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Case Report

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Congenital tuberculosis in a 25-day-old female calf

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Abstract: A female calf, housed in a dairy intensive-system farm, suddenly died 25 days after birth. Routine necropsy revealed multiple variably sized white to yellowish nodules in the lungs. Histopathological diagnosis showed severe granulomatous pneumonia with acid-fast bacilli; *Mycobacterium bovis* was confirmed by polymerase chain reaction and culture isolation. Congenital tuberculosis was diagnosed based on these findings. The calf probably acquired the infection from her mother through aspiration of the amniotic fluid. The aim here is to describe the morphological lesions and complementary confirmation of congenital tuberculosis in a young female calf.

Key words: *Mycobacterium bovis*, congenital transmission, pathological diagnostic

1. Introduction

Bovine tuberculosis (bTB) is an infectious disease of cattle caused by *Mycobacterium bovis*, a gram-positive, acid-fast bacterium; bTB is a zoonosis that represents a major public health problem. In cattle, there are several possible routes of mycobacterial transmission; the usual are respiratory and alimentary, but cutaneous, genital, and congenital infection may also occur. Although genital and congenital transmission are not common, the congenital route is still important in areas where the bTB prevalence is high (1). Usually, bTB is considered a chronic disease of adult animals, but the congenital form can occur in young ones. bTB is characterized by a granulomatous inflammatory process that mainly affects the liver, lungs, and their draining lymph nodes, but other organs can also be affected. Clinical, postmortem, histopathological, immunological, bacteriological, and molecular examinations are used for the confirmation of bTB (2,3). Gross pathological lesions of bTB include white to yellowish, caseous, necrotic, solid nodules surrounded by fibrous tissue. The presence of lesions in specific organs or the affected system indicates the route of entry of *Mycobacterium bovis* (4). Histologic lesions consist of multifocal nodules with central areas of caseous necrosis and calcification, surrounded by macrophages, epithelioid cells, giant cells, few lymphocytes, and plasma cells with an outer connective tissue capsule. Sometimes, acid-fast bacteria may be seen within the cytoplasm of macrophages and giant cells (5). It has been estimated that in Mexico about 86% of the country was in a bTB eradication phase, with prevalence lower than 0.5%; the rest of the country is in a control phase, having a prevalence of 2.5% (http://www.gob.mx/senasica/documentos/situacion-actual-de-tuberculosis-bovina?state=published). Knowledge of the pathology and diagnostic techniques of tuberculosis is an important tool for the recognition and identification of bTB. The aim of this case report is to describe the morphological lesions of congenital tuberculosis in a young female calf, the diagnosis of which was confirmed with complementary tests.

2. Case history

A Holstein female calf from an intensive dairy farm (about 1800 lactating cows) in Aguascalientes, Mexico, with a history of cough, fever, dyspnea, anorexia, and loss of weight died 25 days after birth. Postmortem examination revealed multiple variably sized (0.5–3.0 cm) white to yellowish nodules, occupying more than 80% of the lung parenchyma (Figure 1). The right cranial and medial lung lobes were the most severely affected. Samples of lung tissue were fixed in 10% neutral buffered formalin and sent to the laboratory for the diagnosis of bTB of the

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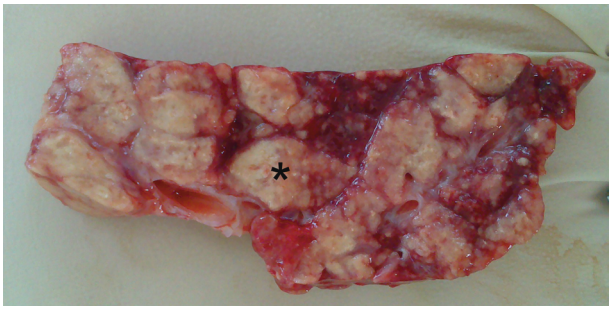

Figure 1. Severe granulomatous diffuse pneumonia. The lungs showed multiple variably sized (0.5–3 cm) white to yellowish nodules (asterisk), occupying more than 80% of parenchyma.

Mexican government (CENID Microbiología, INIFAP); mediastinal lymph nodes were not sent. This laboratory is certified for the diagnosis of bTB by the Mexican Entity of Accreditation. There, fixed samples were paraffin-embedded and cut into 5-µm-thick sections; the slides were stained with hematoxylin and eosin (H&E) and Ziehl–Neelsen (ZN) stain. Additionally, tissue samples were taken for polymerase chain reaction (PCR) and culture isolation (CI). For molecular characterization, DNA was extracted directly from one fresh tissue sample using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. DNA amount and quality were estimated by electrophoresis in ethidium bromide stained gels; DNA was employed for PCR using tuberculosis complex-specific primers that amplify a section of the RD1 region and can distinguish between bacillus Calmette-Guérin (BCG) and field strains; the expected product length is 150 bp for wild-type *Mycobacterium tuberculosis* complex bacteria and 200 bp for BCG (6). Every PCR reaction consisted of 1X buffer, 0.2 mM dNTPs, 48 mM MgCl, 25 nM primers, 2 UI of Taq polymerase (BioTecMol, Mexico), 50 ng of DNA, and molecular biology grade water. Amplification conditions included an initial denaturation cycle of 95 °C/5 min and 35 cycles of 94 °C/30 s, 70 °C/30 s, and 72 °C/30 s, followed by a final extension cycle of 72 °C/5 min. Amplicons were detected by electrophoresis in 2% agarose gels stained with ethidium bromide and using a 50-bp molecular weight marker (Invitrogen, USA), visualized and photographed through UV transillumination with a Molecular Imager Gel Doc XR (Kodak, USA). For microbiological isolation the tissue sample was blended, and half was decontaminated using the modified Petroff method of acid and alkali; the remaining pellet was resuspended and seeded in two Stonebrink tubes and one Löwenstein–Jensen tube. The other half of the blended sample was used for direct smear and the remaining was stored at –20 °C for future reference. Culture slants were kept horizontally under aerobic incubation at 37 °C and checked daily for development of rapidly growing colonies. Caps were tightened after 2 days and tubes remained vertically in the oven for 9 weeks. Mycobacterial identification relied on biochemistry probes (7).

3. Results and discussion

Histologic examination of the lung showed severe granulomatous diffuse pneumonia (Figure 2). Lung lesions were characterized by areas of caseous necrosis surrounded by macrophages, giant cells (Figure 3), lymphocytes, and an outer fibrous capsule; large numbers of acid-fast bacilli were revealed inside the giant cells by ZN staining (Figure 4). Tuberculosis by *Mycobacterium bovis* was confirmed by PCR (Figure 5) and CI. It is noticeable that in this case a

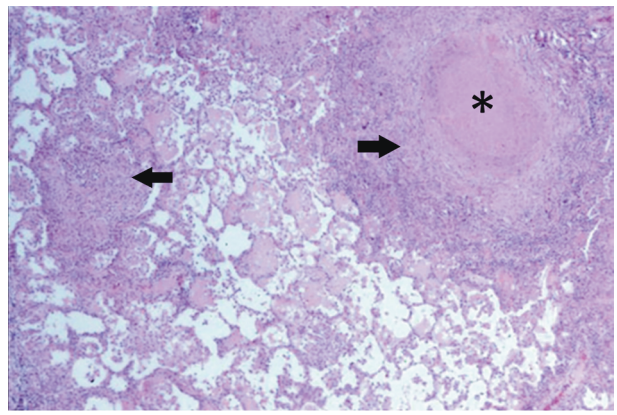

Figure 2. Severe granulomatous diffuse pneumonia. Pulmonary granulomas (arrows) consist of mixtures of cells including macrophages, giant cells, lymphocytes, and fibroblasts surrounding a central area of necrosis (asterisk). H&E, 40×.

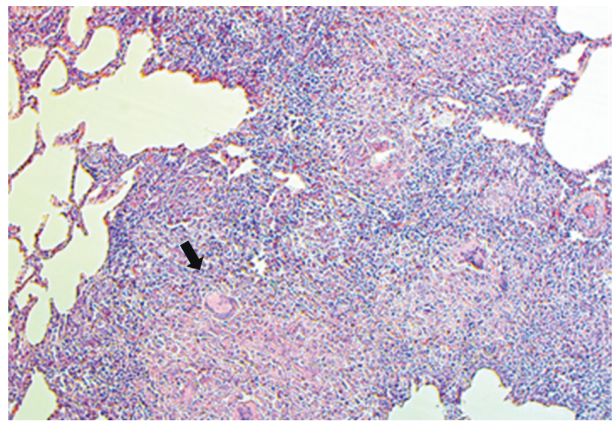

Figure 3. Severe granulomatous diffuse pneumonia. Pulmonary granulomas. Langhans giant cell (arrow) associated with pulmonary tuberculosis. H&E, 100×.

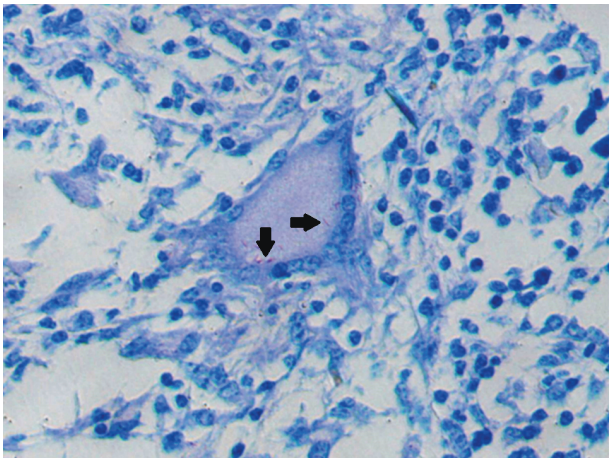

Figure 4. Severe granulomatous diffuse pneumonia. Acid-fast bacilli within giant cells (arrows). ZN, 400×.

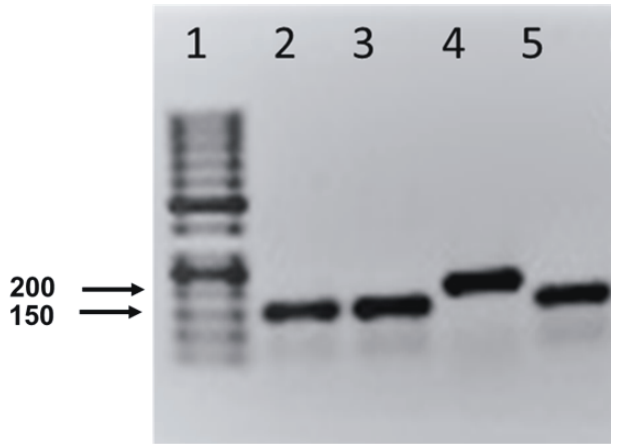

Figure 5. Multiplex PCR RD1. Lane 1: Molecular size marker (bp); Lane 2: Calf sample; Lane 3: Calf sample; Lane 4: Positive control, BCG (ATCC 35734); Lane 5: Positive control, *M. bovis*.

female calf of only 25 days of age was affected. Calves born with congenital infections usually develop generalized tuberculosis in the first weeks or months of life (8,9).

Recognition of the macroscopic and microscopic lesions of tuberculosis in cattle aids the understanding of the disease process. The route of transmission of *Mycobacterium bovis* can be inferred based on the distribution of lesions. In this case, pathological alterations were located within the respiratory tract and no other lesions were observed. Indeed, bTB lesions were observed only in the lungs; thus, the calf probably aspirated contaminated amniotic fluid in utero close to parturition as reported by others (8), which may produce the primary lung lesions. In consequence, the disease developed during the first weeks after birth. It has been reported that bTB lesions may be either localized or generalized depending on the severity or the stage of the disease (9); thus, the calf of our report probably died before bTB would spread to other organs. It is worth mentioning that other implicated organs showed no macroscopic findings, as could have been expected if congenital transmission was hematogenous (5,9).

This case provides evidence for congenital tuberculosis in a 25-day-old female calf. The calf probably acquired the infection from her mother during gestation or at birth. Unfortunately, we have no information on the sanitary status of the mother. Thus, in this case uterine tuberculosis of the mother cannot be ruled out. The prevalence of bTB in the area where this farm is located was about 4% (https://www.gob.mx/senasica/documentos/indicadores-de-la-campana-nacional-contra-la-tuberculosis-bovina?idiom=es), so congenital tuberculosis should not be disregarded. As far as we know, this is the first report of congenital tuberculosis in a calf in Mexico. Congenital tuberculosis in calves is a rare occurrence, but this case shows how more severe disseminated forms of the disease can occur if prevention and control measures are not properly implemented.

Acknowledgments

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