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SVEPM Society for Veterinary Epidemiology and Preventive Medicine
DUBLIN 2014

MAC IN PORTUGUESE SWINE: A PUBLIC HEALTH CHALLENGE

1.INTRODUCTION AND PURPOSE

In Portugal, since the end of 2004, increasing numbers of lesions characteristic of tuberculous lymphadenitis have been detected in regular swine meat inspections (fig.1), and *Micobacterium avium* subsp. *hominissuis* (*Mah*) was identified as the cause of this outbreak.
Although other microorganisms may produce similar and indistinguishable macroscopic lesions detected in abattoir lymph node palpation and incision, *MAC* is considered the main cause of granulomatous lymphadenitis in slaughtered pigs.
The aim of the present study is to investigate and characterize the occurrence of *MAC* in lymph nodes from slaughtered swine in Portugal.

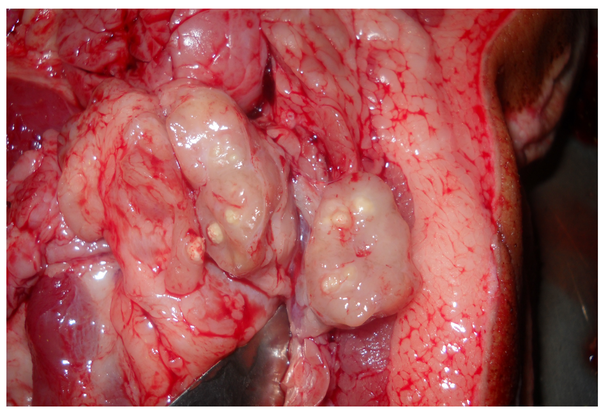

Fig. 1. Granulomatous lymphadenitis detected in swine in *post mortem* inspections (submaxillary lymph nodes).

2.METHODS

Sampling: submaxillary lymph nodes from slaughter pigs approved in *ante mortem* inspection presenting macroscopic granulomatous lesions at abattoir.

#1group: n=47 (7 farms; November 2012-February 2013)

#2 group: n=153 (9 farms; September-December 2013)

Sample processing and *MAC* identification:

a)Samples were divided into two portions (fig.2): one was fixed in 10% buffered formalin for histopathological examination (staining with hematoxylin eosin (HE) and Ziehl-Neelsen (ZN) to detect acid-fast bacilli (AFB) (at the moment n=47) and the other was frozen at -20ºC and then stored at -80ºC for bacterial culture.
b)Microbiology culturing (at the moment n=145): lymph nodes were grinded and decontaminated. Bacterial isolation was performed on LöwensteinJensen®, Coletsos® and BBL™ MGIT™ liquid medium, incubated at 37˚C for up to 6-8 weeks and 42 days, respectively, and observed weekly for growth.
c)The sediment of each processed sample was also stained with ZN and again observed by light microscopy.
d)Genetic characterization:
d1) Differentiation of clinically relevant mycobacteria species (*M. avium* spp. and *M. intracellulare*): Genotype *Mycobacterium* CM (Hain Lifescience GmbH, Nehren, Germany)
d2) Differentiation of *M. avium* subspecies: multiplex PCR (IS901, IS1245, dnaJ).

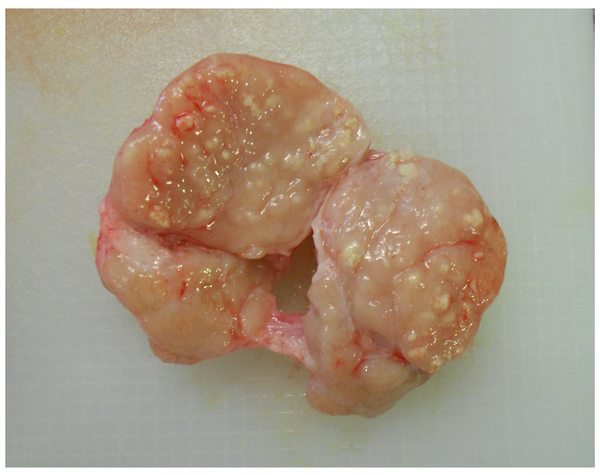

Fig. 2. Submaxillary lymph node showing gross granulomatous lesions.

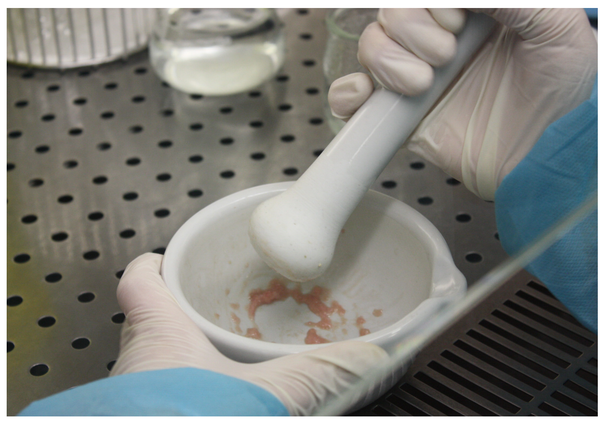

Fig. 3. Sample preparation for culture.

3.RESULTS

#1 group

• All lymph nodes selected revealed lymphadenopathy and 45 (96%) showed gross granulomatous lesions (fig.2).
•Microscopic examination revealed follicular hyperplasia (fig.4) and were identified granulomatous lesions, the typical tubercle lesions with central caseous necrosis (fig.5) surrounded by syncytium of epitheloid cells, other macrophages as well as multinucleated giant cells (fig.6).
• In 9 samples (19%) it was possible to identify AFB with ZN staining.
•Microscopic direct examination of the sediment demonstrated AFB in 18 samples (43%) and culture revealed mycobacteria growth in 24 (57%) (fig.7 and fig.8).
• All the 24 isolates were identified as ***Mah*** by PCR (fig.9).

#2 group (ongoing work)

• Currently, 98 samples were processed and 65 (66%) were positive in cultural examination.
• Microscopic direct examination of the sediment demonstrated AFB in 63 (64%).
• At this moment, we are starting the identification of the positive cultures using PCR.

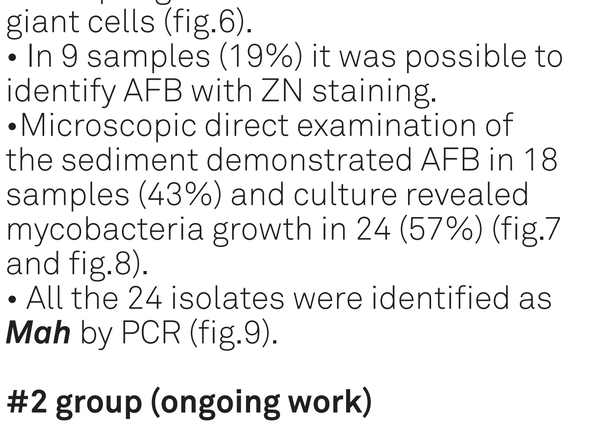

Fig. 4. Histopathological section of lymph node presenting follicular hyperplasia (HE: 20x).

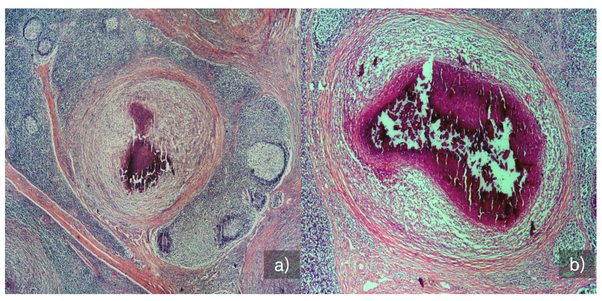

Fig. 5. Granuloma with an area of central necrosis and dystrophic calcification, surrounded by circumferential layers of collagenous connective tissue (HE: a) 20x; b) 40x).

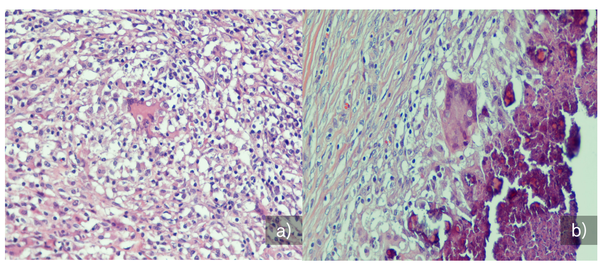

Fig. 6. The caseous necrosis is surrounded by syncytium of epitheloid cells and other macrophages as well as multinucleated giant cells, with horseshoe like nuclei (HE: a)100x; b) 200x).

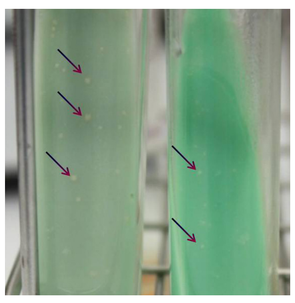

Fig. 7. *M. avium* spp. culture in solid media.

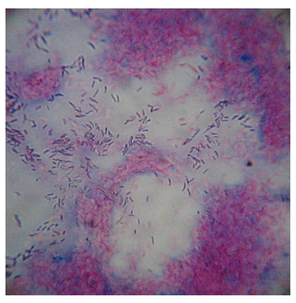

Fig. 8. AFB smears microscopy of *M. avium* spp. positive culture (ZN 1000x).

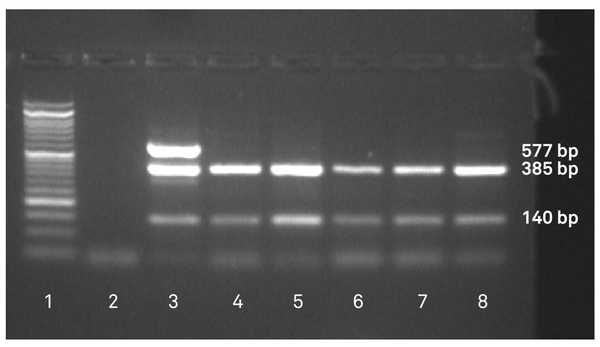

Fig. 9. Multiplex PCR analysis: 1. Ladder; 2. Negative control; 3. Positive control (*Mycobacterium avium avium* DNA); 4. Positive control (*Mycobacterium avium hominissuis* DNA); 5, 6, 7, 8. Tested samples (*Mycobacterium avium hominissuis* DNA).

4.CONCLUSIONS

• Our preliminary results endorse ***Mah*** as the main responsible for granulomatous lymphadenitis in swine in Portugal.
•Although swine can harbor *MAC* before slaughter, infections in pigs have no apparent effect on the health of the animal, and the diagnosis by *ante mortem* examination at the abattoir is usually unmanageable.
• Lymph nodes of slaughter swine must be incised and examined at *post mortem* inspection for granulomatous lesions, but remains essential to identify the etiological agent.
• Sensitivity for detection of the agent during the histopathological exam, with ZN staining, is low.
• Culture, in spite of time consuming, and PCR revealed to be most sensitive methods to identify ***Mah***.
• It remains crucial to clarify the real impact of ***Mah*** in public health and therefore, is essential to implement methods for a rapid and economic identification, in order to prevent the entry in the food chain.

ACKNOWLEDGEMENTS

We gratefully acknowledge the collaboration of Dra. Anabela Costa DAVA-DSAVRC and Doutora Ana Botelho LNIV-INIAV.

FUNDING

PEST/AGR/OE/UO211/2011-14