Rev. peru. biol. 20(2): 165 - 169 (Diciembre 2013)
Facultad de Ciencias Biológicas UNMSM

ISSN-L 1561-0837

TRABAJOS ORIGINALES

Detection of *Bartonella* spp. and *Rickettsia* spp. in fleas, ticks and lice collected in rural areas of Peru

La detección de *Bartonella* spp. y *Rickettsia* spp. en pulgas, garrapatas y piojos recolectados en las zonas rurales de Perú

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Citación:

Cáceres A.G., C.P. Padilla Rojas, J. Arias Stella, G. Huatuco Crisanto, A. Gonzales Pérez. 2013. Detection of Bartonella spp. and Rickettsia spp. in fleas, ticks and lice collected in rural areas of Peru. Rev. peru. biol. 20(2): 165 - 169 (Diciembre 2013)

Abstract

Bartonellosis and rickettsiosis are commonly reported in Peru. In order to detect *Bartonella* sp. and *Rickettsia* sp. in fleas, ticks and lice, specimens from five distinct locations in Peru (Marizagua, Cajaruro, Jamalca, Lonya Grande and El Milagro) were collected and screened for the presence of these bacteria using PCR and later confirmation by DNA sequencing. The specimens collected were distributed in 102 pools (76 *Ctenocephalides felis*, 2 *Ctenocephalides canis*, 16 *Pulex irritans*, 5 *Pediculus humanus*, 2 *Rhiphicephalus sanguineus*, and 1 *Boophilus* spp.), where *Bartonella* was detected in 17 pools (6 of *C. felis*, 9 of *P. irritans*, 1 of *C. canis*, and 1 *P. humanus*). Also, *Rickettsia* was detected in 76 pools (62 *C. felis*, 10 *P. irritans*, 2 *P. humanus*, and 2 *C. canis*). *Bartonella clarridgeiae* was detected in *C. felis*, *C. canis* and *P. irritans* pools at 5.3%, 50% and 12.5%, respectively. *Bartonella rochalimae* was detected in one *C. felis* and two *P. irritans* pools at 1.3% and 12.5%, respectively. Furthermore, *B. henselae* was detected in one *C. felis* pool and one *P. humanus* pool corresponding to 1.3% and 20%, respectively; and *Bartonella spp.* was also found in 5 pools of *P. irritans* at 31.3%. Additionally, *R. felis* was detected in *C. felis*, *C. canis* and *P. irritans* pools at 76.3%, 100% and 37.5%, respectively; and *Rickettsia spp*. was detected in *C. felis*, *P. irritans* and *P. humanus* pools at 5.3%, 25% and 40%, respectively. These results demonstrate the circulation of these bacteria in Peru.

Keywords: *Bartonella*; *Rickettsia*; arthropods; PCR detection; Peru.

Resumen

La Bartonellosis y la Rickettsiosis son enfermedades comúnmente reportadas en Perú. Con el propósito de detectar *Bartonella* sp. y *Rickettsia* sp. especímenes de pulgas, garrapatas y piojos de cinco localidades del Perú (Marizagua, Cajaruro, Jamalca, Lonya Grande and El Milagro) fueron colectadas y analizadas. Para la detección se usó PCR y una posterior confirmación con secuenciamiento de DNA. Los especímenes colectados fueron agrupados en 102 *pools* (76 *Ctenocephalides felis*, dos *Ctenocephalides canis*, 16 *Pulex irritans*, cinco *Pediculus humanus*, dos *Rhiphicephalus sanguineus*, y un *Boophilus* spp.). *Bartonella* fue detectada en 17 *pools* (seis de *C. felis*, nueve de *P. irritans*, uno de *C. canis*, y uno de *P. humanus*). *Rickettsia* fue detectada en 76 *pools* (62 de *C. felis*, 10 de *P. irritans*, dos de *P. humanus*, y dos de *C. canis*). *Bartonella clarridgeiae* fue detectada en *C. felis* (5.3% especímenes), *C. canis* (50%) y *P. irritans* (12.5%). *Bartonella rochalimae* fue detectada en *C. felis* (1.3%) y *P. irritans* (12.5%). Además, se detectó *B. henselae* en *C. felis* (1.3%) y *P. humanus* (20%). *Bartonella* spp. también se encontró en *P. irritans* (31,3%). Además, se detectó *R. felis* en *C. felis* (76.3%), *C. canis* (100%) y *P. irritans* (37.5%), y *Rickettsia* spp. se detectó en *C. felis* (5,3%), *P. irritans* (25%) y *P. humanus* (40%). Estos resultados demuestran la circulación de estas bacterias en el Perú.

Keywords: *Bartonella*; *Rickettsia*; arthropods; PCR detección; Perú.

Presentado: 15/04/2013
Aceptado: 15/09/2013
Publicado online: 09/12/2013

Introduction

Human bartonellosis is caused by various species of *Bartonella*. Nowadays, there are several species of this genus that can infect humans including *B. bacilliformis, B. henselae, B. quintana, B. rochalimae, B. elizabethae, B. vinsonii* subsp. *arupensis, B. vinsonii* subsp. *berkhoffii, B. grahamii, B. koehlerae, B. washoensis*, *B. alsatica,* and *B. tamiae* (Billeter et al. 2008, Daly et al. 1993, Kerkhoff et al. 1999, Roux et al. 2000, Eremeeva et al. 2007, Kosoy et al. 2008). Only *B. bacilliformis*, *B. vinsonii*, *B. quintana*, *B. rochalimae*, *B. elizabethae* and *B. cladrrigeiae* have been reported in Peru (Billeter et al. 2008, Eremeeva et al. 2007, Parola et al. 2002, Roux & Raoult 1999, Raoult et al. 1999). *Bartonella* is believed to be transmitted by an arthropod vector, and infection can result in Carrion's disease, cat scratch disease, trench fever, endocarditis, neuroretinitis, bacillar angiomatosis, hepatic granulomatosis, arthritis, osteomelitis, and sepsis (Billeter et al. 2008, Breitschwerdt & Kordick 2000).

Rickettsia spp. are gram negative coco-bacilli and intracellular pathogens and most of them are transmitted transtadially by a wide variety of arthropod hosts including fleas, lice and ticks. Infections with these pathogens can cause diverse human diseases such as endemic typhus, scrub typhus, erhlichiosis and others rickettsiosis (Raoult & Roux 1997). In Peru, rickettsiosis occurs sporadically along the coast, highlands and jungle. The exposure to rickettsiosis was confirmed by immunofluorescence assay (IFA) in regions of Peru (Raoult et al. 1999, Blair et al. 2004a) and *Rickettsia* species were detected in ticks and fleas (Schoeler et al. 2005, Jiang et al. 2005).

Several arthropods belonging to the orders Diptera (Noguchi et al. 1929, Caceres et al., 1997), Anoplura (Ellis et al. 1999), Siphonaptera (Brouqui et al. 1999; Higgins et al. 1996) and Parasitiformes (Schouls et al. 1999, Angelakis et al. 2010) have been implicated in the transmission of *Bartonella* spp. Previous experimental studies have demonstrated that *Lutzomyia verrucarum* (sandfly) are competent vectors of *B. bacilliformis,* which causes Carrion's disease (Breitschwerdt & Kordick 2000, Maguiña et al. 2009). In order to detect *Bartonella* spp. and *Rickettsia* spp. in fleas, lice and ticks, specimens were captured from humans, domestic and wild animals in five distinct locations in Peru, where Carrion's disease is endemic. The presence of *Bartonella* spp. in these specimens was evaluated by polymerase chain reaction (PCR) and confirmed by DNA sequencing.

Materials and methods

Study sites and specimen collections. Samples were collected from random houses in different places: San Ignacio (Marizagua) of Cajamarca departament; Cajaruro, Jamalca, Lonya Grande and El Milagro of Amazonas departament in July 2000 to September 2001 (Table 1, Fig. 1).

These samples were obtained from different pets such as dogs, cats, and guinea pigs (considered as a food source); moreover, foxes found near the neighborhood were trapped in cages for later sample collection. Specimens such as fleas were collected by hand from animals; but lice were collected from people (head, body and clothes) and ticks were collected only from dogs. These were then stored in vials containing 70% ethanol, and organized according to host and place of collection.

The taxonomic classification was done in the Entomology Laboratory at the "Daniel A. Carrión" Tropical Medicine Institute, of the Universidad Nacional Mayor de San Marcos in Lima, using entomological keys (Jonson 1957, Lewis 1972).

After taxonomic classification, the specimens were grouped into pools of 6 to 20 specimens, each pool from one or two hosts, to reduce the processes for analysis (see Table 1).

DNA extractions from specimens. The whole specimens were grouped in pools and washed with 1X Phosphate Buffered Saline (PBS). They were then triturated with a micro-pestle in 100 µL of PBS. DNA was extracted using a Tissue DNA Purification Kit (QIAGEN) following the standard procedure recommended by the manufacturer.

DNA amplification of the gltA gene by PCR. The *glt*A gene of *Bartonella* was amplified using the BhCS.781p and BhCS.1137 primers (Norman et al. 1995). The same specimens

Figure 1. Localities of the north of Peru where it was collected specimens of arthropods for Bartonellosis and Rickettsiosis study.

Table 1. Arthropods collected from cats, dogs, guinea pigs, foxes and humans from San Ignacio (Cajamarca) and Utcubamba (Amazonas) in Peru.

Department/ Province/ District	Host (Number)	Species	Number of specimens	Number of pools	Capture Date
Cajamarca/ San Ignacio/ San Ignacio (Marizagua)	cat (10)	*Ctenocephalides felis*	119	7	July, 2000
	cat (2)	*Ctenocephalides canis*	34	2	
	dog (45)	*Ctenocephalides felis*	499	26	
	dog (6)	*Pulex irritans*	77	4	
	guinea pig (1)	*Pulex irritans*	92	5	
	guinea pig (1)	*Ctenocephalides felis*	20	1	
Amazonas/ Utcubamba/ Cajaruro	dog (8)	*Ctenocephalides felis*	190	10	September, 2001
	dog (1)	*Pulex irritans*	1	1	
Amazonas/ Utcubamba/ Jamalca	dog (3)	*Ctenocephalides felis*	33	2	September, 2001
	dog (1)	*Rhiphicephalus sanguineus*	5	2	
	dog (1)	*Boophilus spp.*	1	1	
	dog (1)	*Pulex irritans*	1	1	
Amazonas/ Utcubamba/ Lonya Grande	dog (45)	*Ctenocephalides felis*	494	25	June, 2001
	dog (1)	*Pulex irritans*	20	1	
	cat (3)	*Ctenocephalides felis*	35	2	
	human (6)	*Pediculus humanus*	84	5	
	guinea pig (1)	*Ctenocephalides felis*	5	1	
	guinea pig (1)	*Pulex irritans*	12	1	
Amazonas/ Utcubamba/ El Milagro	fox (3)	*Pulex irritans*	32	3	November, 2000
	fox (1)	*Ctenocephalides felis*	4	2	
TOTAL			**1758**	**102**	

were analyzed using the Rp877p and Rp1258n primers, theses primers amplified the *glt*A gene of *Ricketsia* (Regnery et al. 1991).

The amplification reactions consisted of 5 µL of amplification buffer 10X, 5 µL (10 µM) of each primer, 5 µL (25 mM) of magnesium chloride, 2 µL (10 mM) of nucleotide triphosphates, 0.2 µL (5 UµL) of Ampli*Taq* Gold DNA polymerase (Applied Biosystems Inc.) and 5 µL of purified DNA to each tube. The final volume of the amplification reaction was 50 µL. Purified DNA from *B. bacilliformis* strain ATCC 35685 and a *Rickettsia felis* characterized culture were used as positive controls. The amplifications were performed on a 9700 Applied Biosystems thermocycler, and the cycles were as follows: 1 cycle for 95 °C for 10 min; 35 cycles at 95 °C for 0.5 min, 50 °C for 0.5 min, and 72 °C for 1 min; 1 cycle at 72 °C for 5 min; and a final soaking at 4 °C until analysis.

The amplified products were analyzed by electrophoresis in agarose gels, stained with ethidium bromide and visualized in a UV transilluminator.

The amplified products were then purified using QIAQuick Gel extraction Kit (QIAGEN Inc.) and sequenced using a Big Dye DNA terminator Kit (Applied Biosystems Inc.) on an ABI310 sequencer (Applied Biosystems) at the Peruvian National Institute of Health and following standard procedures. The sequences obtained were analyzed with GenBank using BLAST software (www.ncbi.nlm.nih.gov/BLAST). Clustalw software (http://align.genome.jp/) was used for alignment and comparison of sequences.

Results

A total of 1758 specimens were captured from humans, cats, dogs, guinea pigs and foxes (Table 1). Of these, 1399 (79.58 %) samples were identified as *Ctenocephalides felis* (collected from dogs, cats, guinea pigs and foxes). 235 (13.37%) specimens were *Pulex irritans* (collected from dogs, guinea pigs and foxes); 84 specimens (4.78%) were *Pediculus humanus* (collected from humans); 34 specimens (1.93%) were *Ctenocephalides canis* (collected from cats); 5 specimens (0.28%) were *Rhiphicephalus sanguineus* (collected from dogs); and 1 specimen (0.06%) was identified as *Boophilus spp.* (collected from a dog) (Table 1).

One hundred two pools were processed by PCR. Of these, 17 pools (16.67%) were PCR-positive using primers for *Bartonella* genera. These pools were confirmed as *Bartonella* by DNA sequencing.

Bartonella clarridgeiae (accession number AY836149) was detected in 4 pools of *C. felis* collected from three cats and one guinea pig, 1 pool of *C. canis* collected from a cat and 2 pools of *P. irritans* collected from a fox (all 100% homologous with each other). The AY836149 nucleotide sequence was compared with GenBank Database showing 100% homology with *B. clarridgeiae* Houston-2 cat strain (U84386) (Table 2).

Bartonella rochalimae was detected in 1 pool of *C. felis* collected from a cat and 2 pools of *P. irritans* collected from guinea pigs (all 100% homologous to GenBank accession number GU583842). The GU583842 nucleotide sequence was 100%

Table 2. Species of *Bartonella* detected in lice, fleas and ticks from San Ignacio (Cajamarca) and Utcubamba (Amazonas) in Peru.

Arthropod/ bacteria	*C. felis*	*C. canis*	*P. irritans*	*P. humanus*	*R. sanguineus*	Total
Bartonella clarridgeiae	2 cat (MZ), 1 cat (LG) 1 guinea pig (MZ)	1 cat (MZ)	2 fox (EM)	–	–	7
B. rochalimae	1 cat (MZ)	–	2guinea pig (MZ)	–	–	3
B. henselae	1 cat (MZ)	–	–	1 human (LG)	–	2
Bartonella sp.	–	–	4 guinea pig (3 MZ, 1 LG) 1 dog (MZ)	–	–	5

homologous with human isolated *B. rochalimae* BMGH strain (DQ683195) (Table 2).

Bartonella henselae was detected in 1 pool of *C. felis* collected from a cat and 1 of *P. humanus* collected from humans (accession numbers AY840994 and GU583844). Both AY840994 and GU583844 nucleotide sequences showed 100% homology with *B. henselae* strains: BR01 (GU056191), BCF01 (GU056188) and CS3 (FJ832097) (Table 2).

Bartonella spp. was detected in 5 pools of *P. irritans*: four pools collected from guinea pig and one from a dog, all 100% homologous to GenBank accession N° GU583846. The GU583846 nucleotide sequence showed 96.3% and 96.6% homology with *Bartonella* spp. DNA detected in a flea and blood collected from *Sigmodon hispidus* with GenBank accession numbers EF616666 and AF082322, respectively (Table 2).

Seventy-six pools (74.51%) were PCR positive using primers for *Rickettsia*. Of these, 66 pools were positive for *Rickettsia felis* (58 pools of *C. felis*, 2 pools of *C. canis* and 6 pools of *P. irritans*; all 100% homologous to GenBank no. GU583847). The GU583847 nucleotide sequence showed 100% homology with *Rickettsia felis* collected from *C. felis* (EU853837), *Liposcelis bostrychophila* (GQ329877, GQ329873), and *Pulex echidnophagoides* (GU447233) (Table 3).

Ten pools were positive for *Rickettsia* spp. (4 pools of *C. felis*, 4 pools of *P. irritans* and 2 pools of *P. humanus*), where one of the pools was reported as GenBank no. GU583848 and the other nine pools were 100% homologous to GU583849 (Table 3). The nucleotide sequence GU583848 showed 100% homology with *Rickettsia* spp. collected from *Pulex irritans* (EU853838), *Echidnophaga gallinacea* (DQ166938), *C. canis* (AF516333) and *C. felis* (AY953289); also the nucleotide sequence GU583849 showed 100% homology with *Rickettsia* sp. collected from *C. felis* (AY953288) and *C. canis* (AF516331). DNA of *Bartonella* and *Rickettsia* weren't detected in ticks.

Discussion

In this study, only 17 pools were confirmed as *Bartonella* species by DNA sequencing: *Bartonella clarridgeiae* (7 pools), *B. rochalimae* (3 pools), *B. henselae* (2 pools), and uncultured *Bartonella* spp. (5 pools).

Bartonella DNA was also detected in fleas in other regions of Peru. Parola and colleagues (Parola et al. 2002) reported the detection of *B. clarridgeiae-like* (potentially *B. rochalimae*) DNA (Eremeeva et al. 2007) and *B. vinsonii* DNA in fleas near the city of Cuzco. In addition, Raoult and collaborators (Raoult et al. 1997) also reported the detection of *B. quintana* DNA in lice collected in Calca, Cuzco.

In this study, a potentially new species of *Bartonella* in *Pulex irritans* collected from guinea pigs was found. The nucleotide sequence of *Bartonella sp.* showed 96% homology with *Bartonella* sp. DNA, which was detected in a flea and blood collected from *Sigmodon hispidus* (Abbot et al. 2007).

There are also previous reports of *Rickettsia* spp. in Peru. In the region of Sapillica, Piura; *Rickettsia felis* was detected in *C. felis* (2 pools of 59 samples) from dogs and the *Rickettsia massilae* group was detected in *Amblyomma maculatum* (one tick from a dog and one from a horse), *Anocenter nitens* (one tick from a horse) and *Ixodes boliviensis* (one tick from a horse) (Blair et al. 2004b). Also in this area, *Rickettsia* of the spotted

Table 3. Species of *Rickettsia* detected in lice, fleas and ticks from San Ignacio (Cajamarca) and Utcubamba (Amazonas) in Peru. MZ: Marizagua, LG: Lonya Grande, EM: El Milagro, CJ: Cajaruro, JM: Jamalca

Arthropod/ bacteria	*C. felis*	*C. canis*	*P. irritans*	*P. humanus*	*R. sanguineus*	Total
Rickettsia felis	1 guinea pig (MZ) 8 cat (7 MZ, 1 LG) 48 dog (26 MZ, 15 LG, 1 JM, 6 CJ) 1 fox (EM)	2 cat (MZ)	2 dog (MZ) 3 guinea pig (MZ) 1 fox (EM)	–	–	66
Rickettsia sp.	4 dog (2 CJ, 1 JM, 1 LG)	–	2 dog (MZ, LG) 2 guinea pig (MZ, LG)	2 human (LG)	–	10

fever group (SFG) was detected in four febrile patients by PCR analysis; the detected SFG in this study was approximately 95% homologous with *R. conorii* and *R. akari* (Blair et al. 2004b). Other reports indicated the presence of anti-*Rickettsia* antibodies in habitants of Piura, Junin and Cusco (Schoeler et al. 2005). Although a higher proportion of positive pools for *R. felis* (66 positive pools out of 102) were obtained, previous reports are congruent with the results found. In addition, we detected *Rickettsia sp* in Peru, which are 100% homologous with *Rickettsia* spp. detected in fleas from other countries. Also this sequence showed 95% homology with candidatus *R. andeanea* reported in fleas collected from Sullana in northwestern Peru (Blair et al. 2004a).

In conclusion, the results indicate the presence of several *Bartonella* species, and *Rickettsia* in Peru. The clinical and epidemiological significance of possible infections in similar endemic areas in Peru should continue to be investigated and these results should be considered for the epidemiologic and entomologic surveillance. Furthermore, the possible transmission to humans should also be evaluated in the future.

Acknowledgments

This work is dedicated in memory of Dr. Hugo Vizcarra Franco, principal professor of Medicine Faculty of Universidad Nacional Mayor de San Marcos, he was member of our research team and died during project development. We thank villagers from Marizagua, El Ron, Aserradero, Jamalca, El Valor, Yungasuyo, Pucallpa and Danja for permit the sampling in his animals. We specially thank Dr. Michael Kosoy and Sara Billeter from CDC, Colorado USA; and Dr. Audrey Lenhart from Liverpool Scholl of Tropical Medicine of England for critically reading the manuscript. This work was supported by a grant from the Fundacion Hipolito Unanue of Lima, Peru.

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