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PATHOLOGY

Abortion due to *Yersinia enterocolitica* infection in an ewe in Southern Brazil

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ABSTRACT: We described the pathological findings of an abortion associated with *Yersinia enterocolitica* infection in an ewe. A late-gestation aborted ovine fetus and placenta were submitted for necropsy. Pathologic changes were restricted to the chorioallantois. Grossly, cotyledons and intercodilenary regions had areas of mild red discoloration, and cotyledons were covered with a small amount of fibrin. Histological lesions consisted of multifocal, severe necrotizing and fibrinosuppurative placentitis with fibrinoid vasculitis and mineralization. Bacterial culture of lung, abomasum, and placenta samples yielded pure growth of *Y. enterocolitica*. This report described the first case of infection by *Yersinia enterocolitica* causing ovine abortion in Brazil.
Key words: fetal loss, fetuses, ovine, placentitis, yersiniosis.

Aborto causado por infecção por *Yersinia enterocolitica* em uma ovelha no sul do Brasil

RESUMO: Descrevemos os achados patológicos de um aborto causado por infecção por *Yersinia enterocolitica* em uma ovelha. Um feto ovino abortado, em estágio tardio de gestação, e a placenta correspondente foram submetidos à avaliação patológica. As alterações patológicas estavam restritas ao corioalantoide. Macroscopicamente, os cotilédones e as regiões intercotiledonárias estavam avermelhadas e os cotilédones estavam cobertos por pequena quantidade de fibrina. As lesões histológicas consistiam em placentite fibrinosupurativa necrosante multifocal severa, com vasculite fibrinoide e mineralização. A cultura bacteriana de amostras de pulmão, abomaso e placenta revelou crescimento puro de *Y. enterocolitica*. Este relato descreve o primeiro caso de infecção por *Y. enterocolitica* causando aborto em ovino no Brasil.
Palavras-chave: perda fetal, feto, ovino, placentite, yersiniose.

The genus *Yersinia* spp. encompasses gram-negative, coccobacilli bacteria of the family Enterobacteriaceae that cause yersiniosis, a sporadic disease of humans and animals (QUINN, 2011; UZAL et al., 2016). This genus comprises more than ten species, of which *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* are associated with gastrointestinal, reproductive, and/or septicemic disorders in several species (BOTTONE, 1999; PLATT-SAMORAJ et al., 2010). In humans, yersiniosis is considered an important food-borne zoonosis widely distributed and associated with gastrointestinal diseases (BOTTONE, 1999).

Abortions have a high impact on reproductive efficiency in farms, resulting in significant economic losses (MENZIES, 2011). Although, *Y. pseudotuberculosis* is the most pathogenic species for large animals, *Y. enterocolitica* can also cause disease and has been isolated in sporadic cases of abortion in several species, including cattle, pigs, and horses (BREWER & CORBEL, 1983; COSTA et al., 2021; SLEE & BUTTON, 1990). In ewes, reports of spontaneous infections by *Y. enterocolitica* are infrequent and pathological descriptions are limited (BREWER & CORBEL, 1983; CORBEL et al., 1992; VAN DER BROM et al., 2021). We now described the pathological and microbiological findings of a case of ovine abortion associated with *Y. enterocolitica* infection.

A late-term aborted ovine fetus with corresponding fetal membranes (chorioallantois) were submitted for pathological examination to the Setor de Patologia Veterinária at the Universidade Federal do Rio Grande do Sul. The aborted fetus was originally from a farm located in the city of Encruzilhada do Sul (30°31′37″S, 52°31′6″W), state of Rio Grande do Sul, Southern Brazil. The flock was composed of approximately 320 ewes, Texel breed, raised in grasslands. The fetus had mild autolysis and a

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crown-to-rump length of 41cm, consistent with 19-21 weeks of gestation (NJAA, 2012). The gross changes were restricted to the chorioallantois. The cotyledons were moderately irregular and covered with yellow, friable material. The intercotyledorary regions had multifocal areas of dark-red discoloration. Samples of several organs were collected, fixed in neutral-buffered 10% formalin for 48h, routinely processed for histopathology, sectioned (3 µm), and stained with hematoxylin and eosin (HE).

Histopathology of the placenta revealed multifocal areas of necrosis with large amounts of karyorrhectic debris, large numbers of degenerate neutrophils, abundant fibrin exudation, and small amounts of granular basophilic material (mineralization). These necrotic areas affected the chorioallantois, partially replaced and effaced the chorionic epithelium, and extended to the deep stroma (Figure 1). In addition, mainly in the deep stroma, the wall of blood vessels was circumferentially and markedly expanded and partially effaced by a large amount of fibrin, karyorrhectic debris, intact and degenerate neutrophils, and fewer macrophages, lymphocytes, and plasma cells (fibrinoid necrosis). Frequently, affected vessels had fibrin thrombi (thrombosis) (Figure 2). In the lung, the alveolar septa were mildly thickened by a small number of lymphocytes and plasma cells. No histologic lesions were detected in the remaining organs.

A diagnosis of necrotizing fibrinosuppurative placentitis, presumably caused by an infectious agent, was made based on the pathological findings. Fresh samples of the lung, placenta, and abomasal fluid collected during the postmortem examination were submitted for routine microbiological evaluation. The samples were inoculated onto 5% Sheep Blood Agar (Mueller Hinton, Kasvi®) and MacConkey agar (Kasvi®) and incubated at 37 °C. After 24h, pure small non-hemolytic grayish colonies were visualized on Sheep Blood Agar and pure small lactose-negative colonies were visualized on MacConkey agar. The same bacteria growth was identified on all inoculated samples (lung, placenta, and abomasal fluid). The isolates of all samples were submitted to the API 20E (BioMerieux SA, Marcy-l'Etoile, France) strip and also inoculated on Brilliant Green Agar and XLD Agar. The isolates were classified as *Y. enterocolitica* with high percentage of biochemical match by the API identification test. Finally, the colonies grew with green pigment (acid) and yellow pigment (acid) on brilliant green agar and XLD agar, respectively, confirming the species identification. Therefore, a final diagnosis of abortion associated with *Y. enterocolitica* was made.

Y. enterocolitica has been associated with enteritis and septicemia in wildlife and domestic animals, gastroenterocolitis in humans, and sporadic abortion in ewes (QUINN, 2011).

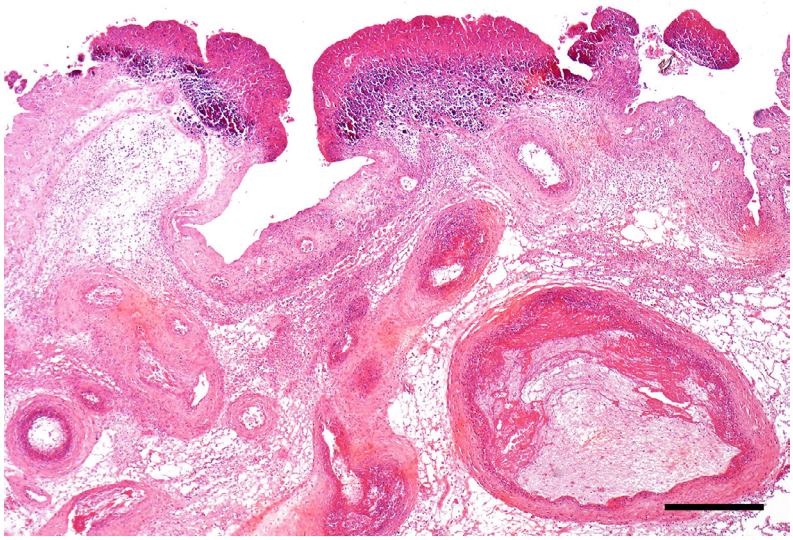

Figure 1 - Placentitis by *Yersinia enterocolitica*, ewe. Placenta. There is a multifocal necrosis of the chorionic epithelium. Diffuse edema, fibrinoid vasculitis, and trombosis are observed in the stroma. H&E stain; bar = 500 µm.

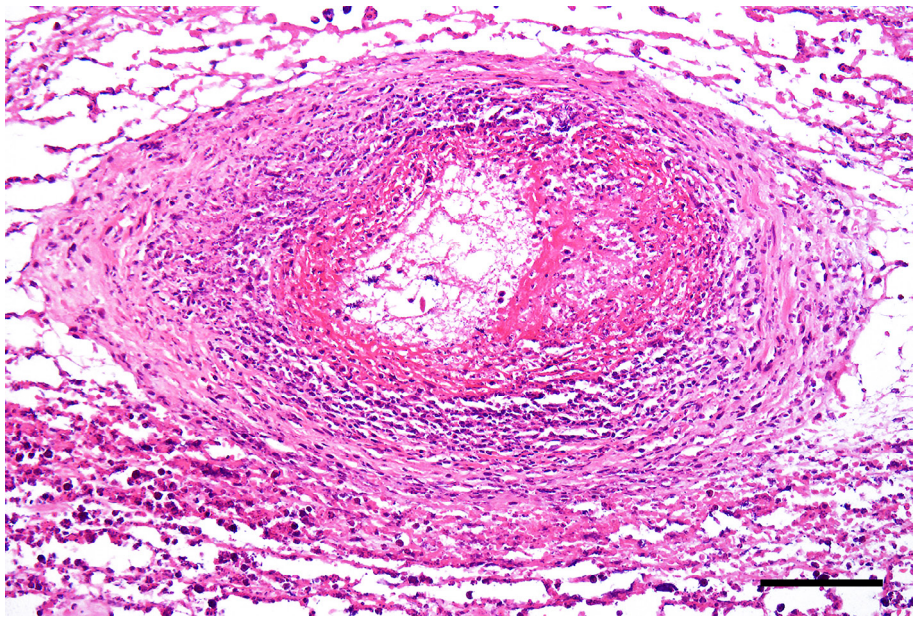

Figure 2 - Placentitis by *Yersinia enterocolitica*, ewe. Placenta. The wall of a blood vessel is expanded by fibrin, karyorrhectic debris, and inflammatory cells. H&E; bar = 200 µm.

Although, the epidemiology of yersiniosis is poorly understood, predisposing factors in farm animals include environmental factors and stressors (UZAL et al., 2016), such as dietary changes, prolonged travel, extreme weather conditions, and handling practices (SARGISON, 2008). These factors were not reported by the farm owner in this case. The infection by *Y. enterocolitica* occurs through the fecal-oral route. After ingestion, the bacteria invade the intestinal epithelium, ultimately leading to transient bacteremia and hematogenous spread to other organs, including the placenta (UZAL et al., 2016). Ascending infections leading to placentitis and abortion due to *Y. enterocolitica* infection are not reported in the literature (BREWER & CORBEL, 1983; CORBEL et al., 1992; VAN DER BROM et al., 2021). Similar to our case, experimental studies in ewes (CORBEL et al., 1992) have indicated that most abortions occur in the last trimester of pregnancy (CORBEL et al., 1992).

Gross lesions by *Y. pseudotuberculosis* infection are normally restricted to the cotyledons; which can be thick and red or tan, surrounded by fibrin and fibrosis (SCHLAFER & FOSTER, 2016). Experimental infection with *Y. enterocolitica* in ewes has been reported to cause gross lesions in the placenta, characterized by dark-red cotyledons and thickened and friable intercotyledonary regions (CORBEL et al., 1992). Hepatic, cardiac, and umbilical gross lesions have been reported in a case of sepsis in an aborted equine fetus (COSTA et al., 2021). In our case, similarly to previous reports, gross lesions were restricted to the placenta, highlighting the importance of the pathological evaluation of the fetal membranes.

In our case, the main histological findings in the placenta were neutrophilic inflammation, necrosis, vasculitis with fibrinoid necrosis, and thrombosis. Similar findings with bacterial colonies have been reported only in experimental cases of ovine abortion associated with *Y. enterocolitica* (CORBEL et al., 1992). In our report, bacterial colonies were not readily observed in tissue sections. In an experimental study of placentitis by *Y.enterocolitica* in ewes, two of five placentas with necrotic and suppurative placentitis had small gram-negative bacilli morphologically resembling *Yersinia* spp (CORBEL et al., 1992). Whereas in sows, necrosis and edema of the epithelium and placental vessels have been reported as the main findings (PLATT-SAMORAJ et al., 2010).

Y. entecolitica is a heterogeneous group with various serotypes classified by biotyping into 6 groups based on phenotypic characteristics. Five serotypes are considered pathogens associated with

different clinical manifestations (MCNALLY et al., 2004). Based on surface antigen (lipopolysaccharide or LPS), they are classified into 57 O serogroups (QUINN, 2011). Biotype 3 (O:5,27) has been reported as the predominant pathogenic biotype carried by ewes and pigs and was the only biotype recovered from cattle in one study (MCNALLY et al., 2004). Serotyping is established by laborious and expensive procedures and PCR-based identification techniques have been developed (QUINN, 2011). Unfortunately, the *Y. entecolitica* biotype was not determined in our case.

Differential diagnoses for *Y. enterocolitica*-associated abortion include other causes of placentitis and abortion in small ruminants, including *Coxiella burnetti and Toxoplasma gondii* (ROBERT & MOELLER, 2012). The lesions caused by *C. burnetti* and *T. gondii* may be easily differentiated using histological and microbiological examinations. In *C. burnetti* infection, the corioallantois is thickened and expanded by neutrophilic and lymphocytic inflammation, and bacteria are easily seen in the cytoplasm of trophoblasts (ROBERT & MOELLER, 2012). In toxoplasmosis, the main gross changes consist of areas of necrosis in the cotyledons with variable numbers of lymphocytes, plasma cells, macrophages and intralesional tachyzoites (ROBERT & MOELLER, 2012). Another differential diagnosis is *Y. pseudotuberculosis* infection, which cannot be microscopically differentiated from *Y. enterocolitica* (UZAL et al., 2016); therefore, bacterial isolation is indispensable for the final diagnosis in these cases.

In conclusion, *Y. enterocolitica* infection may be occasionally associated with abortion in ewes and should be considered as a differential diagnosis, especially in cases of necrotizing fibrinosuppurative placentitis. To the authors' knowledge, *Y. enterocolitica* causing ovine abortion has not been previously reported in Brazil.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled "Abortion associated with *Yersinia enterocolitica* infection in an ewe in Southern Brazil" declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation by the Ethics Committee of the Universidade Federal do Rio Grande do Sul. However, we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assumed full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

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