Infection of Eurasian badgers (Meles meles) with Mycobacterium bovis and Mycobacterium avium complex in Spain

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ABSTRACT

The prevalence, distribution and pathology related to infection with Mycobacterium bovis and other mycobacteria were determined in trapped (n = 36) and road-killed (n = 121) badgers in Spain from 2006 to 2010. The prevalence of M. bovis based on bacteriological culture from road-killed badgers was 8/121 (6.6%) and from trapped badgers was 0/36 (0%). Tuberculosis/M. bovis infection was evident in 15/121 (12.4%) road-killed badgers when bacteriology and histopathology were combined. Mycobacterium avium complex was isolated by culture from the tracheal aspirate of 1/36 (2.8%) trapped badgers and from tissue pools from 8/121 (6.6%) road-killed badgers.

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Introduction

The Mycobacterium tuberculosis complex (MTC; M. tuberculosis, M. bovis, M. caprae, M. pinnipedii, M. africanum and M. microti) causes disease in humans and domestic and wild animals (Grange et al., 1990; Aranaz et al., 1999). Control of bovine tuberculosis (Tb) in cattle can be compromised in areas where a reservoir of infection exists in wildlife. In the United Kingdom (UK) and Republic of Ireland (Irl), Eurasian badgers (Meles meles) are involved in the transmission of M. bovis to cattle (Bourne et al., 2007; Murphy et al., 2010).

The first case of bovine Tb (M. bovis) in a Spanish badger was identified in 2003 in Cabañeros National Park in Central Spain (Sobrino et al., 2008). M. bovis was also isolated from lymph nodes of a badger from sxEo in Northern Spain in 1997 (J.F. García Marín, personal communication). In Doñana National Park in Southern Spain, 23% of badgers were seropositive (Martín-Atance et al., 2006). Elsewhere in continental Europe, M. bovis infection in badgers has been confirmed only in France, with a prevalence of 7.2% (Hars et al., 2010).

Mycobacterium avium complex (MAC) spp. were detected by culture of tissues from 7.4% of badgers in Spain and 0.5% of badgers in the UK (Balseiro et al., 2011). M. avium poratuberculosis (Map) has been isolated from the intestine and mesenteric lymph nodes of a badger in Scotland (Beard et al., 2001). M. intracellulare was isolated from the faeces of two badgers in Ireland (Hughes et al., 1993) and from tissues of a badger in Spain (Sevilla et al., 2005).

In this study we present data on the prevalence, distribution and pathology of M. bovis and other mycobacteria from trapped and road-killed badgers in Spain.

Materials and methods

Collection of samples

Road-killed badgers

From 2006 to 2010, postmortem examinations were performed on 121 badgers (10 cubs and 111 adults; 57 males and 64 females) killed on roads in Spain, mostly from Northern Spain, with smaller numbers from Southern Spain (Fig. 1). Samples of the lungs, intestine and retropharyngeal, submandibular, tracheobronchial, oesophageal and tracheal aspirates were taken from road-killed badgers and submitted for bacteriology. A total of 121 badgers were road-killed and examined from 2006 to 2010. The prevalence of M. bovis infection was evident in 15/121 (12.4%) road-killed badgers when bacteriology and histopathology were combined. Mycobacterium avium complex was isolated by culture from the tracheal aspirate of 1/36 (2.8%) trapped badgers and from tissue pools from 8/121 (6.6%) road-killed badgers.

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Mediastinal, hepatic and mesenteric lymph nodes were collected for bacteriology, molecular studies and histopathology. Serum samples were collected for serology. Samples for culture and real-time PCR were frozen at −20°C before processing.

Trapped badgers

Thirty-six badgers (1 cub and 35 adults; 16 males and 20 females) were captured during trapping operations in Asturias, Northern Spain, from April to July in 2009 and 2010 (Animal Research Ethics Committee of SERIDA register number 041/06-01-2008). Traps were located at active setts in four areas with a high incidence of Tb in cattle (Fig. 1). Trapped badgers were anaesthetised (de Leeuw et al., 2004) and samples of faeces (anal swabs), urine (external palpation of the bladder) and sputum (tracheal aspiration) were collected, along with clotted and heparinised blood samples.

**Bacteriology**

*Mycobacterium tuberculosis complex* and *Mycobacterium avium complex*

Whole blood samples from 31 trapped badgers were used for the detection of M. bovis
Cellular immune response and mesenteric lymph nodes) were also included.
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Identification of isolates

Real-time PCR to identify MTC species was performed on culture isolates using MTC forward primer 5′-TACGATCGATCCAGGAACTAGCTC-3′; MTC reverse primer 5′-CAGTAGATGCCATGGCCTC-3′ and TaqMan probe YY/BHQ5 (Coetzier et al., 2000). Amplification was carried out at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. MAC species and genotypes were identified as described by Balseiro et al. (2011). A triplex PCR targeting IS900 and IS604 was sequenced to screen 7H9 cultures for Map (Sevilla et al., 2009).

MTC isolates were characterised by hybridisation of biotin-labelled PCR products onto a spoligotyping membrane (Isogen Bioscience BV), followed by spoligotyping and IS604 screening for Map (Kamerbeek et al., 1997). Results were recorded in SB code followed by a field of view ( Geoffrey et al., 2011) and a spot for M. bovis Spoligotype Database website.

Immunology

Humoral immune response

Serum samples from one trapped badger from Somiedo and two road-killed badgers (n = 3/121, 2.5%) were positive for antibodies against M. bovis in the Brock (Tb) Stat-Pak test. None of these badgers were M. bovis culture-positive.

Cellular immune response

Blood samples from all 31 badgers tested were negative in the IFN-γ ELISA using the published cut-off (Dalley et al., 2008). Blood samples from 18 badgers were negative in the ELISPOT using a cutoff of 25 spot-forming cells per million cells.

Gross pathology

Gross lesions were observed in one road-killed badger, which had an enlarged mesenteric lymph node with an area of caseous necrosis and mineralisation; tissues from this badger were too autolyzed for histopathological examination.

Histopathology and immunohistochemistry

Tb-like lesions were identified on histological examination in 15/93 (16.1%) road-killed badgers (7 M. bovis culture-positive and 8 culture-negative). Badgers infected with M. bovis had small granulomas in alveolar walls in the lungs, often close to bronchi, with occasional Langhan’s type giant cells (Supplementary Fig. 1A and B). Small granulomas were observed in the tracheobronchial and mediastinal lymph nodes of 6/7 M. bovis culture-positive road-killed badgers. Similar lesions in the lungs and lymph nodes were also observed in the eight culture-negative badgers (7 from Asturias and 1 from Galicia). Sparse AFBs were observed by ZN staining in 6/15 badgers with Tb-like lesions (2/7 culture-positive; 4/8 culture-negative) (Supplementary Fig. 1C). Positive immunolabelling for M. bovis was detected in macrophages within granulomas in all 15 badgers with Tb-like lesions (Fig. 1D). Tb-like lesions were not observed in the intestines or retropharyngeal, submandibular, hepatic or mesenteric lymph nodes of any badger.

Badgers infected with Maa and Mah had small granulomas in the lungs and retropharyngeal, submandibular, tracheo-bronchial, mediastinal and mesenteric lymph nodes. No granulomatous lesions resembling those of Johne’s disease (paratuberculosis) were observed in the mesenteric lymph nodes or intestines of any badger and no positive immunolabelling was observed in these samples when using a specific antibody against M. bovis.

Discussion

This study confirms the presence of mycobacterial infections in badgers in Spain. The prevalence of bovine Tb based on bacteriological culture from road-killed and trapped badgers was 6.6% (n = 8/121) and 0% (n = 0/36), respectively (Table 1). However, the prevalence in trapped badgers may be underestimated, due to

Macroscopic and microscopic changes consistent with M. bovis infection were observed in all 15 badgers with Tb-like lesions (Fig. 1D). These lesions were characterised by the presence of caseous necrosis and mineralisation; tissues from this badger were too autolyzed for histopathological examination.

Results

Culture, identification of isolates and spoligotyping

MTC identified as M. bovis were isolated from pools of tissues by culture and RT-PCR from 8/121 (6.6%) road-killed badgers from Asturias (Table 1). Isolates were characterised by spoligotyping and IS604 screening for Map (Kamerbeek et al., 1997). Pre-immunisation rabbit sera were used as negative controls. Positive and negative control tissues (lungs, intestine and mediastinal lymph nodes) were also included.

Two isolates were not characterised due to insufficient DNA. M. bovis was not isolated from any samples from 36 trapped badgers. Out of 36 trapped badgers tested, one isolate of MAC (2.8%) was identified by PCR as M. avium subsp. avium (Maa) and was cultured from the tracheal aspirate of an animal from Somiedo. MAC were isolated from pools of tissues from 8/121 (6.6%) road-killed badgers (6 from Asturias, 1 from Galicia and 1 from the Pyrenees); of these eight isolates, four were Maa, two were M. avium subsp. hominisuis (Mah) and two isolates could not be identified further.

Cultures and PCR for Map were negative in all cases.

Immunological results

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References


1. www.mbovis.org


the small sample size and the low sensitivity of clinical sampling (Pritchard et al., 1986; Chambers et al., 2002). Combining bacteriology and histopathology data, the estimated prevalence in badgers subjected to postmortem examination increased to 12.4% (n = 15/121) and suggested that we may have missed infected badgers by culture.

The gold standard for diagnosis of Tb in badgers is postmortem examination with bacteriological confirmation of tissues (Pritchard et al., 1986). The sensitivity of culture in this study may have been affected by the number of tissues examined, the incubation period and the pooling of tissues. The sampling of additional tissues increases the sensitivity of detection of Tb in badgers (Crawshaw et al., 2008).

The annual incidence of M. bovis in badgers at Woodchester Park (south west England) in 1990–2004 was estimated to be 2–12% (Vicente et al., 2007). Culture of tissues from badgers culled in 10 other areas (100 km × 100 km) in England yielded M. bovis prevalence estimates of 1.6–37.2% (Bourne et al., 2007). Using an enhanced postmortem examination procedure followed by bacteriological culture, the prevalence of M. bovis in badgers in the RoI was 36.3% (Murphy et al., 2010). However, the incidence of Tb in cattle in the UK and the RoI is substantially higher than in Spain and studies of infection in badgers have focussed on areas of particular high incidence in cattle. In the Asturias region of Spain, where most of the badgers in the present study originated, the incidence of Tb in cattle was 0.21% of herds in 2009 (MARM, 2009). This contrasts with an incidence of Tb in cattle of 6.18% in England (DEFRA, 2009) and 5.09% in the RoI (DAFF, 2009) in the same year.

Only 11 badgers were collected in Mediterranean Spain, a region of higher Tb prevalence in cattle than Northern Spain and with a high prevalence of Tb in wild ungulates (Gortázar et al., 2008; Naranjo et al., 2008). None of these badgers were MTC positive. However, given this limited sample size, the role of badgers in the epidemiology of Tb in Mediterranean Spain remains unclear.

Asturias and Galicia may have been over-represented in our sample because passive and active programmes of wildlife disease surveillance have been in place in these regions since 2001 and 2008, respectively. Nevertheless, we cannot rule out the possibility that the prevalence of M. bovis in this area is influenced by a higher density of badgers. Although there are no data on badger population density in Atlantic Spain, it seems likely that this area may support relatively higher densities than Mediterranean Spain, owing to the more suitable habitat.

Gross lesions were found in 1/8 M. bovis culture-positive badgers, whereas only microscopic lesions were present in the remainder. M. bovis often can be cultured from badgers and other species with no visible lesions (Gallagher et al., 1998; Gavier-Widén et al., 2009). Histological lesions observed in M. bovis culture-positive Spanish badgers resembled those in badgers in England suspected to be at an early stage of infection (Gallagher et al., 1998; Gavier-Widén et al., 2001). Early granulomatus lesions in Spanish badgers were observed in the lungs and tracheobronchial and mediastinal lymph nodes, indicative of the primary focus of infection and suggesting an aerogenous route of infection (Gallagher et al., 1998). Langhans’s type giant cells, generally not observed in badgers (Gallagher et al., 1976; Gavier-Widén et al., 2001), were occasionally present in our study.

No gross lesions were observed in any of the eight MAC culture-positive badgers, similar to previous findings in the UK and Spain (Sevilla et al., 2005; Balseiro et al., 2011). The pathological features of badgers infected with M. bovis and M. avium spp. in our study are consistent with the tissues of the thoracic cavity being the site of choice for seeking evidence of M. bovis and the retropharyngeal and submandibular lymph nodes for M. avium spp.

Badgers and cattle in the same areas of the UK frequently share the same M. bovis strains (Woodroffe et al., 2009). We isolated M. bovis from eight badgers, all of which were from areas of Asturias with a high prevalence of Tb in cattle (see Fig. 1). We also identified four different M. bovis spoligotypes, all of which have previously been identified in cattle in Asturias (Isabel Merediz, personal communication).

MAC, including Maa and Mab, have been isolated previously from badgers and cattle in the UK (Balseiro et al., 2011). In the present study, we also cultured Maa from the tracheal aspirate of a trapped badger. Although a high prevalence of Map has been reported in cattle in Northern Spain (Balseiro et al., 2003), Map was not isolated from any badger in our study, suggesting that badgers are not readily infected with Map and hence are unlikely to be significant reservoir hosts for Map in Spain.

It is uncertain whether badgers are reservoir or spill-over hosts for bovine Tb in Spain. Cattle are very susceptible to respiratory infection with M. bovis and infected badgers could present a risk to cattle if excretion of bacilli is high and persistent (Delahay et al., 2002). Low levels of excretion could be important where badgers and cattle are in close direct or indirect contact, as has been observed in farm buildings in the UK (Garnett et al., 2002).

Conclusions

Relatively little is known about the epidemiology of Tb in badgers in Spain. M. bovis is present in badgers and this species represents a potential source of infection for cattle, as in the UK and the RoI. The aim of future studies should be to improve our knowledge of Tb in badgers in Spain to minimise the risk of spread of Tb from badgers to cattle and from cattle to badgers.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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