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Mycobacterium bovis DETECTION IN SLAUGHTERED PIGS IN THE STATE OF RIO GRANDE DO SUL, BRAZIL

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Abstract

The infection by the *Mycobacterium* genus is important in pig farming due to the economic losses caused by total or partial carcass condemnation in slaughterhouses. The present study investigated the occurrence of a tuberculosis outbreak in pigs, based on the identification of lesions at the slaughter line of a slaughterhouse. At the inspection line of the slaughterhouse, carcasses were identified with viscera containing macroscopic lesions that indicated tuberculosis (granulomatous lymphadenitis). Tracheobronchial, mesenteric, and submandibular lymph nodes were collected, as well as liver samples and their corresponding lymph nodes. The samples were sent to the Federal Agricultural Defense Laboratory (LFDA/RS) and processed for the diagnosis of tuberculosis and the molecular characterization of *Mycobacterium bovis*. Based on the results of post-mortem and laboratory inspections, the occurrence was characterized as a tuberculosis outbreak in pigs, which originated from a farm in the state of Rio Grande do Sul, Brazil. Over three months, three batches, adding up to 2884 animals, were sent to slaughter, of which 102 (3.5%) had tuberculosis-like lesions at the inspection line. Based on these results, the productive process was investigated, assessing the feeding, water supply, and milk whey offered in the diet of pigs. It was concluded that the outbreak was caused by feeding unpasteurized or inadequately pasteurized (insufficient time x temperature relation) whey to the pigs. The use of whey from cheese production is a frequent practice in the state of Rio Grande do Sul and one of the risk factors for granulomatous lymphadenitis in pigs.

Keywords: Pigs. Slaughterhouse. Tuberculosis.

1. Introduction

Mycobacterium bovis is the etiological agent of bovine tuberculosis (TB). Bovine TB is a recrudescent zoonosis whose eradication has eluded some of the least and most developed countries in the world. Swine TB caused by *M. bovis* has been reported in Argentina, South Africa, and West Africa. Pigs can become infected from cattle orally, and outbreaks have been associated with infected yards or buildings contaminated with infected fecal material, feeding piglets with infected cattle milk, and contact with wildlife (Muwonge et al. 2012).

The *Mycobacterium* infection is important in pig farming due to the economic losses caused by the condemnation or change in the destination of carcasses in slaughterhouses (Silva et al. 2002).

Granulomatous lymphadenitis (GL) lesions in pigs are detected during routine inspections in slaughterhouses, and *Mycobacterium avium* (MAC) is the main agent found. It is a chronic disease of slow progression in which the lesions are visually identified only two to four months after contamination, which

prevents identifying the source of infection and consequently its control. The GL occurs mainly in association with poor agricultural and sanitation practices in the herd, higher animal contact with feces, and the lack of biosafety measures in the food served to pigs (Amaral et al. 2004).

M. bovis is the most pathogenic species for animals, which may cause a generalized infection. In pigs, such infections are asymptomatic and mainly detected at slaughterhouses by post-mortem inspections because of their typical granulomatous lesions (Domingo et al. 2014).

This study aimed to report the occurrence of a tuberculosis outbreak in pigs based on the identification of lesions at the slaughter line of a slaughterhouse.

2. Material and Methods

Over three months (September to November 2011), at the inspection procedures in a slaughterhouse, visceral organs containing macroscopic lesions similar to those caused by tuberculosis were identified. This is not a common finding, and it was restricted to one origin, which sent 2884 pigs to slaughter in three batches. From these batches, 787 carcasses were conducted to a final inspection. From these, 102 were condemned and 236 were used in cooked products. Additionally, five samples of the tracheobronchial, mesenteric, and submandibular lymph nodes, as well as two liver samples and their hepatic lymph nodes, were collected and sent to the Federal Agricultural Defense Laboratory (LFDA/RS) owned by the Brazilian Ministry of Agriculture (MAPA).

The samples were processed for the diagnosis of animal tuberculosis using the MET/DDB/PL/001 method, standardized by LFDA for the molecular characterization of *Mycobacterium bovis*. The samples were processed and inoculated in Herrold's medium supplemented with mycobactin. The bacterial isolation technique and real-time PCR were combined to identify the presence of mycobacteria in animals (Sales et al. 2009).

A local investigation was performed at a finishing farm to analyze pig finishing and find out potential sources of animal contamination.

3. Results

From 2884 slaughtered pigs, 787 (27.3%) were examined during routine meat inspections for suspected tuberculous lesions. At the pig slaughter line, 102 carcasses (3.5%) presented macroscopic lesions similar to those found in tuberculosis at two or more sites, in the combinations of mesenteric, tracheobronchial, submandibular, sublingual, epigastric, and hepatic lymph nodes, as well as the liver parenchyma (Figure 1). These carcasses were condemned and sent to rendering. At LFDA, visceral samples were positive for *Mycobacterium bovis* in the caseous material found in the lesions.

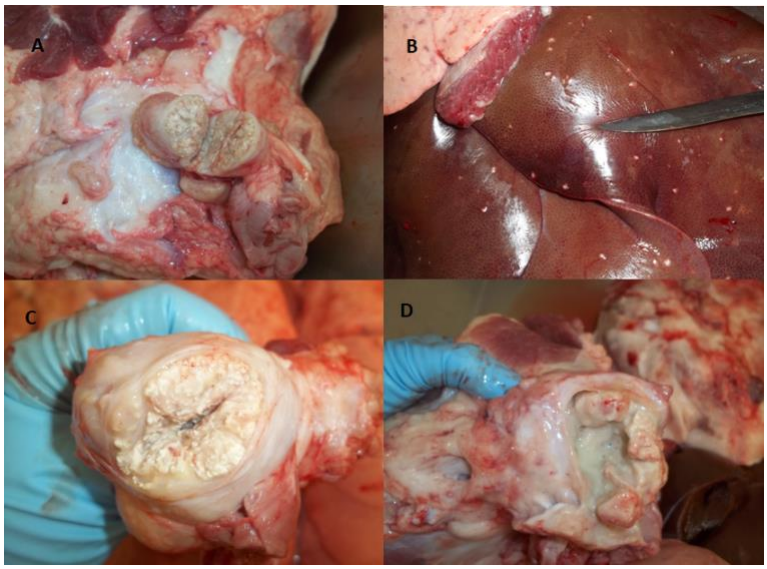

Figure 1. Macroscopic lesions observed in slaughtered pigs, caused by *Mycobacterium bovis*. A – submandibular lymph nodes; B – liver; C – epigastric lymph node; D - lymph nodes.

Based on visual (at inspection procedures) and laboratory results, the occurrence was characterized as a tuberculosis outbreak in pigs.

Based on animal origin and slaughter and laboratory findings, the entire pig finishing process was investigated and assessed, including not only the feeding, water, and whey offered to the pigs but also the cows that produced the milk delivered to the dairy factory. Based on this assessment, it was concluded that the outbreak was caused by feeding unpasteurized or inadequately pasteurized (insufficient time x temperature relation) whey to the pigs.

4. Discussion

Tuberculosis is a chronic swine disease, its main characteristic is the development of tubercles in most organs, and it is caused by *Mycobacterium bovis* and *Mycobacterium avium* complex. The infection occurs primarily by ingestion. The primary complex is incomplete when occurring in the pharyngeal lymph nodes. In this case, the agent is introduced by the tonsils. When bacteria enter through the intestinal mucosa, usually at Peyer's patches, the primary complex includes the mesenteric lymph nodes. Bovine tuberculosis lesions in pigs have a similar appearance to those seen in cattle (Herenda et al. 2000).

The *Mycobacterium* sp. induces granulomatous inflammation in different animal species. *Mycobacterium bovis* and *Mycobacterium avium* are important pathogens to cattle and swine and can also infect humans, especially those immunosuppressed. Production losses, commercial barriers, and carcass condemnations in slaughtering are related to this infection, which implies high economic losses (Holke et al. 2014; Andrezza et al 2015).

Pigs are rather susceptible to *M. bovis*. Progressive lesions are usually observed in the lungs and well-organized tubercles may be present in the liver, spleen, and lymph nodes in thoracic and abdominal cavities. Microscopically, granulomas may contain caseous centers with different degrees of mineralization (Thoen et al. 2009). The diseases caused by the *Mycobacterium* genus are classified into two groups: tuberculous (*M.* bovis and *M. tuberculosis*) and nontuberculous or atypical (*M. avium, M. intracellulare,* and *M. fortuitum*).

In pigs, *M. bovis* causes fibrous lesions in the lymph nodes and internal organs, mainly in the liver, where there are small caseous to caseo-calcareous lesions (Pinto 2003), as observed in the outbreak in question. In the mesenteric lymph nodes, slaughter pigs infected with *M. bovis* presented lesions of a deep yellow color and grit-like texture (Muwonge et al. 2012).

In technified pig farming, the infections caused by *M. bovis*, which is the causal agent of classic zoonotic tuberculosis, are rare, and *M*. *avium* is the etiological agent most frequently identified in macroscopic lesions. However, the visual aspect of granulomatous lesions typical from infections caused by both agents is similar, and the isolation and identification of the agent is the only way to diagnose the causal agent of infections (Kich et al. 2011).

Barandiaran et al. (2015) characterized the epidemiological dynamics of *Mycobacterium avium* complex (MAC) infection in a swine population of Argentina using molecular tools and spatial analysis techniques. Isolates (n = 196) obtained from TB-like lesions were characterized to either *M. bovis* (n = 160) or *M. avium* (n = 16), while the remaining 20 (10·2%) isolates were positive for both *M. bovis* and *M. avium*. The detection of both bacteria suggests a co-infection at the animal level.

In Great Britain, since 2004, it is compulsory to notify any suspected TB lesion in post-mortem inspections to the Animal Health and Veterinary Laboratories Agency (AHVLA). From then to 2012, pigs have become the third most frequent non-bovine domestic species from which *M. bovis* has been isolated (11%) (Broughan et al. 2013). The data suggest that pigs raised outdoors or on holdings with poor biosecurity may be more vulnerable to infection by *M. bovis* (Bailey 2013). In Egypt, Mohamed et al. (2009) detected suspected tuberculous lesions in 14.8% of swine carcasses. From these, suspected mycobacterial isolates were detected in 60% of cases based on PCR genotyping methods. In Poland, authors Lipiec et al. (2019) found *M. bovis* in two pigs maintained on the farm. Also in Poland, the correct and long-term control of cattle has resulted in sporadic cases of bovine tuberculosis in pigs. Tuberculosis in pigs is most often detected in the slaughterhouse during slaughtering, and the same occurs in Brazil.

The main route of infection for mycobacterioses in pigs is through the digestive tract (O'Reilly and Daborn 1995), typically with lesions in the submaxillary and mesenteric lymph nodes (Domingo et al. 2014),

as observed in both the present and a previous outbreak in the city of Viamão, RS, Brazil, at a farm that used restaurant residues as animal feed (Schwarz et al. 2002).

Infection outbreaks are usually found where tuberculosis has been diagnosed in cattle. Yards or buildings contaminated with fecal matter containing viable organisms may serve as a source of infection as milk from infected cows (Thoen et al. 2009). The oral route is the most important for infecting domestic pigs, most frequently caused by feeding milk or milk products from infected cows (Cousins 2001). The use of whey from cheese production, which was identified as the source of infection in pigs in this outbreak, is a frequent practice in the state of Rio Grande do Sul, particularly in regions where handmade cheese is produced with no thermal processing.

The risk factors for the occurrence of lymphadenitis in pigs are associated with the management and maintenance of the premises, the hygiene of water and feed troughs, untreated water, feed management (transportation, storage, and access), and the lack of a quarantine period after disinfection (Amaral et al. 2004).

5. Conclusions

A quick diagnosis and adequate identification of infection sources is necessary to reduce the risk of pig carcass condemnation by tuberculosis. Feeding pigs with whey from unsafe milk and dairy production maintains this disease in the environment, which may lead to pathogen dissemination and consequent harm to human health.

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