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MRSA in carcass abscesses of slaughtered piglets

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Roasted piglets (about 2 months old and 6 to 8 kg live weight) constitute an important gastronomic dish in Portugal, being the production and slaughter of these animals of increasing economic importance. In 2015, 1 148 025 piglets were slaughtered in Portugal. From those, 956 carcasses (0.08%) were totally condemned due to the presence of multiple abscesses, representing the third main cause of piglets´ carcass condemnation at post mortem inspection. One of the pathogens enrolled in the etiology of abscesses it is Staphylococcus aureus and, within this specie, MRSA may represent and additional threat, if present. The main objective of this study was to evaluate the involvement of MRSA in abscesses in piglet carcasses at slaughter. During 12 weeks in the spring 2016, 48 samples of abscesses purulent content were aseptically collected from piglets carcasses condemned at post mortem inspection. Briefly, at laboratory, samples were inoculated in Brain Heart Infusion Broth and after plated in Manitol Salt Agar (OXOID™) agar. Suspicious colonies were identified by Gram staining and catalase test. Those positive to both testes, were plated in ORSAB agar (Oxacillin Resistance Screening Agar Base, OXOID™) and suspicious isolates of MRSA were confirmed by using a multiplex PCR assay targeting the 16S rDNA, nuc and mecA. In this study, MRSA was identified in 23 samples (23/48, 48%), being the first report of MRSA identified in carcass abscesses of piglets in Portugal. Since all analysed samples were from carcasses declared unfit for human consumption, the presence of MRSA can´t be considered a direct food safety issue. Nevertheless, although it is known that asymptomatic slaughtered pigs may be a source of MRSA into the abattoir, the high prevalence (48%) found in carcass abscesses must be take into consideration by FBO as an important and additional source of contamination, requiring provision of adequate decontamination measures to avoid cross contamination. Also, personnel must be aware of the potential risk of exposure during manipulation of these carcasses. More studies should be undertaken, at primary production level, to understand the reason and level of this problem, under One Health perspective.

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Granulomatous lymphadenitis in swine: validation of national data based on identification by the service of federal inspection (SFI)

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Introduction

The granulomatous lymphadenitis (GL) in pigs is mainly caused by *Mycobacterium avium hominissuis* (*MAH*), who belongs to the *Mycobacterium avium* Complex (MAC), considered non-tuberculous mycobacteria (NTM). Although GL does not affect swine zootecnical performance, economic losses occur during the slaughter line by condemning viscera and carcasses. The lesion is characterized by one or more foci of granuloma, which most frequently affect the organs of the digestive tract and peripheral lymph nodes. The main differential diagnosis encompasses *Mycobacterium bovis* (*M. bovis)* and *Mycobacterium tuberculosis* (*M. tuberculosis*), who belongs to the *Mycobacterium tuberculosis* Complex (MTbC), of relevant zoonotic potential. Nevertheless macroscopic examinations and histopathology are insufficient to determine the etiologic agent involved. Federal Meat Inspection had registered the frequency of 0,81% of lymphadenitis in Brazil from 2012 to 2014. The aim of this study was to investigate the etiology of the granulomatous lesions for validation of the national data of inspection and build a database to further risk analysis.

Material and Methods

In 2017 the federal veterinary inspectors collected mesenteric lymph nodes with granulomatous lesions of 399 swine production farms located in eight states (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais, Mato Grosso, Mato Grosso do Sul e Goiás), representing 158 brazilian municipalities. If available, mesenteric lymph nodes with GL of three swine on each farm were sampled, totaling 257 lymph nodes from finisher pigs and 142 from sows/boars. The tissues were submitted to histological examination and bacterial analysis. Mycobacterial isolation and identification were performed according to OIE (2018). Briefly, the isolates from Lowenstein Jensen and/or Stonebrink media, positive to acid-fast bacteria in Ziehl-Neelsen (ZN), were typified by Polymerase Chain Reaction (PCR) protocols (Table 1) for genus and species, supporting the distinction between *MAH*, *Mycobacterium avium avium/silvaticum*, *M. bovis* and *M. tuberculosis*.

Results

Comparing histological findings with macroscopic examination, the Service of Federal Inspection (SFI) correctly identified 85% of granulomatous lesions in individual basis. The rate isolation of mycobacteria was 32,08% (128/399), of which 76,56% (98/128) were positive for *MAH*, 1,56% (02/128) for *M. bovis*, and 21,87% (28/128) only for *Mycobacterium* spp. The identification of *Mycobacterium* species by state is shown in table 2.

Discussion and Conclusion

Overall, the results had shown a good assurance between the evaluation performed in the slaughter line by the veterinarians inspectors and the histopathologic exam. The positive predictive value is higher than 80% when we compare the macroscopic examination

Table 1: Description of the primers used in the Polymerase Chain Reaction (PCR)

Primers	Sequence	Lenght of PCR product	Reference
DNAJ	5´- GGG TGA CGC GAC ATG GCC CA -3´ 3´- CGG GTT TCG TCG TAC TCC TT -5´	236bp	(TAKEWAKI et al., 1993)
IS1245	5´- GCC GCC GAA ACG ATC TAC- 3´ 3´- AGG TGG CGT CGA GGA AGAC -5´	427bp	(GUERRERO et al., 1995)
IS901	M IS901F 5‘- GGATTGCTAACCACGTGGTG -3‘ M IS901R 3‘- GCGAGTAGCTTGATGAGCG -5‘	577bp	(MORAVKOVA et al., 2008)
INS	INS1 5´- CGTGAGGGCATCGAGGTGGC - 3´ INS2 3´- GCG TAGGCGTCGGTGACAAA -5´	245bp	(VAN EMBDEN et al., 1993)
RD4	5‘ AACGCGACGACCTCATATTC 3‘ 3‘ AAGGCGAACAGATTCAGCAT 5‘	400bp	(SALES et al., 2014)

Table 2: Identification of Mycobacterium species by swine production farms as a function of the state of origin

State of origin	MAH	Mycobacterium spp.	M. bovis	Negative	Number of samples
Goiás	01(10.00)	0 (0.00)	0 (0.00)	09 (90.00)	10
Minas Gerais	13 (18.06)	11 (15.28)	0 (0.00)	48 (66.67)	72
Mato Grosso do Sul	0 (0.00)	0 (0.00)	0 (0.00)	03 (100.00)	03
Mato Grosso	0 (0.00)	0 (0.00)	0 (0.00)	4 (100.00)	04
Paraná	17 (31.48)	06 (11.11)	0 (0.00)	31 (57.41)	54
Rio Grande do Sul	15 (28.30)	06 (11.32)	02 (3.77)	30 (56.60)	53
Santa Catarina	42 (24.85)	05 (2.96)	0 (0.00)	122 (72.19)	169
São Paulo	09 (27.27)	0 (0.00)	0 (0.00)	24 (72.73)	33

and the lymph nodes that showed histopathological lesions of granulomatous lymphadenitis. The present study shows a high prevalence of *MAH* causing GL in Brazilian farms, confirming this subspecies as the most prevalent in the swine population as it has been described in other countries. The hypothesis of fecal-oral transmission between animals may justify the permanence and prevalence of *MAH* subspecies in pig farms. The two samples that were positive for *M. bovis* were collected at the same slaughterhouse, but they were from different farms, located in different towns. Both of farms raise pigs and dairy cattle. Anyway the source of the infection was not defined. Nevertheless, due to the disease prevalence in pigs and differences in zoonotic potential between the etiological agents, lesions of porcine granulomatous lymphadenitis should be considered in the definitions of the exams performed by the SFI.

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Pathologic diagnosis of zoonotic parasitosis in slaughter pigs in Brazil

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Introduction

Brazil is the fourth largest swine producer and pork exporter in the world, the slaughter under the federal meat inspection service achieved 37 million pigs in 2017 (https://sidra.ibge.gov.br). Brazilian meat inspection system is under a modernization process and new procedures are just standardized and regulated for pigs reared in farms submitted to health animal service rules. In order to supply the risk analysis for meat inspection modernization, several studies on zoonotic hazards were conducted in Brazil last years. This one was focused on zoonotic parasitosis once that in sanitary *post mortem* examination, the inspectors can identify lesions compatible with cysticercosis (Satyaprakash et al., 2018), hydatidosis (De La Rue, 2008) and sarcosporidiosis (Zainalabidin, et al., 2017).

Cysticercosis is caused by metacestodes of *Taenia solium*. Primarily, cysticercosis is an infection of pigs that act as an intermediate host of *T. solium*. Pigs are infected by ingestion of contaminated water, soil or feed with the eggs of *Taenia solium* expelled from tapeworm carriers. The eggs develop into cysticerci in various organs and musculature causing porcine cysticercosis characterized by small round whitish viscous cyst (7 to 15 mm), located mainly in the lingual muscles, masseters, heart and diaphragm (Satyaprakash et al. 2018).

Cystic echinococcosis is a zoonotic disease caused by the genus *Echinococcus* (*Cestoda: Taeniidae*). Pigs are considered important intermediate hosts of the larval stage by eggs ingestion from contaminated environment with feces of definitive host. The intermediated host develops hydatid cysts in the liver and the parasite cycle can be complete if a definitive host ingest this organ without a heat treatment (De La Rue, 2008).

Sarcosporidiosis is a disease caused by cyst forming coccidian, namely, *Sarcocystis* spp. Pigs can be infected when consuming food contaminated with fecal material of carnivores containing the sporocysts of *Sarcocystis* spp. The whitish filamentous, spindle-shaped, rice-grain-like, macrocyst-forming sarcocyst has been observed in the muscles of pigs, mainly in the heart, tongue and diaphragm (Zainalabidin, et al., 2017). The aim of this study was to validate the macroscopic diagnosis of these lesions detected by veterinary inspection service using histopathology analysis.

Material and Methods

From May 2017 to May 2018 was performed a prospective study with the collaboration of federal meat inspectors, which were asked to collect all lesions suspected of cysticercosis, hydatidosis and sarcosporidiosis found during routine of meat inspection. These samples were sent to animal pathology laboratory of Embrapa Swine and Poultry Research Center. It was analyzed a total of 361 samples, 296 were muscle samples suspected of sarcosporidiosis, 64 cystic livers suspected of hydatidosis and 1 heart sample suspected of cysticercosis. The tissue samples were collected in 10% buffered formalin and sent to the laboratory for processing by the routine histopathology technique.

Results

In 34 (53.1%) liver samples, *Cysticercus tenuicollis*, the larval form of *Taenia hydatigena*, was identified. The macroscopic characteristics of these lesions were single or multiple cysts, colorless fluid, thin membrane and a cephalic invagination corresponding to the scolex (Figure 1A). In the histopathology analysis it was observed that the cysts have a membrane that invaginates in only one scolex (Figure 1B), which has suckers and many hooks. In the other 30 liver samples, there were no parasites inside the cysts and it was not possible to identify the cause of the lesions. *Echinococcus spp.* was not identified. No sarcosporidiosis suspect lesion was found in the finishing pigs. All muscle samples analyzed were from culling sows. In 163 (55%) of these samples, granulomatous myositis (Figure 1C) compatible with *Sarcosporidium* spp. infection was observed. Intact sarcocysts were also observed in some of these samples (Figure 1D). No parasitic lesion was identified in the remaining 45% of the samples. Histopathology was not conclusive in the heart sample suspected of *Cysticerccus* spp. infection. The histologic lesion consisted of a circumscribed area of granulomatous inflammation on the surface of the myocardium.

Discussion and Conclusion

The reports of Brazilian Federal Meat Inspection System in swine slaughterhouses (Coldebella et al., 2017), have shown results of carcass condemnation and trimming data on more than 97 million pigs slaughtered between 2012 and 2014.The zoonosis injuries condemnations/trimming were reported in very low frequency. Among the total of organs and carcass inspected cysticercosis was registered in 0.00092%, sarcosporidiosis in 0.00051% of the cases.