

Comunicación

Unusual pathologic lesions in a heifer affected by *Trueperella pyogenes*

Lesiones patológicas inusuales en una vaquillona afectada por *Trueperella pyogenes*

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ABSTRACT

Four Hereford heifers of approximately 18 months of age presented nodular formations in the uterus detected by transrectal palpation. This pathology had been previously diagnosed in the herd, which led to the early discarding of 1-2% of heifers per year. *Post mortem* examination in a severely affected heifer revealed multiple abscesses in the pelvic and abdominal cavity associated with the uterus, peritoneum, pre stomachs and liver. Microscopically, the abscesses were characterized by a necrotic center with viable and degenerated neutrophils and necrotic debris, surrounded by a well-defined wall of connective tissue. In addition, multifocal neutrophilic ruminitis associated with acidosis was observed. Intralesional gram-positive bacilli were detected in the abscesses. *Trueperella pyogenes* was isolated from the purulent content. It is assumed that rumen acidosis was the origin of the dissemination and abscess formation of *T. pyogenes* in various tissues, including the uterus.

Key words: cattle, infectious diseases, reproductive pathology, ruminants.

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RESUMEN

Cuatro vaquillonas Hereford de aproximadamente 18 meses de edad presentaron formaciones nodulares en el útero detectadas por palpación transrectal. Esta patología había sido previamente diagnosticada en el rodeo, lo que llevó al descarte temprano de 1-2% de vaquillonas por año. El examen *post mortem* en una vaquillona gravemente afectada reveló múltiples abscesos en la cavidad pélvica y abdominal asociados al útero, peritoneo, preestómagos e hígado. Microscópicamente, los abscesos se caracterizaron por un centro necrótico con neutrófilos viables y degenerados y restos necróticos, rodeados por una pared bien definida de tejido conectivo. Además, se observó ruminitis neutrofilica multifocal asociada a acidosis. Se detectaron bacilos grampositivos intralesionales en los abscesos. Del contenido purulento se aisló *Trueperella pyogenes*. Se asume que la acidosis ruminal fue el origen de la diseminación y formación de abscesos de *T. pyogenes* en varios tejidos, incluido el útero.

Palabras clave: bovinos, enfermedades infecciosas, patología reproductiva, rumiantes

INTRODUCTION

Trueperella pyogenes is a Gram-positive, non-motile, facultative anaerobe, rod-shaped bacterium common habitant of mucus membranes in animals (Jost & Billington, 2005; Santos *et al.*, 2010; Rzewuska *et al.*, 2019). *T. pyogenes* infections occur in wild and domestic animals, and is characterized by the infection of any tissue, mainly associated with abscess formation, causing a reduction in productive performance (Ribeiro *et al.*, 2015; Rzewuska *et al.*, 2019).

In cattle, the liver is the main organ where *T. pyogenes*-abscesses can be located, generally as a sequela of ruminal acidosis (Amachawadi & Nagaraja, 2016), also reported in kidneys, lungs, subcutaneous tissue and heart (Kleen *et al.*, 2003; Ribeiro *et al.*, 2015; Abdela, 2016; Rzewuska *et al.*, 2019). This bacterium is an important cause of mastitis and metritis (Azawi, 2008; Sheldon & Owens 2017; Zhang *et al.*, 2017), and can lead to sporadic abortions, usually in the middle and final thirds of pregnancy in cattle (Arainga *et al.*, 2003; Anderson, 2007; Costa *et al.*, 2019; Morrell *et al.*, 2019). Uterine abscesses are rare in animals, though more observed in cattle related to local trauma by

improper use of instruments during uterine manipulation (Schlafler & Foster, 2016). Herein we describe an outbreak of multiple visceral abscesses caused by *T. pyogenes* in a heifer with atypical presentation in the uterus associated to ruminal acidosis.

CASE DESCRIPTION

The cluster of cases occurred in a beef cattle breeding farm located in Buenos Aires province, Argentina. Cases were detected in a paddock of 250, ~18 months old Hereford heifers. Four (1.6%) heifers were detected with multiple firm nodular formations in the reproductive tract during trans rectal palpation. Heifers had been grazing pastures until weaning (~8 months old) and then penned with high-grain diet composed of corn silage (20-30%), grain corn (70-80%) and pellet as protein source for another 10 months. Heifers reaching 18 months of age were transrectally palpated for assessing their reproductive development and then sent to an open paddock. During this procedure, uterine nodular formations had been occasionally detected in previous years in about 3 to 5 heifers per year (~1-2%). Later one, the affected heifers were showing a progressive

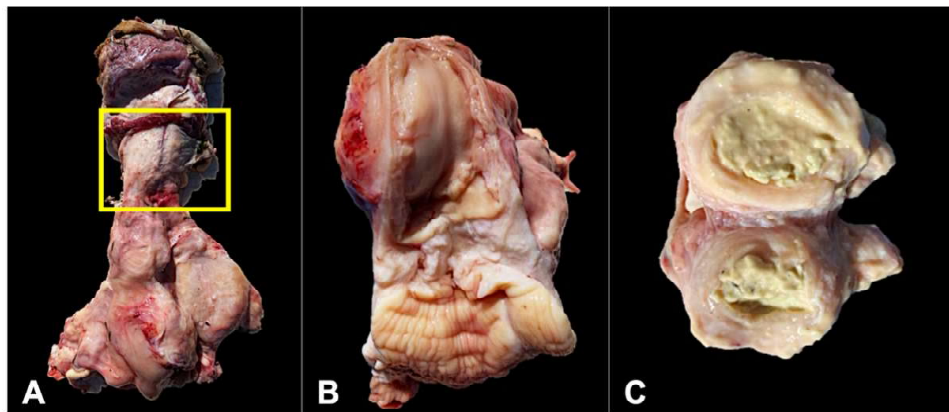


Figure 1. Uterus of an 18-month-old Hereford heifer with multiple visceral abscesses caused by *Trueperella pyogenes*. A) Enlargement of uterine body (yellow frame). B) Rounded intramural formation of 10 cm in diameter at ventral wall of uterine body. C) Cut surface of the intramural abscess, characterized by purulent content surrounding by a thick fibrotic wall

loss of corporal condition, and therefore culled since they were not suitable for reproduction.

One severely affected heifer was euthanized according to the guidelines of the Committee for Care and Use of Experimental Animals of the Instituto Nacional de Tecnología Agropecuaria (INTA) and *post mortem* examination was carried out. Grossly, an intramural nodular abscess of 10 cm length was present in the ventral wall of the uterine body forming a rounded external bulge (Figures 1A,B), that at cut surface it had a thick fibrotic capsule and contained white viscous material defined as pus (Figure 1C). Similar abscesses were also found attached to the omasum, ruminal serosa, peritoneum and mesoduodenum, varying from 2 to 4 cm of diameter. In the diaphragmatic surface of the liver two 1 cm abscesses with irregular edges surrounding by a thin capsule were observed. The mesenteric (duodenum and jejunum) and hepatic lymph nodes were mostly enlarged and edematous.

Tissue samples including the nodular formations, heart, lung, liver, small and large intestine, lymph nodes, spleen, kidney, rumen,

abomasum, central nervous system and uterus were fixed by immersion in 10% neutral buffered formalin and processed by standard techniques to obtain 4-5- μ m sections stained with hematoxylin and eosin (HE) for histologic examination. Microscopically, abscesses from uterus and serosa of omasum and rumen were characterized by a central area of necrosis composed of viable and degenerate neutrophils and macrophages admixed with cellular debris, and surrounded by organizing granulation tissue with macrophages, dense collagen and neovascularization (Figure 2A,B).

The liver showed two foci formed by necrotic center with degenerated inflammatory cells (mainly neutrophils) and necrotic debris, surrounded by fibroblasts and macrophages. Additionally, in the mesentery, diffuse inflammatory infiltrate with macrophages, lymphocytes and neutrophils was present. The rumen showed, multifocal marked cytoplasmic vacuolation of epithelial cells of spinous layer, mixed with moderate number of neutrophils forming micro abscesses (Figure 2C), and additionally the mucosa and submucosa had a diffuse mode-

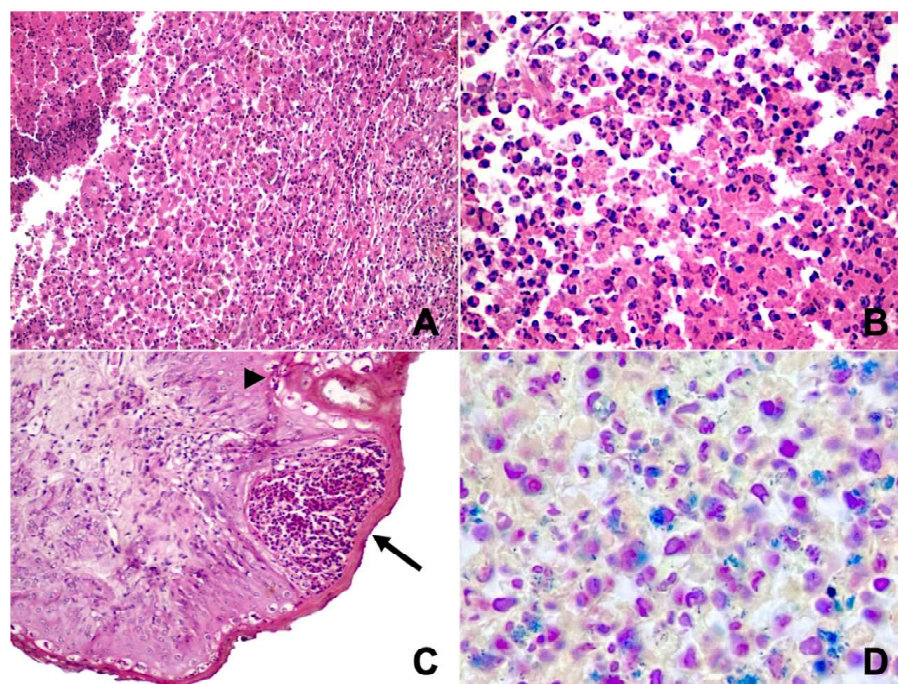


Figure 2. Microscopic lesions in a 18-month-old Hereford heifer with multiple abscesses caused by *Trueperella pyogenes*. A) Abscess from uterus characterized by a central area of necrosis composed of viable and degenerate neutrophils and macrophages admixed with cellular debris (left), surrounded by organizing granulation tissue with macrophages, dense collagen and neovascularization (right) H&E. B) Higher magnification for detail of abundant neutrophils admixed with cellular debris. H&E. C) Ruminal mucosa with micro abscess composed of neutrophils in stratum spinosum (dark arrow) and multifocal marked cytoplasmic vacuolation of epithelial cells of spinous layer (arrowhead). H&E. D) Abundant clusters of Gram-positive short rods from uterine abscess

rate infiltrate of neutrophils. Hepatic and mesenteric lymph nodes had depletion of the germinative centers and showed subcapsular moderate infiltrate composed by macrophages and lymphocytes. Other organs did not present relevant lesions. Direct Gram stain from several tissues (uterine, serosal and liver abscesses), showed clusters of Gram-positive short rods (Figure 2D).

From bacteriological culture, sterile samples of lymph nodes and purulent content of abscesses from uterus and ruminal serosa were collected and inoculated onto Columbia blood agar. After 24 h at 37 °C under aerobic conditions, very small beta hemolytic colonies

developed and gram-positive bacilli and coccobacilli were observed by Gram staining. Colonies were identified as *T. pyogenes* by phenotypic and biochemical characteristics (Rogovskyy *et al.*, 2018).

DISCUSSION

In this report, clinical, pathological and bacteriological findings confirm the occurrence of multiple abscesses by *T. pyogenes*. Abscesses were present in 1.6% of the animals. This occurrence is lower compared with a previous report in which 2 to 20% of cattle from a feedlot were affected

with liver abscesses (Tadepalli *et al.*, 2009). *T. pyogenes* is a commensal bacterium of skin and mucous membranes. During episodes of ruminal acidosis, the bacteria could gain access via bloodstream and spread to the liver, causing abscesses (Nagaraja & Chengappa, 1998; Sheldon & Owens, 2017; Tadepalli *et al.*, 2009; Rzewuska *et al.*, 2019).

In this case, the high concentrate diet after calving, could led to acidosis and rumenitis facilitating the entry of *T. pyogenes* and the dissemination of the bacteria via bloodstream to several tissues causing secondary abscesses (Nagaraja & Chengappa, 1998; Plaizier *et al.*, 2009; Amachawadi & Nagaraja, 2016). Disseminated abscessation by *T. pyogenes* has also been diagnosed in goats and sheep affecting various tissues, suggesting hematogenous spread after immunosuppression related to stress factors (Dughaym, 2004; Lin *et al.*, 2010; Ribeiro *et al.*, 2015).

Hematogenous spread from the gut is a common route of transmission of several infectious microorganisms which can affect, among others, the uterus provoking metritis in cows (Jeon *et al.*, 2017). *T. pyogenes* is considered an important uterine pathogen in postpartum infections of cows causing metritis and endometritis (Sheldon & Owens, 2017; Zhang *et al.*, 2017; Rzewuska *et al.*, 2019) which can lead to late term abortions (Arainga *et al.*, 2003; Anderson, 2007; Costa *et al.*, 2019; Morrell *et al.*, 2019). Reproductive infection by *T. pyogenes* is associated to superficial inflammation of the endometrium and occasionally extending to stromal layer (Bicalho *et al.*, 2012; Ribeiro *et al.*, 2015; Zhang *et al.*, 2017). In this context, *T. pyogenes* can act either as primary agent or synergistically with other bacterial pathogens (*Bacteroides*, *Porphyromonas* and *Fusobacterium*); however, the formation of uterine abscesses is uncommon (Schlafer & Foster, 2016; Sheldon & Owens, 2017; Rzewuska *et al.*, 2019).

In cattle, uterine abscesses are associated to improper uterine manipulation involving instruments such as insemination pipettes and uterine catheters, located in the dorsal wall of the uterine body (Schlafer & Foster, 2016; Sachuk *et al.*, 2020). In this report, the abscess was in the ventral wall of the uterine body and no uterine manipulation was carried out before, discarding a local trauma as well as postpartum contamination given their non-pregnant status.

CONCLUSIONS

- This report describes the presence of nodular formations in the uterus of four heifers, which were detected during transrectal palpation. This could be the result of a possible bacterial dissemination from rumen due to acidosis after high grain diet consumption.
- In one of the affected animals, these lesions were characterized as abscesses being *T. pyogenes* isolated. Though, only one heifer of the outbreak was studied, the similarity of the uterine lesions in the remaining heifers suggests that *T. pyogenes* may be involved as well as other opportunistic agents.

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