [2]one farmer also reported being a student, while other four also reported other parallel activities; [3]trip to a *Leptospira* endemic zone (in preceding 4 weeks); [4]regular contact with livestock and pets; [5]contact in the 4 weeks prior to donation; [6]during the 4 weeks prior to donation. CI: confidence interval.

and worked as a farmer. Regarding the characteristics of *Leptospira* spp. carriers, five cases (62.5%) were reported to have been exposed to a rural environment. Four out of the eight (50%) *Leptospira*-positive donors lived in rural areas, working as farmers or ranchers, while the remaining lived in urban areas, although one was a farmer. Three cases were reported to have travelled to recognised *Leptospira* endemic areas in Cajamarca Department during the preceding 4 weeks (Table I). With regards to the possession of animals, 75% of *Leptospira* spp. carriers had a dog, while 50% had a cat. Regarding asymptomatic *Leptospira* carriers, 62.5% reported contact with backwaters and 100% had had contact with a septic pit latrine in the 4 weeks before blood donation. Meanwhile 62.5% had skin wounds and 75% reported contact with rodents during the preceding 4 weeks (Table I). None of the epidemiological factors analysed showed a statistically significant association with the presence of *Leptospira* (p-value $\geq$0.05).

Discussion

Infections by *Leptospira* spp. and *Bartonella* spp. in humans may be asymptomatic. The lack of evidence of carrier status may be a risk factor for blood donations, especially in areas in which these pathogens are not searched for. In the present study, we found evidence of *Leptospira* spp. and *B. bacilliformis* in 19% and 2.4%, respectively, of the samples from blood donors in Cajamarca. It is important to note that the percentage of samples positive for *Bartonella* may be underestimated, since studies have demonstrated that prior enrichment of the sample may increase positive results by 55% compared to those obtained when testing the original blood sample[2].

Although the authors of a study performed in an area with an increasing incidence of leptospirosis found no previous cases of *Leptospira*-infected transfusions in the region and no cases of acute infection in 485 blood donors studied, they did find evidence of past infections in some donors and proposed that acute bacteraemia in blood donors would provide the potential for transfusion-related transmission[14]. Nonetheless, only one case has been described in India[15], demonstrating the possible presence of viable *Leptospira* in platelets[16]. With respect to *Bartonella*, blood transfusion is considered one of the mechanisms of transmitting *B. bacilliformis* infection, although to our knowledge it has only been described once[17].

Leptospiraemia and bartonellosis occur mainly in rural and deprived urban areas, where they may exacerbate poverty by limiting the productivity of the population and impairing development, causing morbidity in these local populations[18]. These illnesses are not, however, limited to developing countries, being present also in developed countries. Indeed, a high prevalence of *Bartonella* spp. carriers was observed among the homeless[19]. In should be noted that all *Bartonella* spp. can be a risk for transfusions in blood banks, as reported recently[2]. In blood banks, the presence of *Leptospira,* when sought for, is determined by serological techniques, while that of *B. bacilliformis* is determined by thin smear. The PCR method has shown good detection of *Leptospira* in blood donor screening, reducing the risk of infection by blood transfusion[20]. As far as concerns *Bartonella*, although PCR analysis detects a higher number of infections than thin smear studies, the PCR technique seems to have limited value in detecting asymptomatic carriers[21] because DNA amplification from original blood samples is relatively insensitive, primarily because the low level of bacteraemia typically present, especially in asymptomatic subjects, may be below the detection level of the PCR assay[21]. Given that asymptomatic leptospiraemia may not always be detected by routine bacterial culture methods available in rural hospitals, and that the diagnosis of bartonellosis remains a microbiological challenge because of the difficulty of culturing and isolating the causative agent from patients' specimens[22], these bacteria pose a risk to transfusion safety, especially in regions with a high prevalence of *Leptospira*/*Bartonella*. These high-risk regions are associated with climatic phenomena, such as flooding, concentrated in developing countries due to their intrinsic characteristics[22]. In addition, the lack of resources in these countries leads to less stringent screening of samples from blood donors and a consequent increase in the risk of infection by *Leptospira* or *Bartonella*[2,15,21]. In the case of Peru, a middle income country, the national legislation related to blood banks requires that donations are tested for the presence of human immunodeficiency virus, human T-cell lymphotrophic viruses 1 and 2, hepatitis B virus, hepatitis C virus, *Trypanosoma cruzii* and *Treponema pallidum*, while detection of other pathogens such as *B. bacilliformis* or *Plasmodium* spp. is only considered in endemic areas[10,11]. Thus *Leptospira* is not considered in screening, while the risk of a transfusion transmission of *B. bacilliformis* in blood banks within endemic areas is high. The blood bank of Cajamarca is in a *B. bacilliformis*-free urban area, although donors may live outside the city, as in the case of the carrier detected.

The presence of *Leptospira* spp. is usually related to excreta from humans or animals, including rodent urine[22]. Thus, risk factors for leptospirosis infection include skin cuts, abrasions, lack of footwear, and contact with contaminated water, soil or mud either during work or recreational activities[22]. The present study did not show a significant association between the

risk factors considered and the presence of *Leptospira* spp. This may be related to the small sample size of the study, which is the study's main limitation. However it should be considered that the majority of the factors studied are very common in Cajamarca inhabitants, thereby making it difficult to establish a relationship with leptospirosis.

Conclusions

Molecular tools should be routinely implemented in screening for *Leptospira* and *Bartonella* in blood banks, in order to diminish possible post-transfusion infections. The development of more sensitive diagnostic tools and evaluation of the adequacy of current blood service guidelines for the management of leptospirosis and bartonellosis in risk areas are needed.

Acknowledgements

The study was supported by internal funds from the *Universidad Peruana de Ciencias Aplicadas* (Lima, Peru) (to JdV), by a grant from the *Instituto de Salud Carlos III* (Madrid, Spain) (PI11/00983) which includes FEDER funds, and by *Generalitat de Catalunya, Departament d'Universitats, Recerca i Societat de la Informació* (Spain) (2014 SGR 26) (to JR). JR has a fellowship from programme I3, of the ISCIII (grant number: CES11/012). MJP has a post-doctoral fellowship from CONCYTEC/FONDECYT (grant number: CG05-2013-FONDECYT).

Authorship contributions

NU and JdV-M participated in the study design. PL, JS, NU and CT participated in study implementation. MJP and JR analysed and interpreted the data. MJP, JR and JdV made major contributions to writing the manuscript. All the Authors read and approved the final version of the manuscript.

The Authors declare no conflicts of interest.

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Arrived: 31 March 2015 - Revision accepted: 15 September 2015
Correspondence: Juana del Valle-Mendoza
Universidad Peruana de Ciencias Aplicadas
Av San Marcos Cdra 2
LIMA9 Chorrillos, Lima, Peru
e-mail: jdelvall@upc.edu.pe