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Zoonotic tuberculosis in Catalonia, Spain: Phylogenetic insights into *Mycobacterium bovis* and *M. caprae* transmission at the human-livestock interface

Bernat Pérez de Val [a,b,c,*], Enric Vidal [a,b,c], Tod Stuber [d], Jose Luís Sáez [e], María Teresa Tórtola [f,g]

[a] *IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Catalonia, Spain*
[b] *Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, CReSA, Campus de la UAB, Bellaterra, Catalonia, Spain*
[c] *WOAH Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra, Catalonia, Spain*
[d] *National Veterinary Services Laboratories, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Ames, IA, USA*
[e] *Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Dirección General de Sanidad de la Producción Agraria, Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain*
[f] *Microbiology Service, WHO Supranational TB Reference Laboratory, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Research Institute (VHIR), Barcelona, Catalonia, Spain*
[g] *CIBER of Infectious Diseases (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain*

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ABSTRACT

This genomic epidemiology study analyzed *Mycobacterium bovis* and *M. caprae* isolates from animals and humans in Catalonia, Spain, between 2005 and 2023. Of 42 human tuberculosis (TB) cases, five were phylogenetically linked to livestock outbreaks, suggesting zoonotic transmission. The findings specifically indicate goats as a primary source of *M. caprae* transmission to humans. The study also revealed unexpected genetic diversity of mycobacteria within the same outbreak or even within the same patient, highlighting the complexity of tuberculosis transmission dynamics. The results underscore the value of genomic surveillance in understanding zoonotic TB transmission at the human-livestock interface and its role in informing effective control strategies.

1. Introduction

Mycobacterium bovis and *M. caprae*, both animal-adapted ecovars of *M. tuberculosis* (i.e. *M. tuberculosis* var. bovis and *M. tuberculosis* var. caprae, respectively [1]), are the main etiological agents of tuberculosis (TB) in a wide range of mammalians, including livestock and wildlife. Moreover, these are zoonotic pathogens that also can infect humans through inhalation or consumption of livestock products, being clinically or pathologically indistinguishable from TB cases caused by *M. tuberculosis* var. tuberculosis [2].

Livestock-to-human transmission has been identified as a key point to achieve the global goal to end human TB epidemic by 2030 [3]. However, human and animal TB control strategies are rarely addressed as a whole, and zoonotic transmission events often remain undetected or unresolved due to the lack of identification of *M. tuberculosis* variants and the absence of a one-health approach in epidemiological surveys. In Spain, a study of 110 *M. bovis* and *M. caprae* isolates recovered from human patients with TB, conducted between 2004 and 2007, highlighted the relevance of zoonotic transmission through occupational exposure to infected livestock [4].

The aim of the study was to identify the phylogenetic relationships of *M. bovis* and *M. caprae* strains isolated from livestock, wildlife and humans TB patients in Catalonia (Northeastern Spain).

2. Materials and methods

A total of 365 isolates, 267 *M. bovis* and 98 *M. caprae*, were included in the study. Forty-nine of them were isolated from 42 human patients

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[*] Corresponding author at: IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia, Spain.

E-mail address: bernat.perez@irta.cat (B. Pérez de Val).

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with suspected TB clinical signs (two different isolations were carried out in 7 patients) and 316 from animals obtained in the framework of livestock TB eradication programs (193 cattle, 31 goats and 3 sheep), wildlife health surveillance (74 wild boar, 4 red deer and one fox) and 10 laboratory cynomolgus macaques from a natural outbreak [5]. All samples were isolated in Catalonia between 2005 and 2023.

Mycobacteria were isolated in solid media (Löwenstein Jensen with Pyruvate or Coletsos) or by the BACTEC MGIT system. Human and animal isolates were obtained at Vall d'Hebron Hospital (Barcelona, Spain) and IRTA-CReSA (Bellaterra, Spain), respectively. WGS was conducted using Illumina Miseq system at National Veterinary Services Laboratories of the USDA (Ames, IA, USA). Three *M. bovis* reference sequences were included in the analysis: *M. bovis* AN5 (SRR3135071), *M. bovis* Ravenel (SRR3135072) and *M. bovis* BCG (SRR5485728), and all sequences were aligned to the reference genome *M. bovis* AF2122/97 (NC_002945.4). The sequences are available at the GenBank (Bioproject no. PRJNA384785).

Single nucleotide polymorphisms (SNPs) were identified using the vSNP bioinformatics pipeline (https://github.com/USDA-VS/vSNP) using the quality control procedures previously described [6]. Phylogenies were constructed using Randomized Axelerated Maximum Likelihood (RAxML) with aligned whole-genome SNP sequences applying the GTR-CATI model of substitution and a maximum-likelihood algorithm. Sites that fell within Proline-Glutamate (PE) and Proline-Proline-Glutamate (PPE) poly-morphic CG-repetitive sequences (PGRS) were filtered and excluded. The Microreact platform [7] was used for visualizing, annotating, and editing the phylogenetic trees.

Once pairwise SNP distances were analyzed, putative transmission clusters of very closely (≤6 SNPs), closely (≤12 SNPs), or moderately (≤18 SNPs) related isolates were identified (Supplemental file S1), adapting previous data analysis of SNP pairwise distance to predict transmission in TB outbreaks [8–10]. Phylogenies were interpreted together with associated data from epidemiological surveys.

3. Results and discussion

The genome coverage of all analyzed sequences ranged between 98.6 %–99.9 %. Overall, seven major clades were identified (Fig. 1), corresponding to the *M. bovis* European 1, 2 and 3 clonal complexes [11–13], a fourth independent *M. bovis* clade, and three clades of *M. caprae* previously classified as Iberian, Central-East European and Asian [14]. As previously described [15,16], the phylogenetic structures of both *M. bovis* and *M. caprae* isolates in Catalonia revealed a wide genetic diversity, with no clear patterns related to host species, geographic location, or year of isolation. Therefore, low pairwise distances between isolates were indicative of transmission events. This is also supported by the strong correlation between patristic and SNP pairwise distances observed in the likely zoonotic transmission clusters (supplementary file S2).

M. bovis isolates were grouped in 7 subclades (Supplemental file S3) according to previously described classification [15]. However, 14 out of the 39 human *M. bovis* isolates fit into the same subclade (Fig. 2). In addition, three phylogenetic relationships suggesting potential animal-human transmissions of *M. bovis* were identified within this subclade. One human isolate showed a very close relationship (≤ 2 SNPs to the common ancestor) with two sheep isolates (case previously reported in [17]), whereas another human isolate showed moderate phylogenetic relationships (17 SNPs) with them. Despite there was no very close relationship between the two human isolates, the epidemiological investigation revealed that both were isolated in the same year (2017) from the same patient. This patient was the owner of the sheep farm, suggesting direct transmission events between the animals and the farmer, likely in both directions. The strain could have evolved within the herd and/or in the farmer. The third human *M. bovis* isolate showed a close relationship with two bovine isolates (7 SNPs between common ancestors), suggesting a probable common origin followed by a few transmission events (at least one of them interspecific) mediated by untested intermediate individuals. Long-term monitoring of *M. bovis*

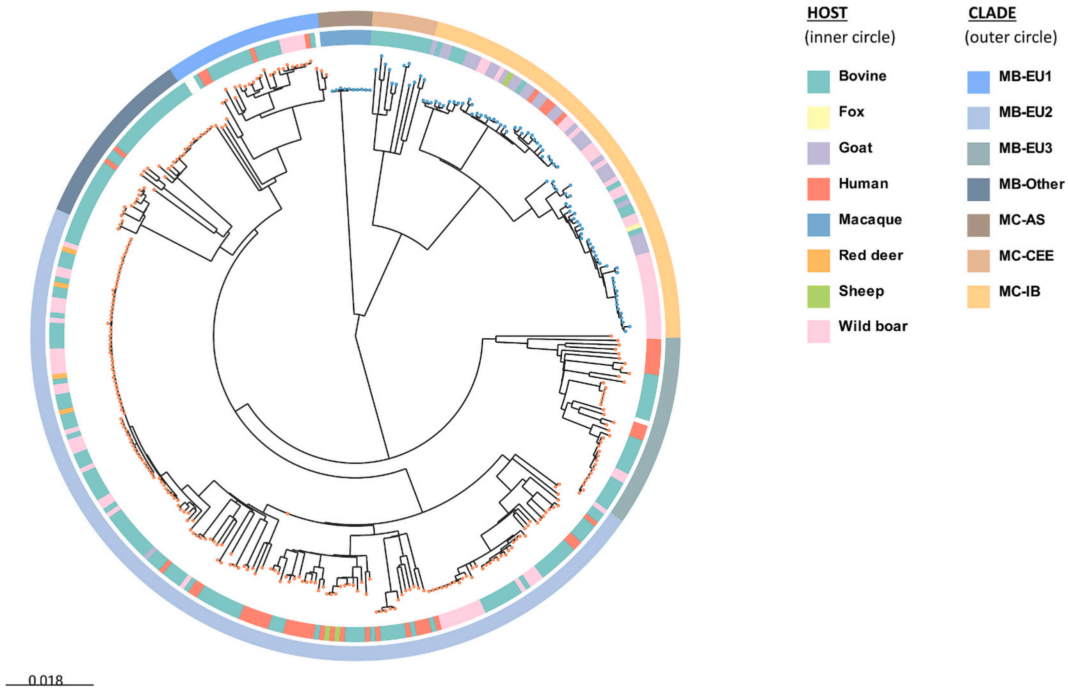

Fig. 1. Maximum-likelihood phylogenetic tree (RAxML) including the 267 *Mycobacterium bovis* and 98 *Mycobacterium caprae* isolates (red and blue tree nodes, respectively), recovered from animals and humans in Catalonia (Spain) between 2005 and 2023. Host type (inner circle) and clades (outer circle) are identified according to the legend. Root: *M. bovis* AF2122/97 reference strain sequence (National Center for Biotechnology. Information accession no. NC_0002945). Scale: Number of nucleotide substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

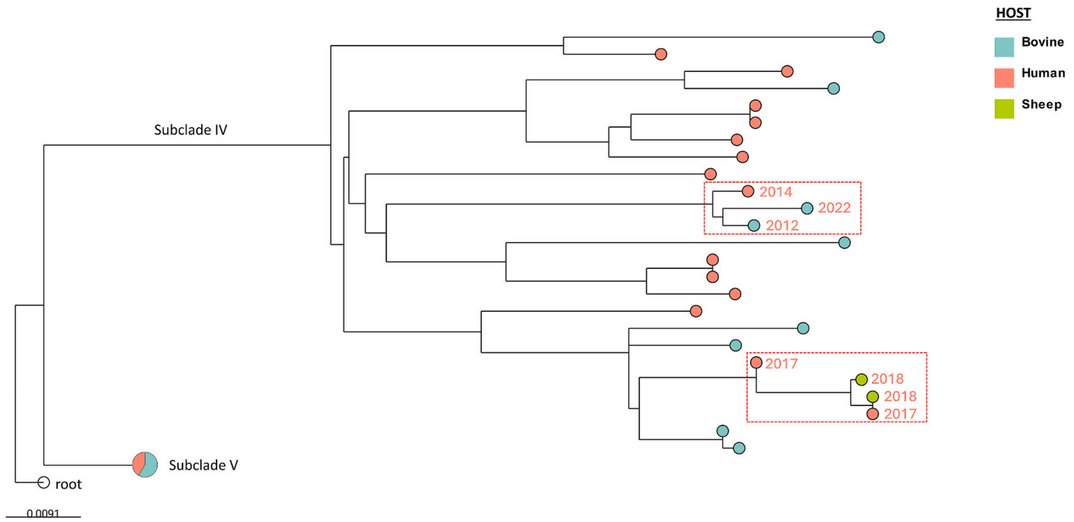

Fig. 2. Phylogenetic tree of *Mycobacterium bovis* isolates belonging to subclades IV ($N = 25$, expanded tree) and V ($N = 12$, collapsed tree). Host type is identified according to the colour legend. Red rectangles identify phylogenetic clusters involving human and animal isolates (≤ 18 SNP). Red numbers beside tree nodes show the year of isolation. Root: *M. bovis* AF2122/97 reference strain sequence (National Center for Biotechnology. Information accession no. NC_0002945). Scale: Number of nucleotide substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

outbreaks in cattle showed that sequences separated by up to 12 SNPs could be related to the same origin [10].

On the other hand, the four human *M. caprae* isolates fit into the same subclade within the Iberian clade (Fig. 3), and phylogenetic relationships suggesting potential animal-human transmissions were identified in three of them. One human *M. caprae* isolate showed a very close relationship (≤ 4 SNPs between common ancestors) with two isolates from a goat herd located less than 20 km from the residence of the human patient. The pairwise distance between the human patient and goat genome sequences were related to such an extent that they could be considered the same strain according to previously described for *M. caprae* outbreaks [16], thus suggesting a direct zoonotic transmission event. Interestingly, although the TB outbreak in the goat herd was detected five years after the human TB case (2021 and 2016, respectively), other close related (<12 SNPs) isolates from outbreaks in goats and sheep herds of the same county were detected one year before (2015, Fig. 3). Unfortunately, no official data was available on the employment of this patient during this period. Two other genome sequences of *M. caprae* isolated from human TB patients showed close to moderate (≤ 9 SNPs and ≤ 13 SNPs between common ancestors, respectively) relationships with goat isolates, suggesting indirect interspecific transmission events mediated by unidentified TB cases.

Remarkably, the high ratio of putative *M. caprae*-related zoonotic cases (phylogenetic relationships with goat isolates were found in three out of four human isolates) highlights the risk of exposure for people working on the small ruminant sector. A recent study revealed that long-term unnoticed circulation of *M. caprae* in goats in southern Spain was a hidden cause of human TB [18]. Another recent long-term phylodynamic study inferred that *M. caprae* host transition events were frequent between goats, wild boars and humans in the Iberian Peninsula [14],

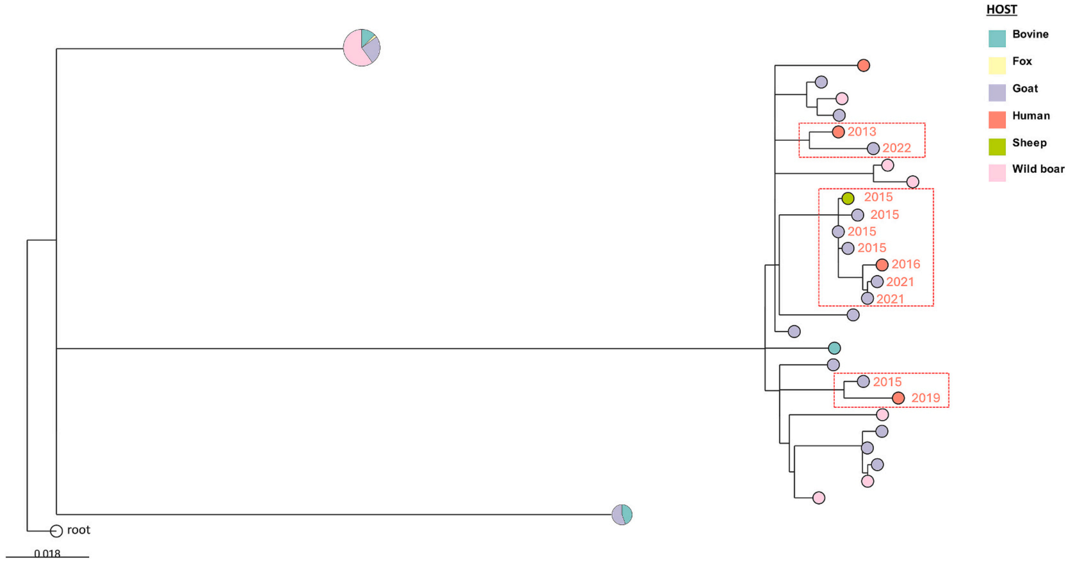

Fig. 3. Phylogenetic tree of *Mycobacterium caprae* isolates belonging to the Iberian clade. One subclade tree includes the four human isolates (shown expanded, $N = 27$), while other two subclade trees are collapsed ($N = 40$ and $N = 9$, respectively). Host type is identified according to the colour legend. Red rectangles identify phylogenetic clusters involving human and animal isolates (≤18SNP). Red numbers beside tree nodes show the year of isolation. Root: *M. bovis* AF2122/97 reference strain sequence (National Center for Biotechnology. Information accession no. NC_0002945). Scale: Number of nucleotide substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

probably as a result of the high goat census, a lack of an official compulsory eradication program, limited farm biosecurity and the type of herd management with close physical contact, factors that enhance the spread of *M. caprae* at multiple scales, ultimately facilitating interspecific transmission.

4. Conclusions

In this study we found evidence of *M. bovis* and *M. caprae* circulation in the livestock-human interface during the last two decades in Catalonia (Spain). Within the forty-two cases of human TB caused by these ecovars, genomic epidemiology analysis suggested a putative zoonotic origin in up to five (12 %), and small ruminants were involved in four of them. In light of these findings, the cooperation between of public and animal health authorities to thoroughly investigate outbreaks and the implementation of specific official TB eradication programs in goats is utterly needed in endemic areas to prevent the occupational exposure to *M. caprae* or *M. bovis* and minimize the risk of zoonotic transmission. Real-time surveillance of TB under a one-health perspective would also benefit from using WGS prospectively to track new TB cases and break transmission chains.

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CRediT authorship contribution statement

Bernat Pérez de Val: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Enric Vidal:** Writing – review & editing, Writing – original draft, Methodology. **Tod Stuber:** Writing – review & editing, Methodology, Formal analysis. **Jose Luís Sáez:** Writing – review & editing, Resources. **María Teresa Tórtola:** Writing – review & editing, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Bernat Perez de Val reports financial support was provided by European Commission. Bernat Perez de Val reports financial support was provided by Government of Spain Ministry of Agriculture Fishing and Food. Bernat Perez de Val reports financial support was provided by Government of Catalonia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2025.100993.

Data availability

Data will be made available on request.

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