Zoonotic Bacteria in Fleas Parasitizing Common Voles, Northwestern Spain

Ruth Rodríguez-Pastor,[1] François Mougeot, Mª Dolors Vidal, Isabel Jado, Rosa M. González-Martín-Niño, Raquel Escudero,[2] Juan José Luque-Larena[2]

Author affiliations: Universidad de Valladolid and Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain (R. Rodríguez-Pastor, J.J. Luque-Larena); Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain (F. Mougeot); Universidad de Castilla-La Mancha, Ciudad Real (M.D. Vidal); Instituto de Salud Carlos III, Madrid, Spain (I. Jado, R.M. González-Martín-Niño, R. Escudero)

DOI: https://doi.org/10.3201/eid2507.181646

We detected *Francisella tularensis* and *Bartonella* spp. in fleas parasitizing common voles (*Microtus arvalis*) from northwestern Spain; mean prevalence was 6.1% for *F. tularensis* and 51% for *Bartonella* spp. Contrasted vector–host associations in the prevalence of these bacteria suggest that fleas have distinct roles in the transmission cycle of each pathogen in nature.

A dynamic prevalence of *Francisella tularensis* and *Bartonella* spp. was reported in irruptive common vole (*Microtus arvalis*) populations during 2013–2015 from agricultural landscapes of northwestern Spain (*1*,*2*). In that area, notifiable tularemia has been endemic since 1997, and human cases periodically occur during outbreaks in voles (*3*,*4*). Prevalence of *F. tularensis* and *Bartonella* spp. in voles increases with vole density (*1,2*), highlighting the key role of fluctuating rodents in shaping zoonoses dynamics (*1*–*4*). Rodent ectoparasites often play a major role in transmitting zoonotic pathogens. In the population studied, ticks rarely infest voles (2% prevalence), whereas fleas are much more prevalent (68%) (*2*). Nevertheless, any potential role for vole fleas in the circulation of *F. tularensis* or *Bartonella* spp. in natural environments remains unknown. To elucidate realistic transmission route scenarios in host-dynamic environments (*5*–*8*), we investigated whether zoonotic bacteria occur concomitantly in voles and fleas.

Our main goal was to study the prevalence of *F. tularensis* in fleas collected from voles previously tested for tularemia (*1*). We screened flea DNA in search of 6 main zoonotic bacteria simultaneously (*Anaplasma phagocytophilum*, *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii*, *F. tularensis*, and *Rickettsia* spp.), following the same molecular procedure (multiplex PCR) (*9*) previously used to screen vole pathogens (*1,2*). Voles and fleas were live-trapped in northwestern Spain during March 2013–March 2015 (Appendix, https://wwwnc.cdc.gov/EID/article/25/7/18-1646-App1.pdf). We collected fleas from each individual vole and identified and grouped them in pools (pool = total fleas/vole). Three flea species parasitize common voles in the area: *Ctenophthalmus apertus*, *Nosopsyllus fasciatus*, and *Leptopsylla taschenbergi* (*2*). We screened monospecific pools (all fleas in a pool belonged to the same species and came from the same vole host), for a sample size of 90 vole hosts (pools) and 191 fleas. We screened 78 *C. apertus* fleas (39 pools) and 113 *N. fasciatus* fleas (51 pools). Among the 90 voles providing fleas, 27 were *F. tularensis* PCR–positive; the remaining 63 were negative (*1*). Of these same 90 voles, 45 were *Bartonella* PCR–positive and 45 were negative. Seventeen were positive for both *F. tularensis* and *Bartonella* spp. (*2*).

Flea pools had an average of 2.12 fleas (range 1–9); however, most (>70%) contained 1 (51%) or 2 (22%) fleas (Table). We did not detect DNA from pathogens other than *F. tularensis* and *Bartonella* spp. in fleas. Three (3%) flea pools harbored *F. tularensis* DNA; we estimated the overall prevalence at 6%. *F. tularensis* prevalence in both flea species was low (1 positive pool of 51 in *N. fasciatus* and 2 of 39 in *C. apertus*). All *F. tularensis* PCR–positive flea pools came from *F. tularensis* PCR–positive voles, and prevalence of *F. tularensis* in fleas was significantly associated with its prevalence in voles (analysis of variance [ANOVA], $R^2 = 0.072$, $F_{0.05, 1, 88} = 6.81$, $p = 0.011$). Of note, all fleas containing *F. tularensis* DNA were collected during July 2014, when vole populations reached top densities and tularemia prevalence peaked among them (33%) (*1*). The low prevalence of *F. tularensis* detected in fleas carried by infected hosts (3 of 27 pools) and the detection of infected flea pools only when abundance of the bacterium in the environment was highest (during vole peaks) (*1*,*4*) suggest that the quantitative role of fleas in the circulation of *F. tularensis* might be modest.

Conversely, the role of fleas in the circulation of *Bartonella* spp. seems much more relevant. We detected *Bartonella* spp. in 28 (37%) flea pools and in both flea species (37% of *N. fasciatus* and 23% of *C. apertus*) (Table). We detected *Bartonella* spp. in fleas collected from *Bartonella* PCR–positive and *Bartonella* PCR–negative voles in nearly equal proportions (51% vs. 44%) (Table). The average prevalence of *Bartonella* spp. in fleas was not associated with its prevalence in voles (ANOVA, $R^2 = 0.006$, $F_{0.05, 1, 88} = 0.53$, $p = 0.467$). We found a higher *Bartonella* spp. prevalence in *N. fasciatus* (65%) than in *C. apertus* (33%).

[1]Current affiliation: Ben-Gurion University of the Negev, Midreshet Ben-Gurion, Israel.

[2]These authors contributed equally to this article.

Table. Detection of *Francisella tularensis* and *Bartonella* spp. in 2 species of fleas from live common voles (*Microtus arvalis*), northwestern Spain, 2013–2015*

		Flea pools			Fleas		
Voles	Flea species	No.	*F. tularensis*–positive, %	*Bartonella* spp.–positive, %	No.	*F. tularensis* prevalence, % (range)	*Bartonella* spp. prevalence, % (range)
All	All	90	3.3	31.1	191	6.1 (3.3–8.8)	51.1([31.1–71.1)
	Nosopsyllus fasciatus	51	2.6	37.3	113	6.9 (3.9–9.8)	64.7 (37.3–92.2)
	Ctenophthalmus apertus	39	3.9	23.1	78	5.1 (2.6–7.7)	33.3 (23.1–43.6)
F. tularensis–negative	All	63			127	0	
	N. fasciatus	32			71	0	
	C. apertus	31			56	0	
F. tularensis–positive	All	27			64	20.4 (11.1–29.6)	
	N. fasciatus	19			42	18.4 (10.5–26.3)	
	C. apertus	8			22	25.0 (12.5–37.5)	
Bartonella spp.–negative	All	45			93		44.4 (26.7–62.2)
	N. fasciatus	21			53		71.4 (38.1–100)
	C. apertus	24			40		20.8 (16.7–25.0)
Bartonella spp.–positive	All	45			98		51.1 (31.1–71.1)
	N. fasciatus	30			60		60 (36.7–83.3)
	C. apertus	15			38		53.3 (33.3–73.3)

*Blank cells indicate that nothing can be calculated for that option.

We identified 3 *Bartonella* species among fleas (*B. taylorii* [17%], *B. grahamii* [14%], and *B. rochalimae* [3%]), as well as mixed infections between them (Appendix). These findings are in accordance with other research showing fleas as a main vector of *Bartonella* spp. (*5*). Although *F. tularensis* and *Bartonella* spp. have been simultaneously detected in ≈13% of voles during population density peaks (*2*), we identified no co-infection among flea pools (ANOVA, $R^2 = 0.011$, $F_{0.05,\,1,\,88} = 0.97$, $p = 0.328$).

Our data show that *F. tularensis* and *Bartonella* spp. occur in the fleas infesting wild common voles in northwestern Spain, with notable differences in prevalence (6% and 51%, respectively) and associations with prevalence in vole hosts. Future studies are needed to determine the role of fleas in the circulation of these pathogens in nature and in particular to ascertain any effective vectoring of *F. tularensis*.

Acknowledgments

We thank Fabio Flechoso for helping with ectoparasite counts and flea identification.

This work was supported by ECOVOLE (Factores ecologicos que influyen en la reproduccion y dinamica poblacional del topillo campesino (*Microtus arvalis*) en medios agrarios; CGL2012-35348), ECOTULA (Ecología de la Tularemia: dinámica espacio-temporal, ciclos ecológicos de transmision y mapas de riesgo en ecosistemas agrarios del NO de España; CGL2015-66962-C2-1-R) and RESERTULA (Microbiología de la Tularemia: circulación de *Francisella tularensis* en los ecosistemas agrarios del NO de España. Estudio d relacines epidemiológicas y filogenéticas; CLG2015-66962-C2-2-R) projects funded by the Government of Spain (lMINECO/FEDER). R.R.P. was supported by a PhD studentship from the University of Valladolid (co-funded by Banco Santander, RR 30/04/2014).

About the Author

Dr. Rodríguez-Pastor is a postdoctoral student at the Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, Israel. Her research interests include the ecology of zoonoses and disease dynamics in animal populations, specifically parasite and infectious pathogen dynamics in wild rodents.

References

1. Rodríguez-Pastor R, Escudero R, Vidal D, Mougeot F, Arroyo B, Lambin X, et al. Density-dependent prevalence of *Francisella tularensis* in fluctuating vole populations, northwestern Spain. Emerg Infect Dis. 2017;23:1377–9. http://dx.doi.org/10.3201/eid2308.161194
2. Rodríguez-Pastor R, Escudero R, Lambin X, Vidal MD, Gil H, Jado I, et al. Zoonotic pathogens in fluctuating common vole (*Microtus arvalis*) populations: occurrence and dynamics. Parasitology. 2019;146:389–98.
3. Luque-Larena JJ, Mougeot F, Roig DV, Lambin X, Rodríguez-Pastor R, Rodríguez-Valín E, et al. Tularemia outbreaks and common vole (*Microtus arvalis*) irruptive population dynamics in northwestern Spain, 1997–2014. Vector Borne Zoonotic Dis. 2015;15:568–70. http://dx.doi.org/10.1089/vbz.2015.1770
4. Luque-Larena JJ, Mougeot F, Arroyo B, Vidal MD, Rodríguez-Pastor R, Escudero R, et al. Irruptive mammal host populations shape tularemia epidemiology. PLoS Pathog. 2017;13:e1006622. http://dx.doi.org/10.1371/journal.ppat.1006622
5. Gutiérrez R, Krasnov B, Morick D, Gottlieb Y, Khokhlova IS, Harrus S. *Bartonella* infection in rodents and their flea ectoparasites: an overview. Vector Borne Zoonotic Dis. 2015;15:27–39. http://dx.doi.org/10.1089/vbz.2014.1606
6. Silaghi C, Pfeffer M, Kiefer D, Kiefer M, Obiegala A. *Bartonella*, rodents, fleas and ticks: a molecular field study on host–vector-pathogen associations in Saxony, eastern Germany. Microb Ecol. 2016;72:965–74. http://dx.doi.org/10.1007/s00248-016-0787-8
7. Hornok S, Földvári G, Rigó K, Meli ML, Gönczi E, Répási A, et al. Synanthropic rodents and their ectoparasites as carriers

of a novel haemoplasma and vector-borne, zoonotic pathogens indoors. Parasit Vectors. 2015;8:27. http://dx.doi.org/10.1186/s13071-014-0630-3
8. Stevenson HL, Bai Y, Kosoy MY, Montenieri JA, Lowell JL, Chu MC, et al. Detection of novel *Bartonella* strains and *Yersinia pestis* in prairie dogs and their fleas (Siphonaptera: Ceratophyllidae and Pulicidae) using multiplex polymerase chain reaction. J Med Entomol. 2003;40:329–37. http://dx.doi.org/10.1603/0022-2585-40.3.329
9. Anda P, Escudero R, Rodriguez-Moreno I, Jado I, Jimenez-Alonso MI. Method and kit for the detection of bacterial species by means of DNA analysis. European patent EP1895015B1. 2012 July 25.

Address for correspondence: Ruth Rodríguez-Pastor, Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 84990, Israel; email: ruth.r.pastor@gmail.com

Mycobacterium bovis Infection in African Wild Dogs, Kruger National Park, South Africa

Roxanne L. Higgitt, O. Louis van Schalkwyk, Lin-Mari de Klerk-Lorist, Peter E. Buss, Peter Caldwell, Leana Rossouw, Tebogo Manamela, Guy A. Hausler, Jennie Hewlett,[1] Emily P. Mitchell,[1] Paul D. van Helden, Sven D.C. Parsons,[2] Michele A. Miller[2]

Author affiliations: Stellenbosch University, Cape Town, South Africa (R.L. Higgitt, G.A. Hausler, P.D. van Helden, S.D.C. Parsons, M.A. Miller); Department of Agriculture Forestry and Fisheries, Skukuza, South Africa (O.L. van Schalkwyk, L.-M. de Klerk-Lorist); South African National Parks, Skukuza (P.E. Buss, L. Rossouw, T. Manamela, J. Hewlett); Old Chapel Veterinary Clinic, Pretoria, South Africa (P. Caldwell); National Zoological Gardens of South Africa, Pretoria (E.P. Mitchell)

DOI: https://doi.org/10.3201/eid2507.181653

[1]Current affiliation: University of Pretoria, Onderstepoort, South Africa.

[2]These authors were co–principal investigators for this article.

We screened African wild dogs (*Lycaon pictus*) in Kruger National Park, South Africa, for *Mycobacterium bovis* infection using an interferon-gamma release assay. We detected *M. bovis* sensitization in 20 of 21 packs; overall apparent infection prevalence was 83%. These animals experience high infection pressure, which may affect long-term survival and conservation strategies.

The African wild dog (*Lycaon pictus*) is an endangered carnivore, occurring in fragmented, small populations (in South Africa, <500 animals). These factors make them susceptible to adverse factors, such as infectious diseases, that may threaten their long-term survival (*1,2*). Of particular concern are diseases caused by multihost pathogens that are capable of persisting in reservoir host species, such as *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB). This pathogen may pose a major threat to the conservation of endangered host populations (*3*).

Since 2012, sporadic cases of wild dogs with macroscopic and histological lesions consistent with tuberculosis (TB) have been recorded in South Africa, specifically in Kruger National Park (KNP; n = 8), uMkuze Game Reserve (n = 1), and Hluhluwe-iMfolozi Park (HiP; n = 2). *M. bovis* infection is endemic in these parks and occurs in multiple species that are preyed upon by wild dogs, such as warthogs, which have an estimated *M. bovis* seroprevalence up to 38% in KNP (*4,5*). In 2 cases from KNP, acid-fast bacilli were associated with granulomatous lymphadenitis, and spoligotype analysis of *M. bovis* isolates from lesions in affected wild dogs from KNP (strain type SB0121) and HiP (strain type SB0130) were the same as those found in local prey (*6*).

M. bovis is a novel pathogen of wild dogs; understanding the impact of bTB disease in wild dogs is imperative to making informed management decisions regarding these animals' conservation. Estimation of prevalence would provide a starting point for this investigation but requires diagnostic tools for accurate detection of *M. bovis* infection. To estimate prevalence in the KNP wild dog population, we assessed sensitization to TB antigens ESAT-6 and CFP-10.

During July 2016–January 2018, we tested blood samples from 77 wild dogs from KNP using an interferon gamma release assay (IGRA) developed by our group (*7*). We tested animals from 21 wild dog packs; 20 of these included ≥1 IGRA-positive animal, indicating widespread exposure to *M. bovis* throughout KNP (Figure). We observed no significant difference in IGRA result based on sex (p = 0.79 by 2-tailed Mann Whitney test). Overall, the apparent prevalence of *M. bovis* infection was 82% (63/77; 95% CI 72%–89% by modified Wald test).

Few reports of active bTB disease and related deaths have been documented in wild dogs, so the high apparent