Mycobacteriosis outbreak caused by Mycobacterium avium subsp. avium detected through meat inspection in five porcine fattening farms

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ANIMAL tuberculosis (TB), caused by Mycobacterium TB complex (MTBC) species, is a chronic zoonotic disease mainly affecting cattle. However, it can also cause disease in humans and a wide range of other animal species (CIE 2009). The wild boar (Sus scrofa) is the third animal species, followed by cattle and goats, in number of MTBC isolates in Spain (Rodríguez-Campos and others 2012). Thus, it is considered to be the main wild reservoir of TB (Naranjo and others 2008, García-Bocanegra and others 2012). The domestic pig (Sus scrofa domestica) accounts for 1 per cent of Spanish MTBC isolates from animals in the period 1996–2011 (Rodríguez-Campos and others 2012). Recent TB outbreaks in domestic pigs due to MTBC have also been reported in Italy (Di Marco and others 2012). There are, however, other opportunistic mycobacteria that can cause TB-like lesions in swine, indistinguishable from those caused by MTBC.

Mycobacterium avium complex (MAC) comprises a number of non-zoonotic pathogenic bacterial species with various degrees of pathogenicity and host preference (Álvarez and others 2011). M. avium is subdivided in four subspecies: M. avium subsp. avium (MAA), M. avium subsp. silvaticum, M. avium subsp. paratuberculosis (MAP), and M. avium subsp. hominisuis (MAH). MAA is known to cause generalised granulomatous lesions in poultry and wild birds; MAP is the causative agent of Johne’s disease in ruminants, while pigs are the primary animal host for MAH (Thorel and others 2001, Mijs and others 2002, Wellenberg and others 2010, Agdestein and others 2011, Álvarez and others 2011). However, pigs may also be infected by MAA, displaying lesions indistinguishable from those caused by mycobacteria belonging to the MTBC (Komijn and others 1999). Therefore, when TB-like lesions are found in pigs at the slaughterhouse, mycobacterial species identification becomes crucial to assess the human and animal health risk posed by the infection.

Following an initiative of the Catalan Health Protection Agency in December 2007, the Slaughterhouse Support Service (Servei de Suport a Escorxadors, SESC) was created within the Animal Health Research Centre (Centre de Recerca en Sanitat Animal, CRESA). Its main objective was to provide continuing education to meat inspectors and contribute to reaching final diagnoses of slaughterhouse findings. Between December 2010 and January 2011, several organs from a total of 20 pigs from five different farms were submitted to SESC for diagnosis. The lesions consisted of multifocal to coalescing whitish nodular lesions with caseous and partially mineralised appearance. Mesenteric lymph nodes (LNs), liver, spleen, mediastinal LNs and/or lung were affected (see Fig 1). While lesions in organs of the abdominal cavity (mainly liver and mesenteric LNs) were observed in all pigs, lesions in the thoracic cavity (lungs and mediastinal LNs) were only present in 12 pigs, coming from three out of the five studied farms.

Histopathological examination of the lesions using H&E routine staining revealed multifocal, necrotising and granulomatous splenitis, hepatitis, pneumonia and lymphadenitis. Numerous multinucleated (Langhans) giant cells were observed. Ziehl Neelsen staining revealed acid-fast bacilli in some of the affected organs (Fig 2), compatible with a mycobacteriosis. Information on each of the outbreaks including the examined organs and the used diagnostic techniques are summarised in Table 1.

Consequently, TB was suspected, the cases were reported to the local Animal and Public Health Authorities, and biosafety measures (laminar air flow, gloves, masks and protective glasses) were implemented for the slaughterhouse personnel when handling pigs from the affected farms.

Even though MTBC infection is not considered a food-borne disease associated with meat consumption, it might pose an occupational hazard to farmers and slaughterhouse personnel. Therefore, ruling out infections caused by zoonotic mycobacteria was established as a priority. Differential diagnosis was performed by means of bacteriological studies to identify the aetiological agent causing the lesions. Isolation was performed on Coletos and Lowenstein-Jensen selective media with pyruvate (bioMérieux España, Madrid, Spain). DNA was then extracted from colonies by boiling them for 10 minutes at 100°C, and identification was performed by means of a multiplex PCR specific for MTBC and MAC (Walton and Cousins 1992) followed by sequencing of the DNA encoding 16S rRNA.

Multiplex PCR identified non-tuberculous mycobacteria belonging to the MAC. Sequencing and subsequent BLAST analysis (Altschul and others 1990) confirmed MAA in all cases.

A subsequent epidemiological investigation suggested that the most likely source of the outbreak was the feed, since it was common between the five farms at least a few months before the outbreak detection. Certain feed contents could have been contaminated with MAA. Unfortunately, the feed samples no longer existed when the outbreak was detected and it was not possible to confirm this hypothesis. Similarly, mycobacteriosis in pigs fed peat naturally contaminated with MAC has been previously described (Matlova and others 2005, Agdestein and others 2011). In those infected pigs, lesions were primarily found in the head and mesenteric LNs. Accordingly, most of the animals studied in the present outbreak had a clear involvement of abdominal LNs and viscera, being strongly consistent with an oral route of infection.

Even though MAA is mainly isolated in birds and MAH is considered a human/porcine type of M. avium (Mijs and others 2002), a recent comparative study of MAA and MAH in experimentally infected pigs did not show significant differences in the ability of both pathogens to infect pigs (Agdestein and others 2012). However, the authors demonstrated that only MAH was isolated from pig faeces, resulting in efficient animal-to-animal transmission by the faecal–oral route, which could explain the higher incidence of infection observed in outbreaks caused by this subspecies in pigs compared with MAA.
Lesions consisting of granulomatous and caseous nodules were observed in (a) the liver, (b) the spleen and (c) mesenteric lymph nodes. In some cases, lesions were also observed in (d) the thoracic cavity.

(a) Granulomatous hepatitis with necrotic foci, abundant macrophages and Langhans cells. (b) Detail of Langhans cells in the splenic parenchyma. (c) In the lung, granulomatous foci were also seen. (d) Ziehl Neelsen stain showed the presence of a few acid-fast bacilli.
(Agdestein and others 2012). Also, if MAA in pigs was not excreted by the faecal route, feed contamination would be the most likely source of MAA infection in the present outbreak.

Pigs are susceptible to MTBC and MAC infections. The zoonotic risk of animals infected with MTBC has been described as an occupational hazard (Rodwell and others 2008, Rodríguez and others 2009, Torres-Gonzalez and others 2013). Additionally, severe MAC infections in humans have also been reported, especially in immunosuppressed individuals (Pavlik and others 2000, Biotus and others 2006). The establishment of a meat inspection support network allowed for rapid management of sample submission, diagnosis and subsequent molecular identification of the mycobacteriosis outbreak, leading to ascertaining its associated risks for public health.

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TABLE 1: Information on the submitted cases and performed diagnostic techniques

| Case     | No. of affected animals | Farm(s) of origin | Reported affected viscera | Diagnostic success |
|----------|-------------------------|-------------------|----------------------------|--------------------|
| Case 1 December 2010 | 1 | A | Liver (1/1) | HP (1/1) |
| Case 2 December 2010 | 1 | B | Liver (1/1) | ZN (1/1) |
| Case 3 January 2011 | 4 | C | Lungs (3/4) | HP (4/4) |
| Case 4 January 2011 | 4 | C and D | Lungs (4/4) | HP (4/4) |
| Case 5 January 2011 | 10 | E | Medial LN (5/10) | HP (10/10) |

A–E, different farms where the cases originated; HP, histopathology; LN, lymph node; ZN, Ziehl Neelsen’s staining