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Short Communication

Molecular detection and identification of *Rickettsia felis* in *Polygenis* (Siphonaptera, Rhopalopsyllidae, Rhopalopsyllinae) associated with cricetid rodents in a rural area from central Argentina

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ABSTRACT

The aim of this study was to detect and identify the presence of *Rickettsia* in fleas associated with cricetid rodents from northeastern Buenos Aires Province, Argentina. Sixteen fleas belonging to three species of *Polygenis* were collected from 56 cricetid rodents and analyzed for the presence of *Rickettsia* performing the conventional polymerase chain reaction (PCR) technique. Only one specimen of *Polygenis (Polygenis) axius axius* collected from *Oxymycterus rufus* was positive for *Rickettsia felis* using the *gltA* gene, and to *ompA* gene. This is the first report of *R. felis* in a Rhopalopsyllidae flea from Argentina, and the first detection of this bacterium in *P. (P.) a. axius*. Since both, *O. rufus* and *P. (P.) a. axius*, are common in areas close to humans, and enzootic cycle of *R. felis* is not fully understood, the results herein obtained might be of epidemiological importance. Further studies are needed in order to analyze the capacity of the species of *Polygenis* to transmit *R. felis*.

1. Introduction

Fleas are common components of mammalian ectoparasite communities, with rodents being their most frequent hosts worldwide (Linardi and Guimarães, 2000). Argentina is not an exception, where about 130 species and subspecies of fleas were reported, and most of them associated with rodents (Lareschi et al., 2016). Fleas are parasites only in the adult stage, feed on host blood, and can be found on them or their nests and burrows (Linardi and Guimarães, 2000). In addition, fleas may be vectors of pathogens, such as *Rickettsia typhi*, which causes the Murine Typhus, and therefore provide a natural means for their dispersal (Peniche-Lara et al., 2015). Most of the epidemiological studies on fleas involve species associated with domestic animals (e.g. Nava et al., 2008; Hornok et al., 2018), while those concerning fleas associated with wild animals are scarce worldwide (Horta et al., 2007). The genus *Rickettsia* comprises a group of Gram-negative intracellular bacteria transmitted by arthropod vectors, such as fleas and ticks, and many species are recognized of medical or veterinary importance (Parola, 2011).

About 45 species and subspecies have been described for *Polygenis*, most of them presented in South America and associated with cricetid rodents (Linardi and Guimarães, 2000; Lareschi et al., 2016). However, there are scarce reports of these fleas associated with zoonotic bacteria (Horta et al., 2007; Peniche-Lara et al., 2015). Herein we analyze the presence of *Rickettsia* in fleas parasitic of cricetid rodents in a semi-urbanized area of La Plata city in central Argentina.

2. Materials and methods

The study was carried out in a semiurbanized area located 14 km south of the city of La Plata (Arana, Partido de La Plata, 35°W′25.28”S, 57°54′33.56”W, 14 m a.s.l.) in the northeast of Buenos Aires Province, Argentina. The area belongs to Humid Pampas ecoregion with steppe or pseudeosteppe of grasses as the dominant vegetation (Burkart et al., 1999). The location compromises about five hectares, surrounding by one permanent living family. The vegetal composition represents a fragmented area composed by typical low bushes such as *Baccharis* sp. and pastureland of *Bromus* sp. with thistles of the genus *Dipsacus* all connected with a no permanent small brooklet.

Samplings were conducted on 4th of July, 4th of September, and 29th of November 2017. The sampling effort was 80 traps per night placed in a distance of five meters one from each other. Rodents were captured alive by using Sherman-like traps baited with oat, anesthetized with sulfuric ether and sacrificed by cervical dislocation. All

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procedures were conducted following the ethical guidelines established by the American Society of Mammalogists (Sikes and The Animal Care and Use Committee of the American Society of Mammalogists, 2016). Fleas were collected in the field, by examining the fur of the hosts using brushes and forceps and were stored in alcohol 96°.

For DNA extraction, an incision at abdominal level was performed on every flea, using a sterile scalpel, to preserve the exoskeleton for further preparation for its identification at optic microscope. Genomic DNA extraction was conducted using Chelex®-100 (Bio-Rad Laboratories, CA, USA) adapting the procedure described by Miura et al. (2017) as it follows: a 5% solution of Chelex 100 resin in sterile distilled water was prepared, 100 µL of the homogenized mixture was placed in a microcentrifuge tube together with the individual flea sample, 5 µL of proteinase K were added; it was left incubating for 18 h at 56 °C, then the enzyme was inactivated at 95 °C for 10 min and once cold it was centrifuged for 5 min at 14000 rpm. In a first instance a conventional PCR targeting the *gltA* gene (citrate synthase) was performed to detect the presence of *Rickettsia* genus, by using primers: CS-239 CTCTTCTCATCCTATGGCTATTAT and CS-1069 CAGGGTCTTCGT GCATTTCTT (Labruna et al., 2004). Samples shown to be positive to *gltA* were used to amplify a ca. 500-bp fragment of the 190-kDa *ompA* (outer membrane protein) to identify *Rickettsia* at the specific level with the primers Rr190.70p: ATGGCGAATATTTCTCCAAAA and Rr190.602n: AGTGCAGCATTCGCTCCCCCT (Regnery et al., 1991). Sequences of the *ompA* gene were chosen because they have enough polymorphism to perform determination to the specific level among the *Rickettsia* species belonging to the Spotted Fever group. For each reaction, a negative control with ultrapure water, and positive control, consisting in DNA of *Rickettsia vini*, were included. Afterwards, PCR products of positive *ompA* samples were purified with Wizard® Genomic DNA Purification kit (Promega®) and sequenced using ABI 3730XLs genetic analizer, Macrogen Inc. (Korea). The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual edition whenever it was necessary and aligned with the program Clustal W (Thompson et al., 1994). DNA sequence obtained of the *ompA* amplicons were subjected to an analysis of comparison of sequences by using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine similarities with species of the genus *Rickettsia*.

After DNA extraction, the exoskeletons of the fleas were washed with distilled water, cleared in 10% KOH, dehydrated in a series of ethanol (80% to 100%), diaphanized in eugenol and mounted in Canada balsam, for their identification at optic microscope (Leica DM 2500), following descriptions and keys provided in Linardi and Guimarães (2000). Fleas were deposited at the Colección de Entomología, Museo de la Plata, Argentina. Rodents were identified by C. Galliari (CEPAVE) and U. Pardiñas (IDEAus, Argentina) and deposited at the Colección de Mamíferos del Centro Nacional Patagónico (CENPAT), Puerto Madryn, Argentina.

3. Results

Fifty six rodents belonging to the following three species were captured (Cricetidae, Sigmodontinae): *Oxymycterus rufus* (Fischer), *Akodon azarae* Fischer and *Oligoryzomys flavescens* (Waterhouse). From the 56 rodents, a total of sixteen fleas were collected and identified as (Siphonaptera, Rhopalopsyllidae, Rhopalopsyllinae) *Polygenis (Polygenis) axius axius* (Jordan & Rothschild), *Polygenis (Polygenis) platensis* (Jordan & Rothschild) and *Polygenis (Neopolygenis) atopus* (Jordan & Rothschild). Number of all flea species collected from each host species per month and their prevalences are detailed in Table 1.

All the sixteen fleas were analyzed, and out of them only one was positive to *gltA* and *ompA* genes. This positive flea was a male identified as *P. (P.) a. axius* (collection number CGf654; 4/VII/2017) collected from the rodent *O. rufus*. Comparison of the *ompA* sequence (444 bp) obtained in this work (Genbank™ accession number: **MT036381**) with those sequences available in GenBank shew that the *ompA* sequence obtained from *P. (P.) a. axius* is 100% similar to *ompA* sequences of *Rickettsia felis* from Mexico (GenBank™ accession number **AJ563398**), Chile (GenBank™ accession number **KY913651**) and Malta (GenBank™ accession number **MG893579**) (99–100% query cover and 0 e-values). The alignment with these four *ompA* sequences can be visualized in the Supplementary file 1.

4. Discussion

Herein we report for the first time *R. felis* associated with fleas of the family Rhopalopsyllidae associated with cricetids from Argentina, extending with *P. (P.) a. axius* the list of arthropod species associated with this bacterium. Previously, *R. felis* was reported from *Polygenis (Neopolygenis) atopus* and *Polygenis (Polygenis) odiosus*, parasitic of marsupials from Brazil and cricetids from Mexico, respectively (Horta et al., 2007; Peniche-Lara et al., 2015), and from the cat flea *Ctenocephalides felis* (Pulicidae) from Rafaela city in Santa Fe Province, Argentina (Nava et al., 2008).

Comparison of the *ompA* sequence obtained during this study shows a high similarity with three *R. felis* sequences from Mexico, Chile and Malta, all isolated from *C. felis* collected from domestic cats (Zavala-Castro et al., 2005; Hornok et al., 2018; Müller et al., 2018). Here, the relationship between the sequences seems to include all fleas. Results herein obtained are in accordance with Horta et al. (2007), who proposes that *Polygenis* might be able to maintain *R. felis* infection in areas where *C. felis* is not present. This horizontal transfer is well described for *R. felis*, resulting in low genetic variability within the species (Hornok et al., 2018).

Rickettsia felis was initially detected three decades ago in tissues of *C. felis* (Parola, 2011). Since then, multiple reports have been documented worldwide associated with these fleas species and further parasitic arthropods such as ticks or mites (Horta et al., 2007; Parola, 2011) suggesting that these arthropods may be implied in the *R. felis* life cycle. Nowadays, the sanitary importance of *R. felis* is still in discussion. While some authors consider this bacterium as a non-pathogenic endosymbiont of fleas (e.g. Billeter and Metzger, 2017), others mention *R. felis* as the causative agent of a human emergent disease called Flea-borne Spotted Fever (e.g. Parola, 2011).

Polygenis (P.) a. axius was reported from central Argentina, southern Brazil and Uruguay, associated with a variety of marsupials and cricetids, *Oxymycterus* species included (Linardi and Guimarães, 2000; Lareschi et al., 2006; Lareschi et al., 2016; Benitez-Ibalo et al., 2020). The low number of fleas reported here agrees with the results obtained from studies carried out in nearby areas in the province of Buenos Aires (Lareschi and Krasnov, 2010). Fleas, including *Polygenis* species, alternate periods in the host with periods in the soil or in the nest of the hosts, where the immature stages occur (Marshall, 1981). Therefore, the characteristics of the locality, such as humidity and hydroperiod, have a strong effect on the abundance of fleas limiting the development of immature stages (Lareschi and Krasnov, 2010).

In addition, *O. rufus* has wide distribution in central and northeast regions of Argentina and it is also reported from Central Brazil (Minas Gerais State), inhabiting grasslands and steppes (De Oliveira and Gonçalves, 2015). It is also very abundant in the marshes from northeastern Buenos Aires Province, close to areas used by humans for activities such agriculture and grazing (Lareschi, 2004; Gómez Villafañe et al., 2012). Thus, the close proximity of *O. rufus* with humans and its wide distribution may be of epidemiological importance. Since the enzootic cycle of *R. felis* is not fully understood, *O. rufus* as well as its fleas, like *P. (P.) a. axius*, may impact in the distribution of the bacteria, especially considering the generalist pattern mentioned above for this flea species. However, further studies are needed in order to analyze the capacity of the species of *Polygenis* to transmit *R. felis*. Considering the wide distribution of *Polygenis* in the neotropics, as well as the scarce studies to determine their association with pathogenic bacteria, the

Table 1
Number of fleas collected with their prevalences in percentage (%). The number of host and fleas is given per month in the study period (J = July, S = September, N = November).

Host species	*Oxymycterus rufus*			*Akodon azarae*			*Oligoryzomys flavescens*		
Month	J	S	N	J	S	N	J	S	N
Number of captured rodents	14	6	5	10	9	3	5	4	0
Flea species									
Polygenis (Polygenis) axius axius	3 (22%)	1 (16%)	2 (40%)	1 (10%)	1 (11%)	1 (33%)	1 (20%)	–	–
Polygenis (Neopolygenis) atopus	2 (15%)	1 (16%)	–	–	1 (11%)	1 (33%)	–	–	–
Polygenis (Polygenis) platensis	–	–	–	–	–	1 (33%)	–	–	–
Total of fleas	5	2	2	1	2	3	1	–	–

presence of *R. felis* in species of this genus should not be underestimated.

Ethical statement

In the manuscript titled "Molecular detection and identification of *Rickettsia felis* in *Polygenis* (Siphonaptera, Rhopalopsyllidae, Rhopalopsyllinae) associated with cricetid rodents in a rural area in central Argentina", authors Mauricio Melis, Mario Espinoza-Carniglia, Ekaterina Savchenko, Santiago Nava, and Marcela Lareschi, the fleas were collected from cricetid rodents according to the general guide of the OIE (Organización Mundial de Sanidad Animal). This study was authorized by Ministerio de Agroindustria (Buenos Aires Province, Argentina) corresponding to disposition 66/17.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2020.100445.

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