Mycobacterium caprae Infection in Livestock and Wildlife, Spain

Sabrina Rodríguez, Javier Bezos, Beatriz Romero, Lucía de Juan, Julio Álvarez, Elena Castellanos, Nuria Moya, Francisco Lozano, M. Tariq Javed, José L. Sáez-Llorente, Ernesto Liébana, Ana Mateos, Lucas Domínguez, Alicia Aranaz, and The Spanish Network on Surveillance and Monitoring of Animal Tuberculosis¹

Mycobacterium caprae is a pathogen that can infect animals and humans. To better understand the epidemiology of *M. caprae*, we spoligotyped 791 animal isolates. Results suggest infection is widespread in Spain, affecting 6 domestic and wild animal species. The epidemiology is driven by infections in caprids, although the organism has emerged in cattle.

M ycobacterium caprae is a cluster within the *M*. tuberculosis complex (online Technical Appendix, www.cdc.gov/EID/content/17/3/532-Techapp.pdf). This pathogen has been recognized mainly in central Europe, where it has been occasionally isolated from tuberculous lesions from cattle (1–5), pigs (4), red deer (*Cervus* elaphus) (4,5), and wild boars (*Sus scrofa*) (3). Its isolation from humans has also been described (3,6); often, a contact with livestock has been suggested as a likely means of transmission (5). To our knowledge, this pathogen has never been isolated outside continental Europe, except from a European patient in Australia (7) and a cow in Algeria (8).

The combination of disease tracing and molecular typing is needed to understand the epidemiology of tuberculosis. This report describes the molecular epidemiology of *M. caprae* infection in Spain compared with other countries. We characterized *M. caprae* isolates from goats and other domestic and wild animals by

Author affiliations: Universidad Complutense de Madrid, Madrid, Spain (S. Rodríguez, J. Bezos, B. Romero, L. de Juan, J. Álvarez, E. Castellanos, N. Moya, F. Lozano, A. Mateos, L. Domínguez, A. Aranaz); University of Agriculture, Faisalabad, Pakistan (M.T. Javed); Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid, (J.L. Sáez-Llorente); and European Food Safety Authority, Parma, Italy (E. Liébana)

DOI: 10.3201/eid1703.100618

spoligotyping (9). The relative contribution of each animal and its role in animal tuberculosis are discussed.

The Study

This study included 791 *M. caprae* isolates from domestic goats (*Capra aegagrus hircus*, n = 542), sheep (*Ovis aries*, n = 2), cattle (*Bos taurus*, n = 229), domestic pigs (*S. scrofa domestica*, n = 2), wild boars (*S. scrofa*, n = 14), red deer (*Cervus elaphus*, n = 1), and a fox (*Vulpes vulpes*, n = 1). The samples originated from skin test– positive animals identified within the national or regional eradication programs, from abattoir surveillance, and from postmortem inspections of wildlife, and were collected from 1992 through June 2009 in different geographic areas in Spain (Figure 1). Spoligotyping was performed as described (9), and authoritative names for spoligotype



Figure 1. Map of Spain showing the distribution of the 2 most frequent *Mycobacterium caprae* spoligotypes and affected animals: C, cattle; D, red deer; F, fox; G, goats; S, sheep; P, pigs; WB, wild boar. A) Spoligotype SB0157. B) Spoligotype SB0416.

¹A list of members of The Spanish Network on Surveillance and Monitoring of Animal Tuberculosis can be found in the online Technical Appendix (www.cdc.gov/EID/content/17/3/532-Techapp. pdf). patterns were obtained from the *Mycobacterium bovis* Spoligotype Database (www.mbovis.org).

Further authentication was achieved by detection of RD4 in the isolates with a 3-primer PCR in a panel of 63 unrelated isolates that included all spoligotyping patterns and animal species. Of the selected isolates, 62 showed the 545-bp product, indicating that they harbor RD4. One isolate from a cow of Eastern European origin repeatedly showed a 340-bp band, and its sequencing could not confirm presence or absence of RD4. For detection of specific *M. caprae* gene polymorphisms, 1 isolate from every spoligotyping pattern was studied. Additional identification was determined by sequencing of the pyrazinamidase A gene, which demonstrated a C at nt 169 that results in the functional wild-type pyrazinamidase A gene, and of the gyrase B gene that showed the G at nt 1311 and a C at position 1410 (online Technical Appendix).

The isolates, which originated from 195 single cases or outbreaks (Table 1), clustered into 15 patterns, which share the features previously described for the species (absence of spacers 1, 3–16, 28, and 39–43). Notably, the Iberian spoligotype cluster lacks spacers 30–33, whereas most *M. caprae* isolates from central Europe belong to spoligotypes that harbor these spacers. The 3 isolates of profiles SB0418 and SB1619 that presented spacers 30–33 originated from cattle imported from southeastern Europe. The 2 predominant spoligotypes, SB0157 and SB0416, were found to be responsible for 60% and 22%, respectively, of the cases and infected different animal species in distant areas, whereas 7 patterns were unique to a single case or outbreak. We calculated the index of discrimination (D) described by Hunter and Gaston (10) using the website of the University of the Basque Country (www.insilico.ehu. es). The result, D = 0.584, is notably lower compared with a parallel research of 252 patterns from 6,215 *M. bovis* isolates (D = 0.87) (11).

Additionally, variable number tandem repeat typing by using loci ETR-A, ETR-B, ETR-D, QUB11a, QUB11b, QUB3232, ETR-E, and MIRU26 (online Technical Appendix) was performed as described by Frothingham and Meeker-O'Connell (*12*) on a selection of 20 isolates (Table 2). The isolates originated from 10 properties (6 goat herds and 4 cattle farms), each with 2 different spoligotypes detected at a time. At 5 farms, the loss of spacers 25–27, 29, and 34–38, which can be explained by a single deletion event, had caused a change of the spoligotype pattern. This loss changed SB0157 to SB1081 and SB1084 to SB1889, while the variable number tandem repeat profiles within the same farm remained identical.

The routine application of molecular diagnosis and typing techniques in clinical laboratories has enabled its real role as a pathogen for several species to be recognized. In Spain, *M. caprae* represents 7.4% of all *M. tuberculosis* complex isolates from domestic and wild animals. Seventy-five of the 197 outbreaks (38.1%) involved goats (Table 1). This species showed the highest diversity among *M. caprae* with 12 patterns identified, 6 of them exclusive to caprine herds. The association of *M. caprae* with goats in Spain may be due to 2 reasons. First, the microorganism seems to be highly pathogenic for the goats in Spain, based on the

Table 1. Spoligotyping results of 791 Mycobacterium caprae isolates and their distribution within different animal species, Spain, 1992–2009*

		No. animals (no. outbreaks)						
						Wild	Red	
Ref	Spoligotyping pattern†	Goats	Sheep	Cattle	Pigs	boar	deer	Fox
SB0157		204 (44)	1	133 (67)	1	4 (4)	1	
SB0415		37 (4)	1	8 (4)		4 (3)		
SB0416		80 (18)		58 (21)		5 (3)		1
SB0866		1		1	1			
SB0973		1						
SB1077				17 (4)		1		
SB1078		1		1				
SB1079		1						
SB1080		2 (1)						
SB1081		1		8 (4)				
SB1084		211 (1)						
SB1889		2 (1)						
SB1872		1						
SB0418				2 (2)				
SB1690				1				
Total		542	2	229	2	14	1	1
		(75)	(2)	(105)	(2)	(11)		

*Ref, reference. Numbering according to www.Mbovis.org.

†■, presence of spacer ; □, absence of spacer.

			No. alleles at locus								
Farm	Animal	Spoligotype	ETR-A	ETR-B	ETR-D	QUB3232	QUB 11a	QUB 11b	MIRU 26	MIRU 31	
1	Goat	SB0416	4	4	4	8	7	2	5	2	
		SB0866	5	3	3	8	7	4	2	4	
2	Goat	SB0416	4	3	4	8	7	2	4	2	
		SB0157	4	3	4	8	7	2	4	2	
3	Goat	SB0416	4	5	5	7	6	4	5	5	
		SB0415	5	1	3	8	7	3	5	5	
4	Cattle	SB0157	3	3	4	8	7	2	5	2	
		SB1081	3	3	4	8	7	2	5	2	
5	Cattle	SB0157	4	3	4	3	7	2	5	2	
		SB1081	4	3	4	3	7	2	5	2	
6	Goat	SB0157	4	3	4	8	7	2	5	2	
		SB1078	4	3	4	8	7	2	5	2	
7	Goat	SB1084	5	1	3	9	5†	3	5	4	
		SB1889	5	1	3	9	5†	3	5	4	
8	Cattle	SB0157	4	3	4	8	7	2	5	2	
		SB1081	4	3	4	8	7	2	5	2	
9	Cattle	SB0416	5	3	3	8	6	4	2	3	
		SB0157	4	3	4	8	7	2	5	2	
10	Goat	SB0973	4	3	-	-	-	-	_	-	
		SB0157	4	3	4	9	-	2	5	-	
*- no am											

Table 2. Variable number tandem repeat analysis of isolates from 10 farms that presented mixed *Mycobacterium caprae* infection (different spoligotype patterns), Spain, 1992–2009*

†Gel band of ≈1,800 bp. Sequencing showed that insertion sequence IS6110 is inserted within the third repetition of QUB11a.

disseminated tuberculous lesions that it produces and its fast transmission within a herd. Second, caprine herds have not been included in the national eradication campaign (except when coexisting with cattle or as part of some regional programs). Therefore, *M. caprae* infection can spread easily through animal movements, such as purchase for replacement or genetic improvement.

The emergence of this pathogen in cattle has been observed. Cattle were involved in 106 outbreaks (53.3%) during the study period. Since 2004, cattle from 2,218 herds identified in the eradication program have been inspected by bacteriology. The number of cattle properties infected with M. caprae represented 0.85%-6.67% of the total number of herds diagnosed with bovine tuberculosis. Temporal trend of M. caprae isolates cultured over time was assessed by using the software WINPEPI 9.4 (13). The proportion of *M. caprae* isolated from bovine samples has increased consistently during 2004-2009, showing a significant positive trend (p = 0.009, by Mantel trend test) (Figure 2). We observed more *M. caprae* infections in cattle in regions with a high goat density. However, an analysis of the type of farm production shows that 86.7% of M. caprae-infected cattle have been raised in farms without any contact with small ruminants. This fact indicates recirculation of the pathogen within and between cattle herds. In countries that are virtually free of animal tuberculosis such as Germany, Austria, and the Czech Republic, a large number of cases in cattle and red deer are caused by M. caprae.

Identification of isolates from human patients has shown *M. caprae* as a human pathogen (3,6,14). A recent study suggests that *M. caprae* causes 0.3% of the cases of human tuberculosis in Spain, with SB0157 also being the most dominant spoligotype (14). The role of the pathogen as a public health risk is highlighted by lesions that can



Figure 2. Proportion and regression lines of *Mycobacterium caprae* isolates (black dots, continuous line) and *M. caprae*–infected herds (white triangles, dashed lines) of the total number of *M. tuberculosis* complex isolates and *M. tuberculosis* complex–infected herds identified in cattle during 2004–2009. Error bars indicate 95% confidence intervals.

also be found in the mammary glands of infected goats; thus, consumption of unpasteurized dairy products remains a concern (15).

Conclusions

Compelling evidence indicates that *M. caprae* poses a serious health risk not only for goats, but also for other domestic and wild animal species and humans. Our results indicate that *M. caprae* infection is widespread in Spain and that the epidemiology is driven by caprine infections. Considering the role of *M. caprae* in animal tuberculosis, relevant legislation should be considered to address the infection as was done for *M. bovis*.

Acknowledgments

We thank the National and Regional Animal Health authorities, especially L. Carbajo, for their continuous encouragement. We are grateful to T. Alende, A. Gutiérrez, C. Viñolo, L. Guijarro, J. Gimeno, N. Álvarez, N. Montero, C. Lozano, L. Pazos and S. González for technical help. We acknowledge the staff of SADNA (Centro de Investigaciones Biológicas, Madrid) for sequencing.

This research was supported by European Union project TB-STEP (KBBE-2007-1-3-04, no. 212414), the Ministry of Environment and Rural and Marine Affairs, the Comunidad de Madrid, and the Junta de Castilla y León.

Ms Rodríguez is a PhD candidate in the Departamento de Sandidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid and received predoctoral research fellowship AP2006-01630 from the Spanish Ministry of Education. Her research focuses on molecular characterization of *M. tuberculosis* complex isolates and its application in epidemiology of these pathogens.

References

- Boniotti MB, Goria M, Loda D, Garrone A, Benedetto A, Mondo A, et al. Molecular typing of *Mycobacterium bovis* strains isolated in Italy from 2000 to 2006 and evaluation of variable-number-tandemrepeats for a geographic optimized genotyping. J Clin Microbiol. 2009;47:636–44. DOI: 10.1128/JCM.01192-08
- Duarte EL, Domingos M, Amado A, Botelho A. Spoligotype diversity of *Mycobacterium bovis* and *Mycobacterium caprae* animal isolates. Vet Microbiol. 2008;130:415–21. DOI: 10.1016/j. vetmic.2008.02.012
- Erler W, Martin G, Sachse K, Naumann L, Kahlau D, Beer J, et al. Molecular fingerprinting of *Mycobacterium bovis* subsp. *caprae* isolates from central Europe. J Clin Microbiol. 2004;42:2234–8. DOI: 10.1128/JCM.42.5.2234-2238.2004

- Pavlik I, Dvorska L, Bartos M, Parmova I, Meliciiarek I, Jesenska A, et al. Molecular epidemiology of bovine tuberculosis in the Czech Republic and Slovakia in the period 1965–2001 studied by spoligotyping. Vet Med (Praha). 2002;47:181–94.
- Prodinger WM, Eigentler A, Allerberger F, Schonbauer M, Glawischnig W. Infection of red deer, cattle, and humans with *Mycobacterium bovis* subsp. *caprae* in Western Austria. J Clin Microbiol. 2002;40:2270–2. DOI: 10.1128/JCM.40.6.2270-2272. 2002
- Kubica T, Rüsch-Gerdes S, Niemann S. *Mycobacterium bovis* subsp. *caprae* caused one-third of human *M. bovis*–associated tuberculosis cases reported in Germany between 1999 and 2001. J Clin Microbiol. 2003;41:3070–7. DOI: 10.1128/JCM.41.7.3070-3077.2003
- Sintchenko V, Jelfs P, Dally M, Crighton T, Gilbert GL. A case of urinary tuberculosis due to *Mycobacterium bovis* subspecies *caprae*. Pathology. 2006;38:376–8. DOI: 10.1080/00313020600821391
- Sahraoui N, Müller B, Guetarni D, Boulahbal F, Yala D, Ouzrout R, et al. Molecular characterization of *Mycobacterium bovis* strains isolated from cattle slaughtered at two abattoirs in Algeria. BMC Vet Res. 2009;5:4. DOI: 10.1186/1746-6148-5-4
- Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol. 1997;35:907–14.
- Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol. 1988;26:2465–6.
- Rodríguez S, Romero B, Bezos J, de Juan L, Álvarez J, Castellanos E, et al. High spoligotype diversity within a *Mycobacterium bovis* population: clues to understanding the demography of the pathogen in Europe. Vet Microbiol. 2010;141:89–95. DOI: 10.1016/j. vetmic.2009.08.007
- Frothingham R, Meeker-O'Connell WA. Genetic diversity in the Mycobacterium tuberculosis complex based on variable numbers of tandem DNA repeats. Microbiology. 1998;144:1189–96. DOI: 10.1099/00221287-144-5-1189
- Abramson JH. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiol Perspect Innov. 2004;1:6. DOI: 10.1186/1742-5573-1-6
- Rodríguez E, Sánchez LP, Pérez S, Herrera L, Jiménez MS, Samper S, et al. Human tuberculosis due to *Mycobacterium bovis* and *M. caprae* in Spain, 2004–2007. Int J Tuberc Lung Dis. 2009;13:1536– 41.
- Rodwell TC, Moore M, Moser KS, Brodine SK, Strathdee SA. Tuberculosis from *Mycobacterium bovis* in binational communities, United States. Emerg Infect Dis. 2008;14:909–16. DOI: 10.3201/ eid1406.071485

Address for correspondence: Alicia Aranaz, Facultad de Veterinária, Universidad Complutense de Madrid, Departamento de Sanidad Animal, Avda. Puerta de Hierro s/n, Madrid 28040, Spain; email: alaranaz@vet. ucm.es

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Search past issues of EID at www.cdc.gov/eid