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Short Communication

Mycobacteriosis outbreak caused by *Mycobacterium avium* subsp. *avium* involving five porcine fattening farms detected through slaughterhouse surveillance

Bernat Pérez de Val[1], Llorenç Grau-Roma[2], Joaquim Segalés[1,2], Mariano Domingo[1,2], Enric Vidal[1,*]

[1]Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA 08193 Bellaterra, Barcelona, Catalonia, Spain

[2]Servei de Diagnòsti de Patologia Veterinària (SDPV), Departament de Sanitat i d'Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Catalonia, Spain

Bernat Pérez de Val, BSc, MSc,

Llorenç Grau-Roma, DVM, PhD, DipECVP

Joaquim Segales, DVM, PhD, DipECVP

Mariano Domingo, DVM, PhD, DipECVP

Enric Vidal, DVM, PhD

E-mail for correspondence: enric.vidal@cresa.uab.cat

Abstract

Between December 2010 and January 2011 a number (n=20) of cases were submitted to the Slaughterhouse Support Service (Servei de Suport a Escorxadors, SESC-CReSA), consisting of grossly nodular granulomatous and caseous lesions in pig carcasses from five different farms. Lesions involved lymph nodes, lungs, liver and spleen.

Histopathological examination showed multifocal to coalescent, granulomatous and necrotizing splenitis, hepatitis, pneumonia and lymphadenitis. The presence of acid-fast bacilli in some cases revealed that it was a mycobacteriosis.

Bacteriological analysis was performed to confirm the diagnosis and identify the aetiological agent (to rule out it was from the *M. tuberculosis* complex mycobacteria, which includes species causing human and animal tuberculosis). The identification of culture isolates by PCR confirmed the growth of *M. avium* complex. Further sequencing analysis determined it was *M. avium.* subsp. *avium*.

The most likely source of the outbreak was considered to be the feed which shared the five farms, which might have been contaminated with *M. avium* subsp. *avium* (common pathogen in poultry and other birds). The fact that most of the animals presented a clear involvement of abdominal viscera is consistent with an oral route of infection.

Keywords: mycobacteriosis, tuberculosis, pigs, Mycobacterium avium, slaughterhouse

Animal tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC) species, is a chronic zoonotic disease mainly affecting cattle, but also can cause disease in a wide range of animal hosts and humans (OIE 2009). The wild boar (*Sus scrofa*) is the third animal species after cattle and goats in the number of MTBC isolates in Spain (Rodriguez-Campos and others 2012), where is considered to be the main wild reservoir of TB (Naranjo and others 2008; Garcia-Bocanegra and others 2012). In addition, domestic pigs (*Sus scrofa domestica*) represented the 1% of Spanish MTBC isolates from animals in the period 1996-2011 (Rodriguez-Campos and others 2012). Moreover, recent TB outbreaks in domestic pigs due to MTBC have been also reported in Italy (Di Marco and others 2012). There are, however, other non-tuberculous mycobacteria that are non-zoonotic pathogens but can be opportunistic causing similar pathologies in swine.

M. avium complex (MAC) comprises a number of bacterial species that are non-zoonotic pathogens but with a different degree 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 generalized granulomatous lesions in poultry and wild birds, MAP is the causative agent of Johnes Disease in ruminants, while pigs are the primary animal host for MAH (Thorel and others 2001; Mijs and others 2002; Agdestein and others 2011; Álvarez and others 2011). However, pigs may also play a role as reservoirs of MAA infection causing indistinguishable lesions from TB (Komijn and others 1999). Therefore, mycobacterial species identification becomes crucial to determine the zoonotic nature of outbreaks in pig farms with animals presenting TB-like lesions.

In December 2007, as an initiative of the Catalan Government's Health Protection Agency, the Slaughterhouse Support Service (Servei de Suport a Escroxadors, 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 in reaching final diagnoses of slaughterhouse findings. Between December 2010 and January 2011, several organs from a total of 20 pig cases coming from 5 different farms were submitted to SESC. The lesions consisted of multifocal to coalescing whitish nodular lesions with caseous and partially mineralized appearance, and affected mesenteric lymph nodes (LN), liver, spleen, mediastinal LN and lung (see Figure 1). While lesions in organs of the abdominal cavity (mainly liver and mesenteric LN) were observed in all pigs, lesions in the thoracic cavity (lungs and mediastinic LN) were present in 12 pigs, coming from only 3 out of the 5 studied farms.

Histopathological examination of the lesions using haematoxylin and eosin (HE) routine staining revealed multifocal, necrotizing and granulomatous splenitis, hepatitis, pneumonia and lymphadenitis. Numerous multinucleated (Langhans) giant cells were observed. Ziehl-Neelsen (ZN) staining revealed, in some of the cases, the presence of acid-fast bacilli indicating that it was a mycobacteriosis (see Figure 2). Information on each of the outbreaks including the organs examined and the different diagnostic techniques used are summarized in Table 1.

Consequently, a suspected TB was reported to local Animal and Human Health Authorities, and biosafety measures (latex gloves and facial masks) were implemented for slaughterhouse personnel.

Ruling out the infection caused by zoonotic mycobacteria was established as a priority. Differential diagnosis was performed by means of bacteriological studies to identify the ethological agent causing the lesions. Isolation was performed on Coletsos and Lowenstein-Jensen selective media with pyruvate (bioMérieux España, Madrid, Spain). Thereafter DNA was extracted from colonies by boiling them 10 min. at 100° C, and identification was performed by means of a multiplex PCR specific for MTBC and MAC (Wilton 1992) followed by sequencing of the DNA encoding 16S rRNA.

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The multiplex PCR of these colonies identified a non-tuberculous mycobacteria belonging to the MAC. Sequencing and subsequent Basil Local Alignment Search Tool (BLAST®) analysis (Altschul and others 1990) confirmed MAA in all cases.

Subsequent epidemiological investigation suggested that the most likely source of the outbreak was the feed which was shared between the five different farms a few months before the outbreak detection. Certain feed contents could have been contaminated with MAA. Mycobacteriosis in pigs fed peat naturally contamined with MAC has been previously described (Matlova and others 2005; Agdestein and others 2011). In these infected pigs, lesions were primarily found in the head and mesenteric LN. Accordingly, most of the animals studied in the present outbreak showed a clear involvement of abdominal LN 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 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, causing a major animal-to-animal transmission by the faecal-oral route, which could explain the higher incidence of infection caused by this subspecies in pigs as compared to MAA (Agdestein and others 2012). Also, if MAA in pigs is 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 both MTBC and MAC infections. The zoonotic risk of animals infected with MTBC has been widely described (Rodwell and others 2008; Rodríguez and others 2009; Torres-Gonzalez and others 2013). Nevertheless, severe MAC infections in humans have been also reported, especially in immunosupressed individuals, (Pavlik and others 2000; Biet and others 2005; Mobius and others 2006). SESC proved to be an effective tool that allowed a rapid diagnosis and molecular identification of the mycobacteriosis outbreak, leading to know its associated risks for public health.

Acknowledgements

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Figures:

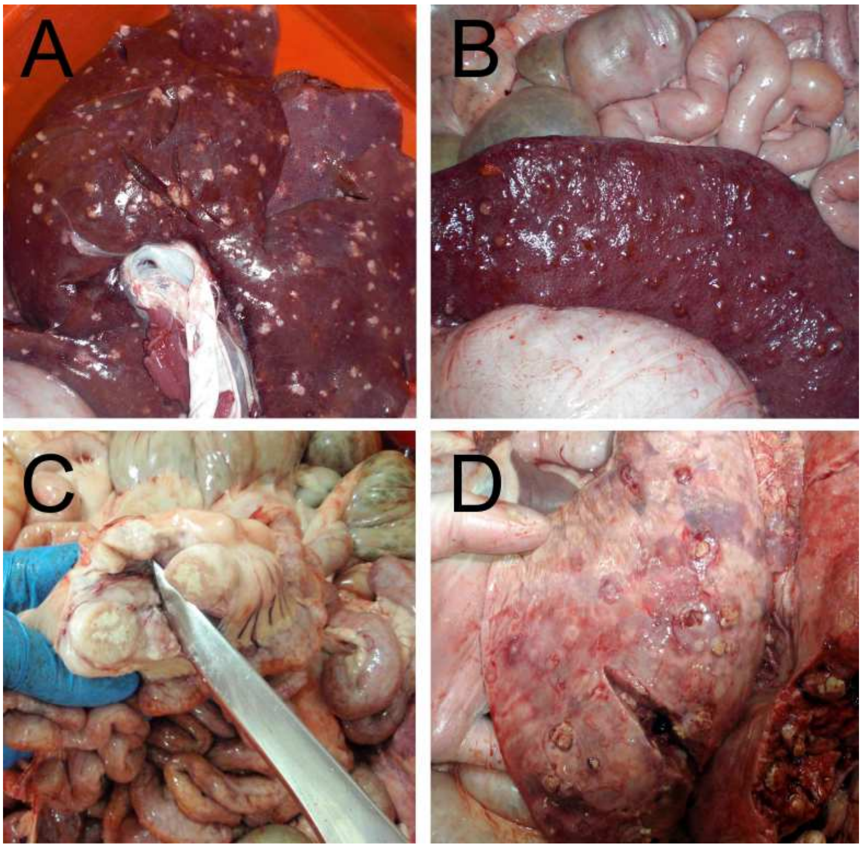

Figure 1. Lesions consisting on granulomatous and caseous nodules, were observed in the abdominal viscera affecting (A) liver, (B) spleen and (C) mesenteric lymph nodes. In some cases, lesions were also observed in (D) thoracic cavity.

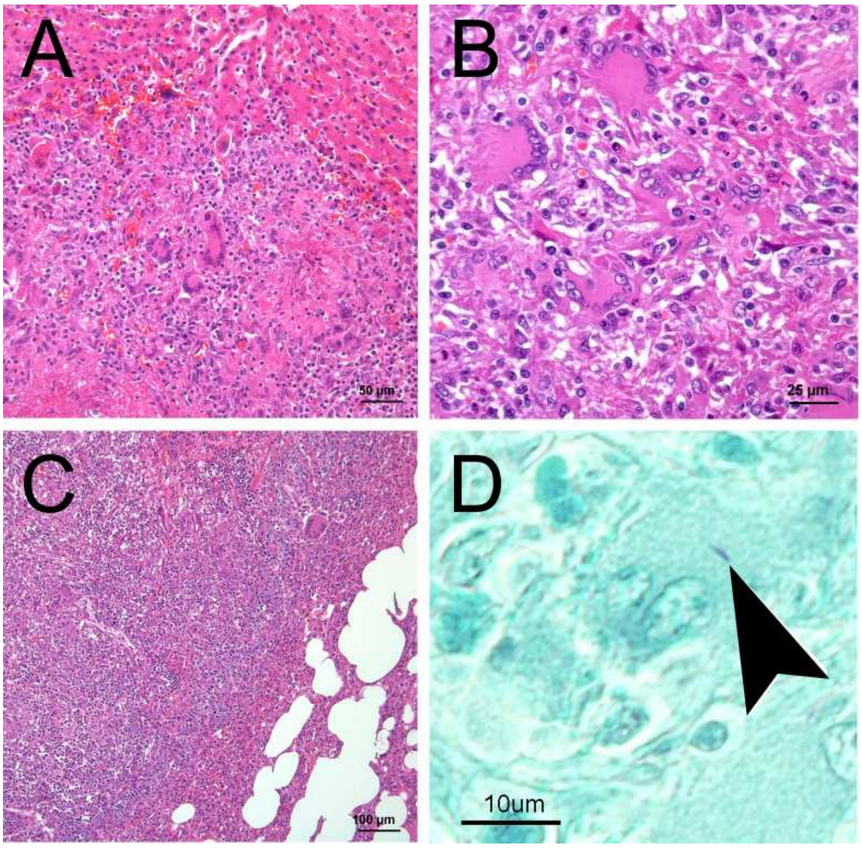

Figure 2. (A) Granulomatous hepatitis with necrosis foci, abundant macrophages and Langhans cells. (B) Detail of Langhans cells in the splenic parenchyma. (C) In the lung, granulomatous foci of inflammatory infiltrate were also appreciated. (D) Ziehl-Neelsen stain showed the presence of a few acid-fast bacilli.

Tables:

Table 1: Information on the cases submitted and diagnostic techniques performed.

CASE	**NO. OF AFFECTED ANIMALS**	**ORIGIN**	**REPORTED AFFECTED VISCERA**	**DIAGNOSTIC RESULTS**
Case 1 December 2010	1	B	Liver (1/1) Mesenteric LN (1/1)	HP + (1/1) ZN + (1/1) Culture + (0/1)
Case 2 December 2010	1	A	Liver (1/1)	HP + (1/1) ZN + (1/1) Culture + (1/1)
Case 3 January 2011	4	C	Lungs (3/4) Spleen (2/4) Liver (4/4) Mesenteric LN (1/4)	HP + (4/4) ZN + (2/4) Culture + (4/4)
Case 4 January 2011	4	C and D	Liver (4/4) Spleen (4/4) Lungs (4/4) Mesenteric LN (4/4) Mediastinal LN (4/4)	HP + (4/4) ZN + (1/4) Culture (4/4)
Case 5 January 2011	10	E	Lungs (2/10) Mediastinal LN (5/10) Spleen (3/10) Liver (9/10) Mesenteric LN(3/10)	HP + (10/10) Culture + (10/10)

HP: Histopathology. ZN: Ziehl Neelsen's staining. LN: Lymph nodes. A to E: different farms where the cases where originate.

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