

# *Mycobacterium bovis* in Egyptian mongoose, Spain

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## Abstract

Tuberculosis caused by *Mycobacterium bovis* and other related mycobacteria has been reported in a wide range of mammals worldwide. In the case of the Herpestidae family, *Mycobacterium mungi* and *M. bovis*, both belonging to the *Mycobacterium tuberculosis* Complex, have been reported in banded mongooses (*Mungos mungo*) in Africa and in Egyptian mongooses (*Herpestes ichneumon*) in Portugal, respectively. Thus, we hypothesized that Tuberculosis may occur in Egyptian mongooses from Spain. Twenty-five found dead Egyptian mongooses were necropsied in order to detect macroscopic TB-compatible lesions and mandibular lymph nodes and lungs were cultured onto mycobacteria-specific growth media. We isolated *M. bovis* in 3/25 Egyptian mongooses (12.00%, IC95: 4.17–29.96%) and identified spoligotypes SB0121 (2/3) and SB0134 (1). No macroscopic TB-compatible lesions were observed. To the best of our knowledge, this is the first report of *M. bovis* in Egyptian mongoose in Spain, as well as the only study that includes spoligotyping in this species. Although the absence of visible lesions suggests a minor role of the Egyptian mongoose in Tuberculosis epidemiology, further research thereon is encouraged.

## KEYWORDS

bacteriological culture, Egyptian mongoose, *Mycobacterium bovis*, spoligotype, tuberculosis

## 1 | INTRODUCTION

Animal Tuberculosis (TB), caused by *Mycobacterium bovis* and other mycobacteria of the *Mycobacterium tuberculosis* Complex (MTC), affects a wide range of mammals, including humans. Hence, TB still constitutes a major challenge not only in livestock breeding and wildlife conservation, but also in public health (Rodríguez et al., 2010). In parts of Africa, a novel pathogen belonging to the MTC, named *Mycobacterium mungi*, has been reported to cause high-mortality outbreaks in banded mongooses (*Mungos mungo*; Alexander et al., 2010). The Egyptian mongoose (*H. ichneumon*),

which also belongs to the Herpestidae family, is the only mongoose species that occurs naturally in Europe (Gaubert et al., 2011). It is a social carnivore, as it often forms groups of up to five individuals (Palomares & Delibes, 1993). This species is currently expanding its range and coexists with other predators, such as the European badger (*Meles meles*) and the red fox (*Vulpes vulpes*; Soto & Palomares, 2015). Although its main preys are European rabbits (*Oryctolagus cuniculus*), small mammals, reptiles, amphibians, small birds and insects, Egyptian mongooses do also consume carrion (Palomares, 1993). The first case of *M. bovis* infection in Egyptian mongoose was reported by Matos et al. (2013) in Portugal. Subsequently, Matos

Elisa Ferreras-Colino and Esther Descalzo contributed equally to this work.

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et al. (2016) isolated *M. bovis* from one individual and detected *M. bovis* DNA in two others, obtaining a total prevalence of 20% by culture or PCR. Thus, we hypothesized that infection with MTC may occur in Egyptian mongooses from Spain.

## 2 | MATERIALS AND METHODS

Between 2018 and 2021, we collected 25 Egyptian mongoose carcasses in Castilla-La Mancha region (central Spain; Figure 1). Hunting the species is banned, so all samples derived from roadkills. During necropsy, mandibular, mediastinal and tracheobronchial lymph nodes (LN), tonsils, lungs and spleen were visually examined in order to detect macroscopic TB-compatible lesions. Further, 2 g of mandibular LN and 2 g of lung tissue from each animal were stored at  $-20^{\circ}\text{C}$  until further analysis. Samples were decontaminated using 0.75% (w/v) hexadecyl pyridinium chloride for 1 h, and cultured onto Coletsos and 0.2% (wt/vol) pyruvate-enriched Löwenstein-Jensen media (Difco, Madrid, Spain) at  $37^{\circ}\text{C}$  for a maximum of 3 months. Culture was considered positive when isolates were identified as MTBC by conventional PCR and /or DVR-spoligotyping (Lorente-Leal et al., 2019).

## 3 | RESULTS

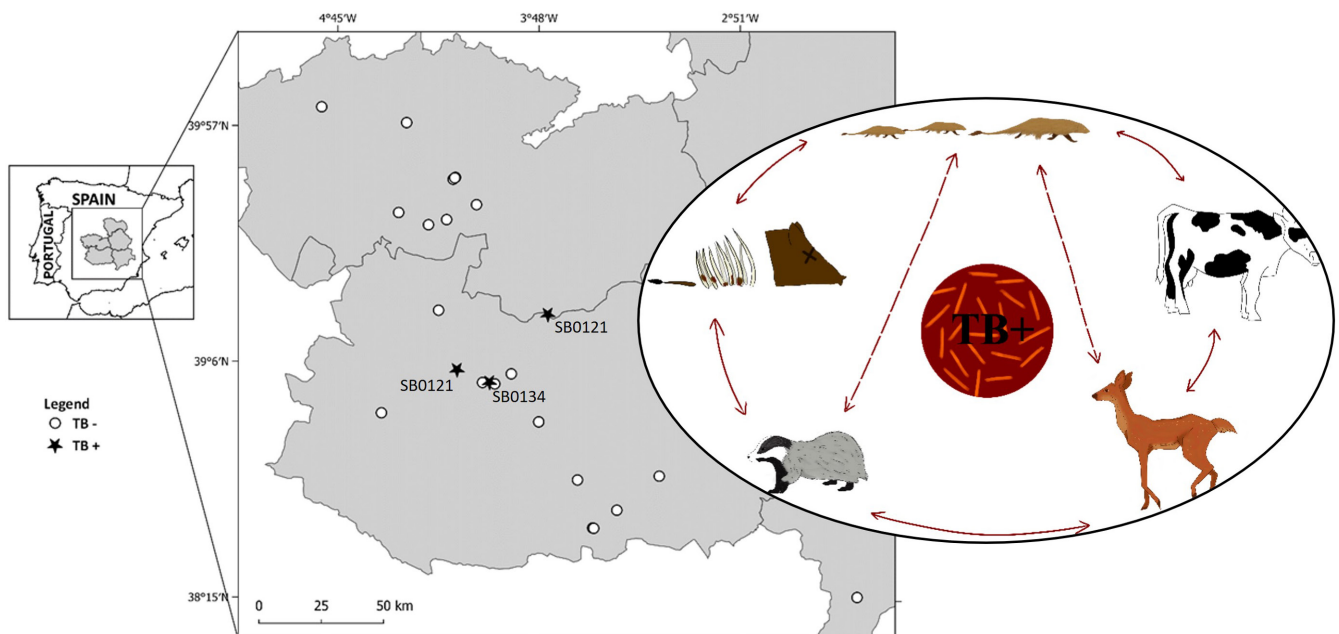
*Mycobacterium bovis* was isolated from three individuals (12.00%, IC95: 4.17–29.96%) and the isolates were identified and characterized by spoligotyping with the two profiles SB0121 (two cases) and SB0134 (one case), according to the [mbovis.org](http://mbovis.org) nomenclature. No visible macroscopic TB-compatible lesions were observed.

## Impacts

- We confirmed that Egyptian mongooses (*Herpestes ichneumon*), the only mongoose species naturally present in Europe, are susceptible to infection with Tuberculosis caused by *Mycobacterium bovis*, a major pathogen due to its zoonotic risk and economic importance.
- We identified *M. bovis* strains in the Egyptian mongoose that have already been reported in other wild and domestic species in the study region and that display high frequency among both livestock and wildlife in Spain. This suggests the participation of the Egyptian mongoose, along with the coexisting wildlife and livestock reservoirs, in the wildlife-domestic Tuberculosis circulation established in Mediterranean ecosystems in Spain.
- We did not observe any macroscopic lesions compatible with tuberculosis (i.e. granuloma) in any organ from the Egyptian mongooses. Therefore, only a minor role can be attributed to the Egyptian mongoose in Tuberculosis transmission and maintenance so far.

## 4 | DISCUSSION

Our results confirm previous observations from Portugal, where Matos et al. (2016) reported a close 20% MTC infection frequency in Egyptian mongoose, also without visible lesions. However, in Portugal *M. bovis* had been isolated only from two mongooses without reporting the spoligotype (Matos et al., 2013, 2016). Our



**FIGURE 1** Location of Egyptian mongoose (*Herpestes ichneumon*) samples analysed by culture for *Mycobacterium tuberculosis* complex in Spain (open circles, negative; black stars, positive).

data further confirm that the Egyptian mongoose, a native and expanding carnivore, can harbour MTC in the Iberian Peninsula. Frequency estimation based on bacteriological isolation may underestimate true prevalence, as culture displays excellent specificity but poor sensitivity (60%; Cousins & Florisson, 2005). Further, tissues might have suffered degradation from freezing as well. Thus, true prevalence may be higher. The two identified spoligotypes are the most prevalent ones reported in domestic and wild ungulates in Spain (Rodríguez et al., 2010) and have already been detected in cattle from Castilla-La Mancha (Gomez-Buendia et al., 2021). The confirmed presence of MTC infection in Egyptian mongooses from Spain suggests that further research of its role in MTC epidemiology is advisable (Corner, 2006). However, the absence of visible lesions leads to think of a minor role of the Egyptian mongoose in TB dynamics.

### AUTHOR CONTRIBUTIONS

Esther Descalzo collected the carcasses when notified of roadkills. Elisa Ferreras-Colino and Esther Descalzo performed the necropsies and the sampling. Beatriz Romero carried out the bacteriological cultures and spoligotyping. Christian Gortázar and Pablo Ferreras designed and supervised the study. Elisa Ferreras-Colino wrote the first draft of the manuscript and all authors contributed to the writing of the manuscript.

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### CONFLICT OF INTEREST STATEMENT

The authors also declare that they have no conflict of interest.

### ETHICS STATEMENT

The present study did not involve purposeful killing of animals. All animals were found dead due to roadkills. Protocols, amendments and other resources were done according to the guidelines approved by the Autonomous government following the R.D.1201/2005 of the Ministry of Presidency of Spain. Sample collection was being performed by professional personnel for routine procedures before the design of the study in compliance with the Ethical Principles in Animal Research. Thus, no ethical approval was deemed necessary. The authors declare that this work has not been published before, it is not under consideration for publication anywhere else, and it has

been read and approved by all co-authors and by the responsible authorities at the institute where the work has been carried out.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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