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Detection of *Bartonella* and *Rickettsia* in small mammals and their ectoparasites in México

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Fleas and sucking lice are important vectors of multiple pathogens causing major epidemics worldwide. However these insects are vectors of a wide range of largely understudied and unattended pathogens, especially several species of bacteria's of the genera *Bartonella* and *Rickettsia*. For this reason the aim of the present work was to identify the presence and diversity of *Bartonella* and *Rickettsia* species in endemic murine typhus foci in Hidalgo, México. A cross-sectional study was carried out to collect small mammals and their associated ectoparasites during October, 2014. Samples of liver and ear of hosts, and ectoparasites were fixed in absolute ethanol and examined to identify the presence of *Bartonella* and *Rickettsia* DNA by the amplification of specific fragments of the *gltA* and *ompB* genes using conventional PCR. The recovered sequences were compared with those deposited in GenBank, and phylogenetic analyzes were carried out to identify the position of the pathogens detected with respect to the valid species previously reported worldwide. A total of 47 fleas and 172 sucking lice, belonging to five families (Ceratophyllidae, Leptopsyllidae, Ctenophtalmidae, Hoplopleuridae, Polyplacidae) and related to six species were collected from 40 rodents of four species and one shrew. Only four hosts (two *P. beatae*, and two *R. norvergicus*) were positive to *Bartonella elizabethae*, *Bartonella vinsonii* and *Rickettsia typhi*. In the case of ectoparasites, 23 specimens of two flea species (*Peromyscopsylla hesperomys* and *Plusaetis mathesoni*) tested positive for *B. vinsonii*. No evidence of *Bartonella* or *Rickettsia* was detected in any lice. Our findings represent the first record of *Bartonella elizabethae* a confirmed zoonotic pathogen causing endocarditis in México and several new associations of *Bartonella* with Mexican flea species, which highlight the importance of the establishment of active entomological surveillance in wildlife.

Las pulgas y los piojos son vectores de patógenos causantes de epidemias de importancia histórica. Sin embargo, estos insectos son vectores de una amplia gama de patógenos poco estudiados y no atendidos, especialmente varias especies de bacterias de los géneros *Bartonella* y *Rickettsia*. Por este motivo, el objetivo del presente trabajo fue identificar la presencia y diversidad de las especies de *Bartonella* y *Rickettsia* en un foco de tifus murino en el estado de Hidalgo, México. Se realizó un estudio transversal para recolectar hospederos y sus ectoparásitos durante octubre de 2014. Las muestras de hígado y oreja de los hospederos y los ectoparásitos se fijaron en etanol absoluto y se examinaron para identificar la presencia de ADN de *Bartonella* y *Rickettsia* mediante la extracción de DNA y amplificación de fragmentos específicos de los genes *gltA* y *ompB*. Las secuencias obtenidas fueron comparadas con aquellas depositadas en GenBank y se realizaron análisis filogenéticos para identificar la posición de los patógenos detectados respecto a las especies válidas previamente reportadas a nivel mundial. Se recolectaron un total de 47 pulgas y 172 piojos chupadores, pertenecientes a seis especies de cinco familias (Ceratophyllidae, Leptopsyllidae, Ctenophtalmidae, Hoplopleuridae, Polyplacidae) asociados con 40 roedores de cuatro especies y una musaraña. Sólo cuatro hospederos (dos *P. beatae*, y dos *R. norvergicus*) resultaron positivos para *Bartonella elizabethae*, *Bartonella vinsonii* y *Rickettsia typhi*. En el caso de los ectoparásitos, 23 ejemplares de dos especies de pulgas (*Peromyscopsylla hesperomys* y *Plusaetis mathesoni*) fueron positivos para *B. vinsonii*. No se detectó evidencia de ninguno de los dos patógenos en los piojos analizados. Nuestros hallazgos representan el primer registro de *Bartonella elizabethae*, un patógeno zoonótico confirmado que causa endocarditis en México y varias asociaciones nuevas de *Bartonella* con especies de pulgas mexicanas, lo cual resalta la necesidad de implementar vigilancia entomológica activa para el monitoreo de estos patógenos en animales silvestres.

Keywords: *Bartonella elizabethae*; emerging diseases; *Rickettsia typhi*; small mammals; vectors.

Introduction

Fleas and sucking lice are important vectors of multiple pathogens causing major epidemics worldwide, such as plague (*Yersinia pestis*) and epidemic typhus (*Rickettsia prowazekii*). Despite the historical importance of both diseases, this group of ectoparasites has been little studied with respect to other vectors such as mosquitoes or ticks (Gillespie *et al.* 2009; Bitam *et al.* 2010; Eisen and Gage 2012). However, these groups of insects are hosts for a wide range of largely understudied pathogens, especially several species of bacteria of the genera *Bartonella* and *Rickettsia* (Bitam *et al.* 2010). The genus *Bartonella* includes at least 33 species of Gram-negative, intracellular and slow-growing coccobacilli with complex life cycles including multiple vertebrate hosts and vectors, such as *B. elizabethae* and *B. vinsonii arupensis,* declared pathogens causing endocarditis in humans and dogs (Breitschwerdt and Kordick 2000; Tsai *et al.* 2011; Kosoy *et al.* 2012; Regier *et al.* 2016). On the other hand, *Rickettsia* encompasses 26 species of obligate intracellular bacteria which are transmitted by different groups of hematophagous arthropods such as ticks, lice and fleas (Fournier and Raoult 2009; Merhej *et al.* 2014). *Rickettsia* species are classified into four groups, two of which are pathogens for man: members of the Spotted Fever group [SGF] (*R. conorii*, *R. massiliae*, *R. rickettsii* and *R. parkeri*) and Typhus group [TG] (*R. prowazekii* and *R. typhi*), this latter group is transmitted exclusively by lice and fleas, which cause epidemic and murine typhus (Fournier *et al.* 2003; Fournier and Raoult 2009).

In recent decades with the advent of molecular biology techniques, the number of species or strains of both bacteria genera has increased exponentially (Merhej *et al.* 2014; Regier *et al.* 2016). Particularly, fleas and sucking lice associated with rodents are the groups in which more studies have focused for the detection of pathogens, with the identification of 16 validated species of *Bartonella*, nine of *Rickettsia* and more than 17 new linages near to several validated taxa (but which require isolation for formal identification) for both genera, associated with 45 flea species and seven sucking lice which are also associated with 42 species of rodents in 24 countries around the world (Table 1).

Table 1. *Bartonella* and *Rickettsia* species detected in fleas and sucking lice associated with rodents worldwide

Bacteria species	Flea	Host	Country	References
B. birtlesii	*Ctenophtalmus andorrensis catalanensis*	*Apodemus sylvaticus*	Spain	Cevidanes *et al.* 2017
	Leptopsylla taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al.* 2017
B. coopersplainsensis	*Stephanocircus pectinipes*	*Rattus fuscipes*	Australia	Kaewmongkol *et al.* 2011
B. doshiae	*Xenopsylla cheopis*	*Rattus* sp.	Afghanistan	Marie *et al.* 2006
B. elizabethae	*Leptopsylla segnis*	*Mus spretus*	Algeria	Bitam *et al.* 2012
	Synosternus cleopatrae	*Gerbillus pyramidum*	Israel	Morick *et al.* 2010
	Synopsyllus fonquerniei	*Rattus rattus*	Madagascar	Brook *et al.* 2017
	X. cheopis	*Rattus norvergicus*	Algeria	Bitam *et al.* 2012
			USA	Frye *et al.* 2015
		R. rattus	Algeria	Bitam *et al.* 2012
		Rattus tanezumi	Indonesia	Winoto *et al.* 2005
		Rattus sp.	Afghanistan	Marie *et al.* 2006
			Nigeria	Kamani *et al.* 2013
B. grahamii	*Ctenophthalmus agyrtes*	ND	Lithuania	Lipatova *et al.* 2015
	Ct. andorrensis catalanensis	*A. sylvaticus*	Spain	Cevidanes *et al.* 2017
	Ctenophthalmus nobilis	*Myodes glareolus*	England	Bown *et al.* 2004
	Megabothris turbidus	ND	Lithuania	Lipatova *et al.* 2015
	Megabothris walkeri	ND	Lithuania	Lipatova *et al.* 2015
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al.* 2015
	Xenopsylla ramesis	ND	Israel	Rzotkiewicz *et al.* 2015
B. henselae	*X. ramesis*	ND	Israel	Rzotkiewicz *et al.* 2015
		Meriones tristrami	Israel	Morick *et al.* 2010
B. koehlerae	*Xenopsylla gerbilli*	*Meriones lybicus*	Afghanistan	Marie *et al.* 2006
B. phoceensis	*X. cheopis*	*R. tanezumi*	Indonesia	Winoto *et al.* 2005
B. queenslandensis	*X. cheopis*	*Rattus* sp.	Thailand	Klangthong *et al.* 2015
B. quintana	*X. gerbilli*	*Meriones lybicus*	Afghanistan	Marie *et al.* 2006
B. rattaustraliani	*Stephanocircus dasyure*	*R. fuscipes*	Australia	Kaewmongkol *et al.* 2011
B. rattimassiliensis	*X. cheopis*	*R. tanezumi*	Indonesia	Winoto *et al.* 2005
B. rochalimae	*X. cheopis*	*R. norvergicus*	USA	Frye *et al.* 2015
B. taylorii	*Ct. agyrtes*	ND	Lithuania	Lipatova *et al.* 2015
	Ct. andorrensis catalanensis	*A. sylvaticus,* *C. russula, M. spretus*	Spain	Cevidanes *et al.* 2017
	Ct. nobilis	*M. glareolus*	England	Bown *et al.* 2004
	Ctenophthalmus uncinatus	ND	Lithuania	Lipatova *et al.* 2015
	Hystrichopsylla talpae	ND	Lithuania	Lipatova *et al.* 2015

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Bacteria species	Sucking lice	Host	Country	References
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	M. turbidus	ND	Lithuania	Lipatova *et al*. 2015
	M. walkeri	ND	Lithuania	Lipatova *et al*. 2015
	X. gerbilli	*M. lybicus*	Afghanistan	Marie *et al*. 2006
B. tribocorum	*Ctenophtalmus* sp.	*ND*	Nigeria	Kamani *et al*. 2013
	X. cheopis	*R. norvergicus*	USA	Reeves *et al*. 2007a; Frye *et al*. 2015
		R. rattus	Algeria	Bitam *et al*. 2012
		R. tanezumi flavipectus	China	Li *et al*. 2007
		Rattus sp.	Thailand	Klangthong *et al*. 2015
B. vinsonii	*Polygenis bohlsi bohlsi*	*Thrichomys fosteri*	Brazil	de Sousa *et al*. 2018
	Polygenis gwyni	*Sigmodon hispidus*	USA	Abbot *et al*. 2007
B. vinsonii arupensis	*Malareus sinomus*	*Peromyscus eremicus*	México	Zapata-Valdés *et al*. 2018
	Orchopeas leucopus	*P. eremicus*		
		Peromyscus leucopus, Peromyscus maniculatus		Fernández-González *et al*. 2016
	Pleochaetis exilis	*Onycomys torridus*		Zapata-Valdés *et al*. 2018
B. vinsonii vinsonii	*Ctenophthalmus pseudagyrtes*	*Microtus* sp.	USA	Reeves *et al*. 2007a
	Meringis parkeri	*Onychomys arenicola, Onychomys leucogaster*	México	Fernández-González *et al*. 2016
	Orchopeas sexdentatus	*Neotoma albigula*	México	Fernández-González *et al*. 2016
	Pleochaetis exilis	*N. albigula, O. arenicola, O. leucogaster, P. maniculatus*	México	Fernández-González *et al*. 2016
B. washoensis	*Orchopeas hirsuta*	*Cynomys* sp.	USA	Stevenson *et al*. 2003; Reeves *et al*. 2007b
		Cynomys ludovicianus	México	Zapata-Valdés *et al*. 2018
	Orchopeas howardi	*Sciurus carolinensis*	USA	Durden *et al*. 2004
	Oropsylla montana	*Otospermophilus beecheyi*	USA	Osikowicz *et al*. 2016
	Pulex sp.	*C. ludovicianus*	México	Fernández-González *et al*. 2016
	Thrassis fotus	*Cynomys* sp.	USA	Reeves *et al*. 2007b
Bartonella near *birtlesii*	*O. howardi*	*S. carolinensis*	USA	Reeves *et al*. 2005b
Bartonella near *clarridgeiae*	*Ctenophthalmus lushuiensis*	*Eothenomys* sp.	China	Li *et al*. 2007
	L. segnis	*R. rattus*	Egypt	Loftis *et al*. 2006
	P. gwyni	S. hispidus	USA	Abbot *et al*. 2007
Bartonella near *doshiae*	*Ct. andorrensis catalanensis*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
Bartonella near *elizabethae*	*Ct. andorrensis catalanensis*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	Leptopsylla algira	ND	Israel	Rzotkiewicz *et al*. 2015
		Mus musculus	Israel	Morick *et al*. 2010
	L. taschenbergi amitina	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	Ornithophaga sp.	*M. spretus*	Portugal	De Sousa *et al*. 2006
	Stenoponia tripectinata	*M. spretus*	Portugal	De Sousa *et al*. 2006
		R. rattus	Portugal	De Sousa *et al*. 2006
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al*. 2015
		G. pyramidum	Israel	Morick *et al*. 2010
	X. cheopis	*Rattus* sp.	Thailand	Klangthong *et al*. 2015
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *grahamii*	*Meringis altipecten*	*O. arenicola, O. leucogaster, Dipodomys merriami*	México	Fernández-González *et al*. 2016
	Meringis arachis	*O. arenicola, O. leucogaster, D. merriami*	México	Fernández-González *et al*. 2016
	M. parkeri	*O. arenicola, O. leucogaster, D. merriami*	México	Fernández-González *et al*. 2016
	Nosopsyllus fasciatus	*Rattus surifer*	Thai-Myanmar Border	Parola *et al*. 2003

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Bacteria species	Sucking lice	Host	Country	References
	P. exilis	*O. arenicola, O. leucogaster*	México	Fernández-González *et al*. 2016
	Sy. cleopatrae	*Meriones sacramenti*	Israel	Morick *et al*. 2010
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *henselae*	*Or. howardi*	*Glaucomys volans*	USA	Reeves *et al*. 2007a
	Sy. cleopatrae	*Gerbillus andersoni allenbyi*	Israel	Morick *et al*. 2010
Bartonella near *phoceensis*	*X. cheopis*	*R. norvergicus, R. rattus*	Egypt	Loftis *et al*. 2006
Bartonella near *quintana*	*Or. howardi*	*S. carolinensis*	USA	Durden *et al*. 2004
Bartonella near *rochalimae*	*L. taschenbergi amitina*	*A. sylvaticus*	Spain	Cevidanes *et al*. 2017
	X. cheopis	*R. norvegicus*	Algeria	Bitam *et al*. 2012
	X. ramesis	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella near *taylorii*	*Ct. lushuiensis*	*Eothenomys* sp.	China	Li *et al*. 2007
Bartonella near *tribocorum*	*X. cheopis*	*R. rattus*	Benin	Leulmi *et al*. 2014
Bartonella near *vinsonii arupensis*	*Sy. cleopatrae*	ND	Israel	Rzotkiewicz *et al*. 2015
Bartonella sp.	*Echinophaga gallinacea*	*Dipodomys spectabilis*	México	Fernández-González *et al*. 2016
	Ct. andorrensis catalanensis	*C. russula*	Spain	Cevidanes *et al*. 2017
	M. arachis	*D. spectabilis*	México	Fernández-González *et al*. 2016
	M. altecpin	*D. spectabilis, O. arenicola*	México	Fernández-González *et al*. 2016
	Or. hirsuta	*Cynomys* sp.	USA	Reeves *et al*. 2007b
	Sy. cleopatrae	ND	Israel	Rzotkiewicz *et al*. 2015
	Thrassis aridis	*D. spectabilis*	México	Fernández-González *et al*. 2016
	X. cheopis	*R. norvegicus*	Algeria	Bitam *et al*. 2012
		R. rattus	Algeria, Israel	Morick *et al*. 2010; Bitam *et al*. 2012
R. conorii	*Stivalius aporus*	*Mus caroli*	Taiwan	Kuo *et al*. 2016
R. felis	*Acropsylla episema*	*Apodemus agrarius*	Taiwan	Kuo *et al*. 2016
	Anomiopsyllus nudata	*N. albigula*	USA	Stevenson *et al*. 2005
	Ctenocephalides felis	*Peromyscus yucatanicus*	México	Peniche Lara *et al*. 2015
		R. norvegicus	Cyprus	Psaroulaki *et al*. 2006
		R. rattus	Cyprus	Psaroulaki *et al*. 2006
	Ct. agyrtes	*Apodemus flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
	Ctenophthalmus calceatus calceatus	*Lophuromys aquilus*	Tanzania	Leulmi *et al*. 2014
	Ctenophtalmus sp.	*R. norvegicus*	Portugal	De Sousa *et al*. 2006
	H. talpae	*Micromys minutus*	Lithuania	Radzijevskaja *et al*. 2018
	L. segnis	*Mus* sp.	Algeria	Bitam *et al*. 2009
	Polygenis odiosus	*Ototylomys phyllotis*	México	Peniche Lara *et al*. 2015
	S. aporus	*M. caroli*	Taiwan	Kuo *et al*. 2016
	X. cheopis	*R. norvegicus*	Cyprus	Christou *et al*. 2010
		R. rattus	Cyprus, Madagascar	Christou *et al*. 2010; Rakotonanahary *et al*. 2017
		Rattus sp.	Afghanistan, Algeria	Marie *et al*. 2006; Bitam *et al*. 2009
R. helvetica	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
	M. turbidus	*A. flavicollis*		
		M. minutus		
	M. walkeri	*A. flavicollis*		
R. japonica	*S. aporus*	*M. caroli*	Taiwan	Kuo *et al*. 2016
R. monacensis	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
R. raoultii	ND	*A. flavicollis, Myodes glareolus*	Germany	Obiegala *et al*. 2016
R. typhi	*Ctenophthalmus congeneroides*	*A. agrarius*	South Korea	Kim *et al*. 2010
	L. segnis	*R. norvegicus*	Cyprus	Christou *et al*. 2010
		R. rattus	Cyprus, Egypt, Portugal	De Sousa *et al*. 2006, Loftis *et al*. 2006; Christou *et al*. 2010
	Rhadinopsylla insolita	*A. agrarius*	South Korea	Kim *et al*.2010
	Xenopsylla brasiliensis	*Mastomys natalensis*	Tanzania	Leulmi *et al*. 2014

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Bacteria species	Sucking lice	Host	Country	References
		R. rattus	Tanzania	Leulmi *et al*. 2014
		Rattus sp.	Democratic Republic of the Congo	Leulmi *et al*. 2014
	X. cheopis	*R. norvegicus*	Cyprus, Egypt	Loftis *et al*. 2006; Christou *et al*. 2010
		R. rattus	Benin, Cyprus, Egypt, Madagascar	Loftis *et al*. 2006; Christou *et al*. 2010; Leulmi *et al*. 2014, Rakotonanahary *et al*. 2017
		Rattus sp.	Argelia	Bitam *et al*. 2009
Rickettsia prowazekii	*Or. howardii*	*G. volans*	USA	Sonenshine *et al*. 1978
Candidatus Rickettsia Asemboensis	*E. gallinacea*	*R. rattus*	Egypt	Loftis *et al*. 2006
	S. cleopatrae	ND	Israel	Rzotkiewicz *et al*. 2015
	X. ramesis	*Gerbillus dasyurus, Meriones tristrami, M. musculus*	Israel	Rzotkiewicz *et al*. 2015
Rickettsia felis-like	*X. ramesis*	ND	Israel	Rzotkiewicz *et al*. 2015
Rickettsia near *monacensis*	*Oropsylla hirsuta*	*Cynomys* sp.	USA	Reeves *et al*. 2007b
Rickettsia sp. Oh16	*Or. howardi*	*S. carolinensis*	USA	Reeves *et al*. 2005
Rickettsia sp. TwKM01	*S. aporus*	*A. agrarius*	Taiwan	Kuo *et al*. 2016
Rickettsia endosymbiont of Eucoryphus brunneri	*Ct. agyrtes*	*A. flavicollis*	Lithuania	Radzijevskaja *et al*. 2018
B. henselae	*Neohaematopinus sciuri*	*S. carolinensis*	USA	Durden *et al*. 2004
B. phoceensis	*Hoplopleura pacifica*	*R. norvegicus*	Egypt	Reeves *et al*. 2006
	Polyplax spinulosa	*R. norvegicus*	Taiwan	Tsai *et al*. 2010
	Polyplax sp.	*R. rattus*	Madagascar	Brook *et al*. 2017
		Rattus sp.	Thailand	Klangthong *et al*. 2015
B. rattimassiliensis	*Hoplopleura pacifica*	*R. norvegicus*	Egypt	Reeves *et al*. 2006
	Polyplax spinulosa	*R. norvegicus*	Egypt, Taiwan	Reeves *et. al*. 2006; Tsai *et al*. 2010
	Polyplax sp.	*R. rattus*	Madagascar	Brook *et al*. 2017
		Rattus sp.	Thailand	Klangthong *et al*. 2015
B. tribocorum	*Polyplax spinulosa*	*R. norvegicus*	Taiwan	Tsai *et al*. 2010
B. vinsonii	*Hoplopleura hirsuta*	*S. hispidus*	México	Sánchez-Montes *et al*. 2016b
B. washoensis	*Neohaematopinus sciuri*	*S. carolinensis*	USA	Durden *et al*. 2004
Bartonella near *tribocorum*	*Polyplax spinulosa*	*R. norvegicus*	Egypt	Reeves *et al*. 2006
Bartonella near *washoensis*	*Hoplopleura sciuricola*	*S. carolinensis*	USA	Durden *et al*. 2004
Bartonella sp.	*Polyplax* sp.	*Thrichomys apereoides*	Brazil	Fontalvo *et al*. 2017
R. prowazekii	*Neohaematopinus sciuropteri*	*G. volans*	USA	Sonenshine *et al*. 1978
	*Polyplax spinulosa**	*R. norvegicus*	México	Mooser *et al*. 1931
R. typhi	*Enderleinellus marmotae*	*Marmota monax*	USA	Reeves *et al*. 2005
	Hoplopleura pacifica	*R. norvegicus*	Egypt	Reeves *et al*. 2006

In México, nine taxa of fleas (*Ctenocephalides felis*, *Malareus sinomus, Meringis parkeri*, *Orchopeas hirsuta*, *O. leucopus*, *O. sexdentatus*, *Pleochaetis exilis, Pulex* sp., and *Polygenis odiosus*) and two species of sucking lice (*Hoplopleura hirsuta* and *Polyplax spinulosa*) tested positive for at least one of four validated species of *Bartonella* (*B. vinsonii* and *B. washoensis*) and *Rickettsia* (*R. felis* and *R. prowazekii*). Additionally new lineages of *Bartonella* have been registered in six more flea species (*Echinophaga gallinacea*, *Meringis altipecten*, *M. arachis*, *M. parkeri, Pleochaetis exilis, Thrassis aridis*, Table 1). These records came from isolated studies carried out in wildlife from the southeast and northern parts, lacking data regarding central México where there is a report of human cases of murine typhus (Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades 2018; Sánchez-Montes *et al.* 2019). Additionally, for México, 172 species of fleas and 44 species of sucking lice, have been recorded, then, the inventory of species of both bacteria genera is still far from complete (Sánchez-Montes *et al.* 2013; Acosta-Gutiérrez 2014).

Due to the great diversity of potential vectors and the historical presence of human cases of murine typhus in the centre of the country; the purpose of this study was to identify the presence and diversity of *Bartonella* and *Rickettsia* species in a focus of murine typhus in Hidalgo, México.

Material and Methods

During August to September 2014, we sampled in two private ranches from Mineral del Monte and Tulancingo de Bravo (Figure 1), in the state of Hidalgo, México, close

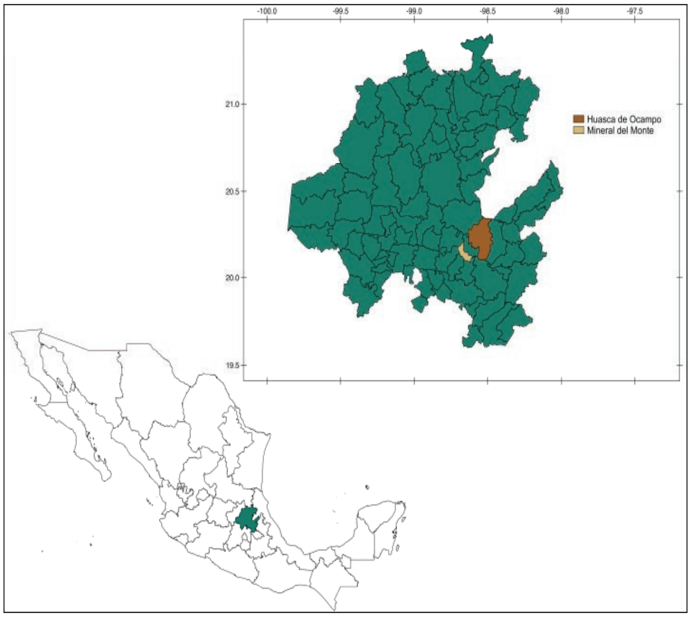

Figure 1. Sampling sites along the state of Hidalgo, México. Green: State of Hidalgo; Brown: Huasca de Ocampo; Yellow: Mineral del Monte.

to sites where human murine typhus cases have been reported (CENAPRECE 2016). This study was approved by the Ethics and Research Committee of the Medical Faculty of the Universidad Nacional Autónoma de México [FMED/CI/JMO/004/2012].

In order to identify the presence of several flea-borne and louse-borne pathogens (*Rickettsia* and *Bartonella*) in small mammals and their associated ectoparasites, we trapped small mammals using Sherman traps following Romero-Almaraz *et al.* (2007), under permission FAUT-0170 from the Secretaría del Medio Ambiente y Recursos Naturales. All mammals were sacrificed in accordance with the Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research (Sikes *et al.* 2016). We performed the necropsy of each animal, extracting a portion of liver and ear which were fixed in 96 % ethanol until its processing in the laboratory. Additionally, fleas and lice were recovered from host's bodies by manual inspection and fixed in absolute ethanol. Hosts and fleas were identified and deposited at the Mammal Collection and the Flea Collection of the Museo de Zoología "Alfonso L. Herrera" Facultad de Ciencias (MZFC) and Colección del Centro de Medicina Tropical, Facultad de Medicina (CMTFM), both belonging to Universidad Nacional Autónoma de México.

For morphological determination, fleas and lice were mounted on slides using the modified techniques of Kim *et al.* (1986) and Wirth and Marston (1968). Species were identified using specialized taxonomic keys such as Kim *et al.* (1986) for lice and Acosta and Morrone (2003), Hastriter (2004), Hopkins and Rothschild (1971), Morrone *et al.* (2000), and Traub (1950) for fleas.

From collected ectoparasites and hosts tissues, we extracted DNA with the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). As an endogenous internal control and for molecular identification of the ectoparasites, we amplified a

Table 2. Oligonucleotide primers used in this study.

Gen	Primers	Sequence (5´-3´)	Length (bp)	Reference
		Fleas and lice		
COI (Cytochrome oxidase subunit I)	L6625	CCGGATCCTTYTGRTTYTTYGGNCAYCC	400	Hafner *et al.* 1994
	H7005	CCGGATCCACNACRTARTANGTRTCRTG		
		Rickettsia sp.		
gltA (Citrate synthase)	RpCS.415	GCTATTATGCTTGCGGCTGT	806	de Souza *et al.* (2006)
	RpCS.1220	TGCATTTCTTTCCATTGTGC		
ompB (Outer membrane protein B)	120-M59	CCGCAGGGTTGGTAACTGC	862	Roux and Raoult, 2000
	120–807	CCTTTTAGATTACCGCCTAA		
		Bartonella sp.		
gltA (Citrate synthase)	BhCS781.p	GGGGACCAGCTCATGGTGG	379	Norman *et al.* 1995
	BhCS1137.n	AATGCAAAAAGAACAGTAAACA		

fragment of 400 bp of Cytochrome Oxidase Subunit I (COI) gene. For pathogens detection, we amplified a fragment of *gltA* and *ompB* genes specific for each group using primers and temperature conditions previously reported (Table 2).

The reaction mixture consisted of 12.5 µL of GoTaq® Green Master Mix, 2X of Promega Corporation (Madison, WI, USA), the pair of primers (100 ng each), 6.5 µL nuclease-free water and 30 ng DNA in a final volume of 25 µL (Sánchez-Montes *et al.* 2016a, b).

PCR products were resolved in 2 % agarose gels using TAE buffer at 85 V during 45 minutes and visualized using an ODYSSEY CLx Imaging System (LICOR Biosciences). Purified amplification products were submitted for sequencing at Macrogen Inc., Korea.

Sequences were analysed and edited using Bioedit version 5.0.9 Sequencing Alignment Editor Copyright © program and deposited in GenBank under accession numbers (MG952757 to MG952772). In order to identify the species of *Bartonella* and *Rickettsia*, we used the similarity criteria of the *gltA* and *ompB* genes proposed by La Scola (2003), Fournier and Roult (2009) and Fournier *et al.* (2003). Global alignments were done using Clustal W (Thompson *et al.* 1994) and the best substitution model was selected based on the lowest BIC (Bayesian Information Criterion) score for each gene using MEGA 6.0 (Tamura *et al.* 2011; Sánchez-Montes *et al.* 2016c). Additionally phylogenetic reconstruction was done using Maximum Likelihood also in MEGA 6.0 and branch support was evaluated over 10,000 bootstrap replications.

Results

We collected 40 rodents from four species (*Mus musculus*, *Peromyscus beatae*, *Rattus norvergicus*, and *Reithrodontomys sumichrasti*), and one shrew (*Sorex ventralis*), which are deposited in the MZFC under the following catalogue numbers LRR001 to LRR040. We detected the presence of *Bartonella* DNA in four samples of liver of two *P. beatae* (2/26 = 7.69 %) and two *R. norvergicus* (2/4 =50 %). Sequences recovered from *P. beatae* exhibited a similarity of 98 % with *B. vinsonii vinsonii* (a member of the *Bartonella vinsonii* complex) and those from *R. norvergicus* corresponded in a 100 %, respectively with *B. elizabethae* (Figure 2). In the case of

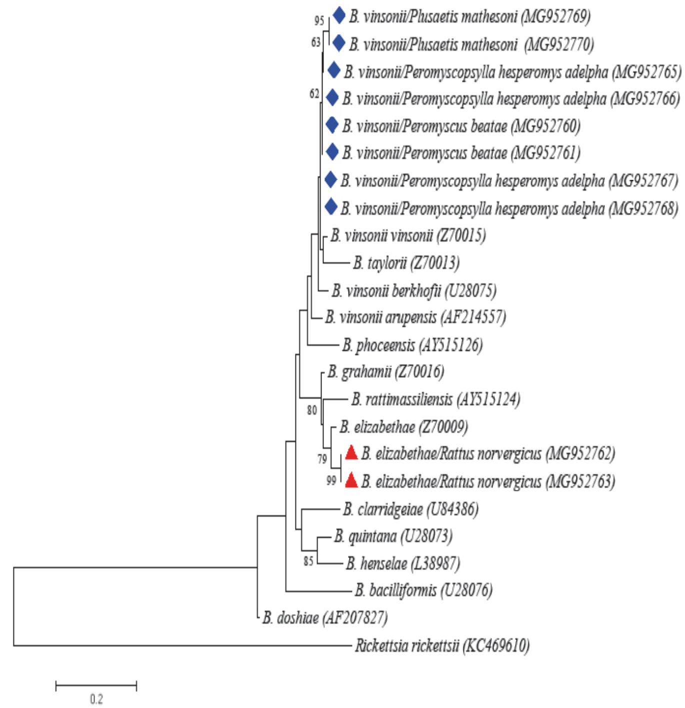

Figure 2. Maximum likelihood (ML) phylogenetic tree generated with *gltA* gene (300 bp) from several members of the genus *Bartonella*. The nucleotide substitution model was the Tamura three parameter model (T92) with discrete Gamma distribution (+G). Bootstrap values higher than 50 are indicated at the nodes. Sequences recovered in the study are marked with blue rhombuses and red triangles.

Rickettsia detection, a single specimen of *R. norvergicus* (1/4 = 25 %) tested positive in samples from liver and ear; we recovered sequences of *gltA* and *ompB* genes which exhibited a similarity of 99 % and 100 % with *R. typhi* (Accesion number AE017197) deposited in GenBank (Figure 3). A single *R. norvergicus* specimen presents co-infection between *B. elizabethae* and *R. typhi*.

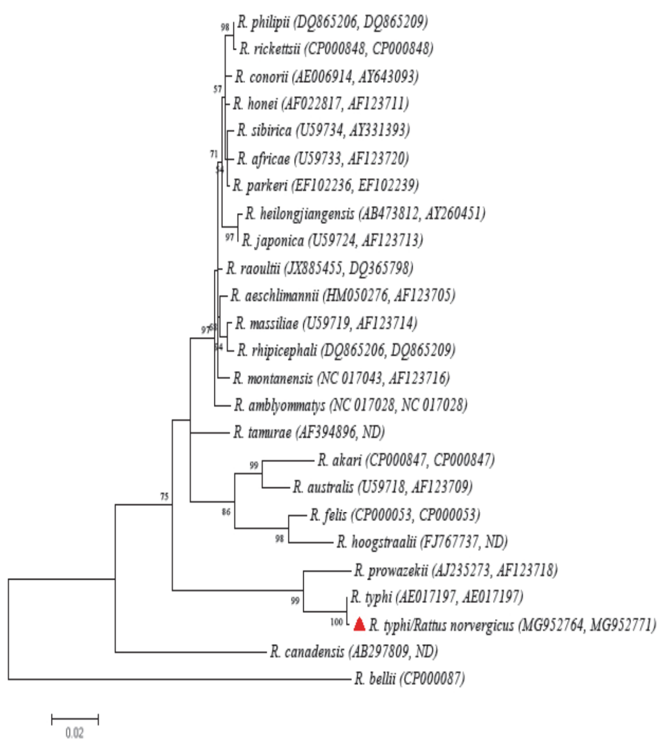

Figure 3. Maximum likelihood (ML) phylogenetic tree generated with *gltA* and *ompB* genes concatenated (1547 bp) from several members of the genus *Rickettsia*. The nucleotide substitution model was the Tamura three parameter model (T92) with discrete Gamma distribution (+G). Bootstrap values higher than 50 are indicated at the nodes. Sequences recovered in the study are marked with red triangles.

Hosts were infested by 47 fleas (18 females, 29 males), and 172 sucking lice (60 females, 39 males, 73 nymphs), distributed in six taxa, five species belonging to five families and six genera (Table 3). No fleas or lice were recovered from *M. musculus* and *S. ventralis*. After morphological identification was done, we amplified a fragment of 400 bp of Cytochrome oxidase subunit I (COI) in all ectoparasites recovered, in order to corroborate the identification of all samples, especially of those damaged specimens and nymphal stages. DNA sequences of the COI for four of the six species analysed were deposited in GenBank with the following accession numbers: *C. tecpin* (MG952757), *P. hesperomys adelpha* (MG952758); *P. mathesoni* (MG952759), *P. spinulosa* (MG952772) and *H. reithrodontomydis* (KT151126). No complete sequences were obtained for *J. b. breviloba*. We detected the presence of the same *Bartonella* lineage previously refereed in *P. beatae*, in two flea species (six *P. hesperomys adelpha* and 17 *P. mathesoni*) recovered from the two hosts which tested positive and from three others that were negative (Table 3). Sequences from fleas and hosts shape a single cluster within our phylogenetic analysis (Fig. 1). None of the flea or sucking lice species analysed was positive for *Rickettsia* DNA.

Discussion

We report for the first time the presence of two species of *Bartonella* and one of *Rickettsia* in the state of Hidalgo, México. The first *Bartonella* species is a member of the *B. vinsonii* complex, closely related with previous sequences detected in Cricetid rodents and fleas of the northern México (Rubio *et al.* 2014; Fernández-González *et al.* 2016). Also, this is the first study to report the presence of a *Bartonella* in the fleas *P. hesperomys adelpha* and *P. mathesoni* and in the host *P. beatae* (Table 1). Our phylogenetic analysis grouped sequences of *B. vinsonii* from *P. hesperomys adelpha*, *P. mathesoni* and *P. beatae* in a single cluster, then, our inference is that both flea species could be the potential vectors of these. Additionally, positive *P. hesperomys adelpha* were recovered from negative hosts, suggesting that these fleas may disseminate the pathogen in non-infected individuals among the rodent population bacteria (Kosoy *et al.* 1997; Morick *et al.* 2010). However, it is necessary to carry out tests to verify their vectorial capacity. Both species of fleas have a restricted distribution in México, which extend along the northeastern and central parts of the country, parasitizing several cricetid species such as *Peromyscus levipes*, *P. maniculatus*, *Reithrodontomys megalotis* (*P. mathesoni*) and *P. difficilis* (*P. hesperomys adelpha*), so it is not unexpected that this strain of bacteria is widely distributed in the country (Ponce-Ulloa and Llorente-Bousquets 1993; Hoffman *et al.* 1989; Whitaker and Morales-Malacara 2005; Acosta and Fernández 2015).

Table 3. Ecological parameters of *Bartonella* and *Rickettsia* species detected in fleas, sucking lice and small mammals in Hidalgo, México.

Host						Ectoparasite									
Family	Species	n	HI	%	BAD	**Family**	**Species**	**HP**	**EA**	**%**	**A**	**II**	**EI**	**%**	**BAD**
Ranch 1 Tulancingo de Bravo															
Cricetidae	*Peromyscus beatae*	20	2	10	*Bartonella vinsonii*	Ceratophyllidae	*Jellisonia breviloba breviloba*	2	3	10	0	2	0	0	ND
							Plusaetis mathesoni	10	27	5	1	3	17	57	*Bartonella vinsonii*
						Ctenophtalmidae	*Ctenophtalmus tecpin*	2	3	10	0	2	0	0	ND
						Leptopsyllidae	*Peromyscopsylla hesperomys adelpha*	4	7	20	0	2	6	86	*Bartonella vinsonii*
	Reithrodontomys sumichrasti	2	0	0	ND	Hoplopleuridae	*Hoplopleura reithrodontomydis*	1	4	50	2	4	0	0	ND
Soricidae	*Sorex ventralis*	1	0	0	ND	NR	NR	0	NR	(-)	(-)	(-)	NR	NR	ND
Ranch 2 Mineral del Monte															
Cricetidae	*Peromyscus beatae*	6	0	0	ND	Ceratophyllidae	*Plusaetis mathesoni*	1	3	17	1	3	0	0	ND
Muridae	*Mus musculus*	8	0	0	ND	NR	NR	0	NR	(-)	(-)	(-)	NR	NR	ND
	Rattus norvergicus	4	2	50	*Bartonella elizabethae*	Polyplacidae	*Polyplax spinulosa*	4	172	100	43	43	0	0	ND
			1	25	*Rickettsia typhi*										

n: Host collected; **HI:** Number of hosts infected; **%:** Prevalence; **BAD:** Bacterial agents detected; **HP:** Host parasitized; **EA:** Ectoparasites collected; **A:** Mean abundance; **II:** Intensity of infestation; **EI:** Ectoparasites infected; **NR:** Not recovered; **ND:** Not detected.

We also report for the first time the presence of *B. elizabethae* in México, a zoonotic bacterial that may causes endocarditis and neuroretinitis in humans. This agent was reported for the USA in the 1990's, however, is has become an emerging problem in several countries of Southeast Asia, Portugal and France (Regier *et al.* 2016; Tay *et al.* 2016). *Bartonella elizabethae* is mainly transmitted by the rat flea *Xenosylla cheopis* (Table 1); however, in our study we did not recovered any fleas from the four *R. norvergicus* analysed. The higher prevalence of *B. elizabethae* in collected murid rodents suggests the presence of this flea or other competent vector in the area (Bitam *et al.* 2012). Additionally, we compiled evidence for the first time of the presence of *R. typhi* in rodents of the state of Hidalgo. This *Rickettsia* produces febrile cases with a wide range of severity that can lead to systemic failure in less than 5% percent of cases (Zavala-Castro *et al.* 2009). In the state of Hidalgo, three cases of murine typhus had been reported between 2005 to 2010, nevertheless, in 2015 there was an outbreak with 12 cases (Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades 2018).

Only one rat reported coinfection by *B. elizabethae* and *R. typhi*, a phenomenon that has been previously reported, probably because both pathogens are transmitted by the same flea species (Table 1). This reinforces the hypothesis of the presence of this vector in the study area (Marie *et al.* 2006; Bitam *et al.* 2012; Frye *et al.* 2015). The presence of positive Norway rats for these two zoonotic pathogens is a risk to human health, because this rodent species invade suburban and urban areas, live and thrive in human settlements and could carry fleas that can feed on human hosts and produce urban outbreaks. Our findings represent the first record of several confirmed zoonotic pathogens that can cause murine typhus and endocarditis in México, which highlight the importance of the establishment of active entomological surveillance in wildlife.

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