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Case Report

Suppurative meningoencephalitis secondary to a *Trueperella pyogenes*-induced mandibular abscess in a free-ranging gray brocket deer (*Mazama gouazoubira*)

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Abstract

Trueperella pyogenes is an opportunistic pathogen, most commonly affecting ruminants and pigs. A female free-ranging gray brocket deer (*Mazama gouazoubira*) had an abscess in the masseter muscle associated with an osteolysis and osteomyelitis in the adjacent right mandibule, with a fistulous tract to the orbit and through the optic foramen into the skull, causing a suppurative meningitis. Histologically, the abscess was characterized by a neutrophilic and histiocytic inflammatory infiltrate with myriad of Gram-positive rod-shaped bacteria, and a focally extensive suppurative and histiocytic leptomeningitis of the ventral portions of the brain. Aerobic microbiologic culture, MALDI-ToF, and PCR identified *T. pyogenes*.

Key words: Cervidae, osteomyelitis, meningitis, *Trueperella pyogenes*, Cervidae.

Introduction

Trueperella (*Arcanobacterium*) *pyogenes* is a Gram-positive, non-motile, non-spore-forming, short, rod-shaped actinomycete bacteria (24). It is commonly isolated from mucous membranes, oropharynx, upper respiratory, urogenital, and gastrointestinal tracts of ruminants. *T. pyogenes* is an important opportunistic pathogen, since it potentially infects any animal tissue, usually resulting in a suppurative mastitis, pneumonia, and endometritis. *T. pyogenes* infections may also result in septicemia, pyometra, orchitis, pericarditis and encephalitis, particularly in cattle and pigs (13, 24, 29). Unlike ruminants, *T. pyogenes* infection is infrequently described in dogs and cats, probably because it is not considered to be a normal inhabitant of the mucous membranes of small animals (11).

The gray brocket deer (*Mazama gouazoubira*) is a small to medium size neotropical deer. It is largely distributed in Brazil, Uruguay, Argentina, Paraguay, and Bolivia. *M. gouazoubira* is the most common Cervidae species in these areas, occupying a large variety of biomes (4, 7). This species has a solitary behavior. In Brazil, its habitats vary from rainforests to savannas, and it can occupy areas used for agriculture (10). Therefore, despite a growing anthropogenic activity with continuous expansion of agriculture frontiers, the gray brocket deer has adapted to modified environments due to its high ecological plasticity (10).

Trueperella pyogenes is an emerging pathogen of captive or free-ranging white-tailed deer (*Odocoileus virginianus*) (3, 7, 14, 31, 32). This bacterium has been isolated as the etiological agent associated with chronic purulent infections in other species of deer, including the red deer (*Cervus elaphus*), key deer (*Odocoileus virginianus clavium*), fallow deer (*Dama dama*), forest musk deer (*Moschus berezovskii*), roebuck (*Capreolus capreolus*), and mule deer (*Odocoileus hemionus*) (1, 15, 18, 21, 33, 34, 35). However, despite being an important opportunistic pathogen for various species of ruminants, including deer, to the best of our knowledge *T. pyogenes* infection had not been reported in neotropical deer. We describe an abscess in the masseter in a free-ranging *M. gouzimbira* that fistulated to adjacent tissues, reaching the brain with isolation of *T. pyogenes*.

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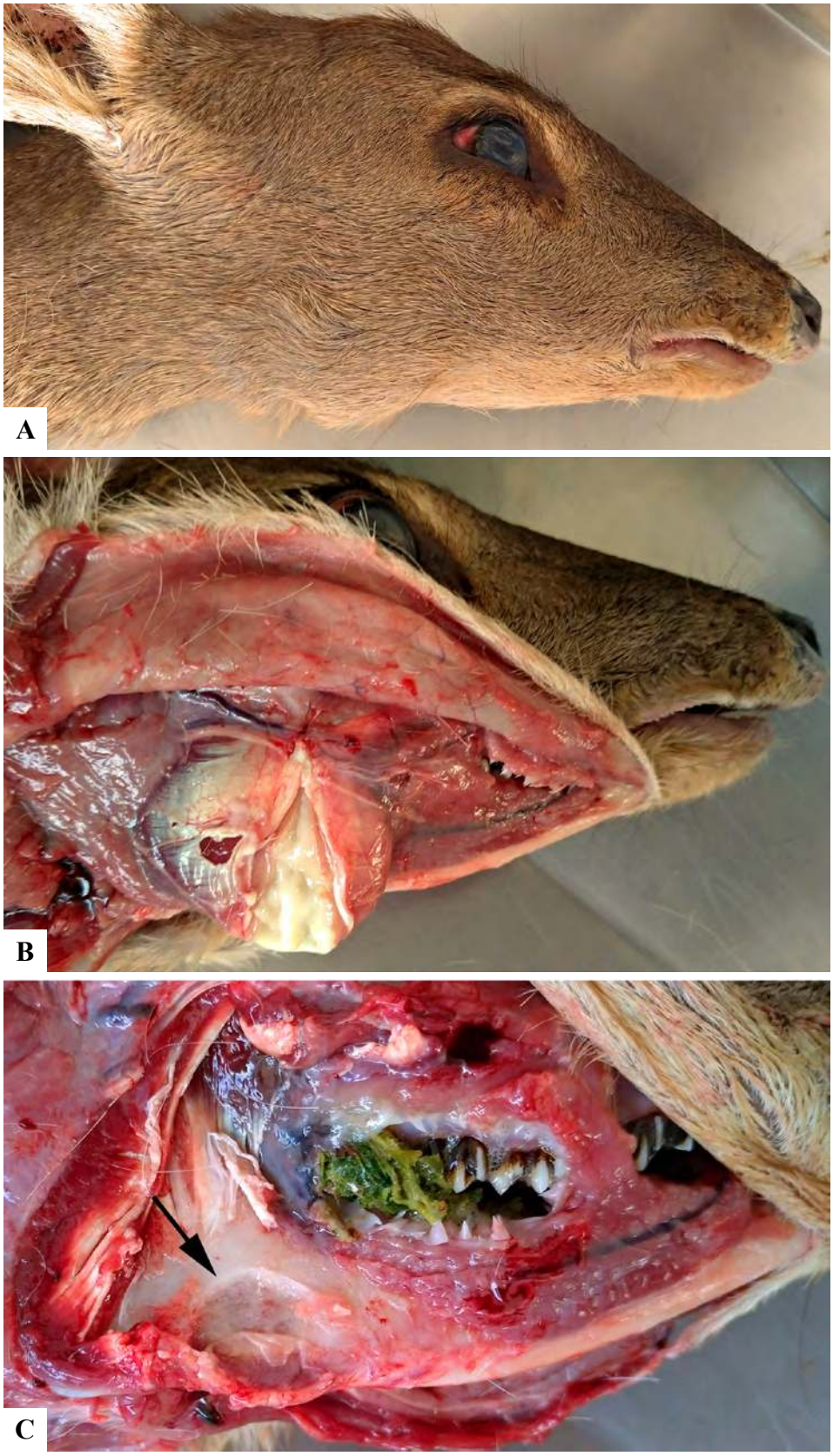

Figure 1. Abscess in the masseter muscle associated with osteolysis in a gray brocket deer. **A.** right lateral view of the head of adult gray brocket deer with a focally extensive swollen area with 5.0 x 5.0 x 3.0 cm in the mandibular region. **B.** Abscess in masseter muscle draining purulent exudate. **C.** A focal area of depressed bone tissue in the adjacent mandibular bone (arrow) compatible with osteolysis.

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Case report

An adult female free-ranging gray brocket deer living at the Mangabeiras Park in Belo Horizonte (Brazil) was rescued presenting a good body condition, severe dehydration, and altered state of consciousness with marked apathy. Presumptive clinical differential diagnosis included traumatic brain injury, capture myopathic or a neurologic disease. The animal died a few hours later, and was sent to the Veterinary Hospital at the Belo Horizonte Zoological Garden for necropsy.

At necropsy, a 5.0 x 5.0 x 3.0 cm abscess with large amounts of purulent exudate was observed in the right masseter (Fig. 1B). The adjacent mandible had a focal area of depression and bone loss which was interpreted as focal osteolysis. No signs of trauma with absence of fractured bones or cutaneous laceration or abrasions.

The oral mucosa, adjacent to the abscess, had a small fistula. The abscess also had a fistulous tract to the right orbit (Fig. 2A), with purulent exudate in the retrobulbar space, , muscles and sclera of the right eye, extending through the optic foramen into the skull (Fig. 2B). The leptomeninges were diffusely congested, and in the ventral brain, from the region of the optic chiasm to the ventral surface of the brainstem, had an accumulation of purulent exudate, compatible with a focally extensive suppurative meningitis (Fig. 2C). In addition, there was moderate pulmonary edema and mild liver congestion. The deer was pregnant, with one fetus with a gestational age estimated as the final third of pregnancy. No gross changes were observed in the fetus or the placenta.

Samples of masseter muscle, mandibular bone, oral mucosa, brain, heart, kidney, lungs, and salivary gland were collected from the adult female. Fetal tissues, including the lung, heart, kidney, and brain were also sampled. Tissues were routinely processed for histologic evaluation.. Bone fragments were decalcified in 24% formic acid solution after fixation.

Microscopically, there was a focally extensive area of inflammatory infiltrate composed by a central core of intact or degenerate neutrophils surrounded by macrophages. There was a myriad of gram-positive rod-shaped intralesional bacteria (Fig. 3). The mandibular bone had a focal area with extensive osteolysis associated with a neutrophilic infiltrate. The skeletal muscle, connective tissue and adipose tissues adjacent to the right eye had a similar inflammatory process as observed in the masseter muscle associated with fibrinous thrombi. The leptomeninges in the ventral portion of the brain had an inflammatory infiltrate composed by neutrophils and macrophages, which was more intense in the region of ventral metencephalon and telencephalon of temporal lobe, characterizing a focally extensive meningitis (Fig. 4). The inflammatory process extended caudally to the brainstem, where there were lymphocytes, plasma cells, and some neutrophils multifocally distributed in neuropil, with some Gitter cells. There was also a lymphocytic and plasmacytic perivascular infiltrate. The lungs had alveolar septa with multifocal areas of neutrophilic infiltrate, often associated with intravascular thrombi. In the alveolar lumen there was marked edema and a focal area with a large number of foamy macrophages. Samples of liver, salivary gland, heart, placenta, uterus, ovary, kidney, urinary bladder, tongue, rumen and abomasum had no histologic changes. Fetal tissues including brain, spleen, kidney, lung, liver had a few areas of mild multifocal hemorrhage.

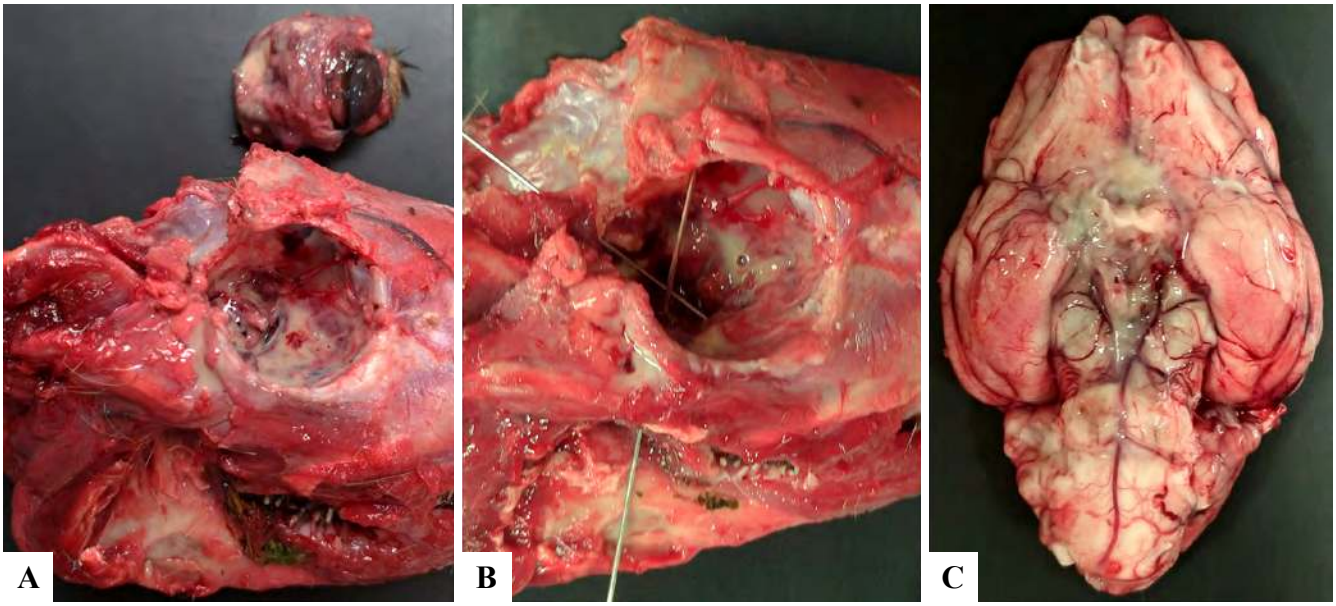

Figure 2. Abscess fistulation to the right orbit and through the optic foramen in a gray brocket deer. **A.** Purulent exudate in the right orbit, retrobulbar muscle, and sclera. **B.** Indication of the fistulous tract thought the orbit and optic foramen. **C.** The ventral surface of the brain, with a focally extensive accumulation of purulent exudate extending from the optic chiasm to the brain stem, characterizing a suppurative meningitis.

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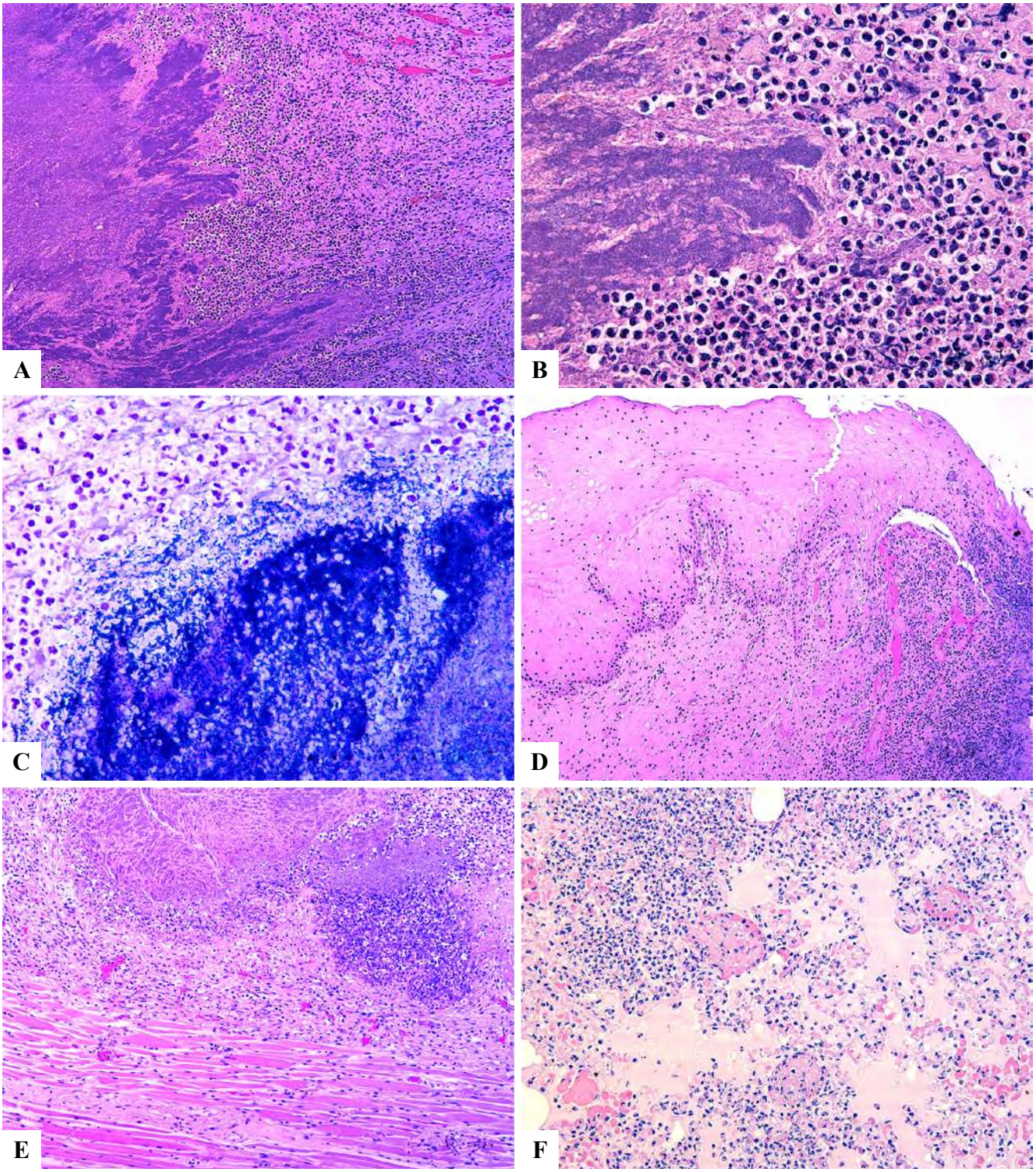

Figure 3. *Truerella pyogenes*-induced lesions in a in a gray brocket deer. **A.** abscess in the masseter muscle, with a focally extensive area of inflammatory infiltrate composed by macrophages in the periphery and intact or degenerated neutrophils in the center. Hematoxylin and eosin (HE), 100x. **B. & C.** Neutrophilic infiltrate with myriad of intralesional Gram-positive rod-shaped bacteria in the abscess. Gram stain, 400x. **D.** Oral mucosa with a focal area of ulceration with a neutrophilic and histiocytic infiltrate. Hematoxylin and eosin (HE), 100x. **E.** Retrobulbar muscle, connective and adipose tissues with marked neutrophilic and histiocytic infiltrate and intralesional bacteria. Hematoxylin and eosin (HE), 200x. **F.** Multifocal areas of neutrophilic infiltrate in the lungs, often associated with thrombi, characterizing a thromboembolic pneumonia. Hematoxylin and eosin (HE), 200x.

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Swabs from the abscess and meninges were collected for microbiological culture. Samples were plated on Mueller Hinton agar (MH; Kasvi, Italy) supplemented with 5% horse blood and MacConkey agar (MC; Kasvi, Italy), subsequently incubated at 37°C for 48 hours under aerobic or anaerobic conditions, resulting in a pure culture. Isolates were characterized by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS, Bruker Daltonics, Germany), which demonstrated that the isolates had a profile compatible with *Trueperella pyogenes*.

Samples of the abscess in the masseter muscle, brain and lungs were collected during the necropsy, and stored at -20°C for DNA extraction, which was performed using the guanidine method described by Bloom et al. (1990) (5). PCR was performed for identification of *T. pyogenes* genomic DNA sequences, using the following primers (Jost et al., 2002): GGCCCGATGTCACCGC and AACTCCGCCTCTAGCGC. PCR reactions contained 13 µL of PCR supermix (Thermo Fisher Scientific, USA), 1.0 µM of each primer (25 mM), 4.0 µL of template DNA, and supplementation with 1.0 U of Taq DNA polymerase (Thermo Fisher Scientific, USA). Amplification parameters were 95°C for a 5 min, 40 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a 7 min final extension step at 72°C. A 270 bp amplicon was demonstrated using a 1.5% agarose gel electrophoresis, which demonstrated specific *T. pyogenes* amplicons from template DNA extracted from swabs obtained from the meninges and lung. No amplification was obtained from negative controls without template DNA.

Discussion

Here we describe a case of a *T. pyogenes*-induced abscess that led to a case of fatal suppurative meningitis in an adult female gray brocket deer (*M. gouazobira*). The etiologic diagnosis in this case was accomplished through

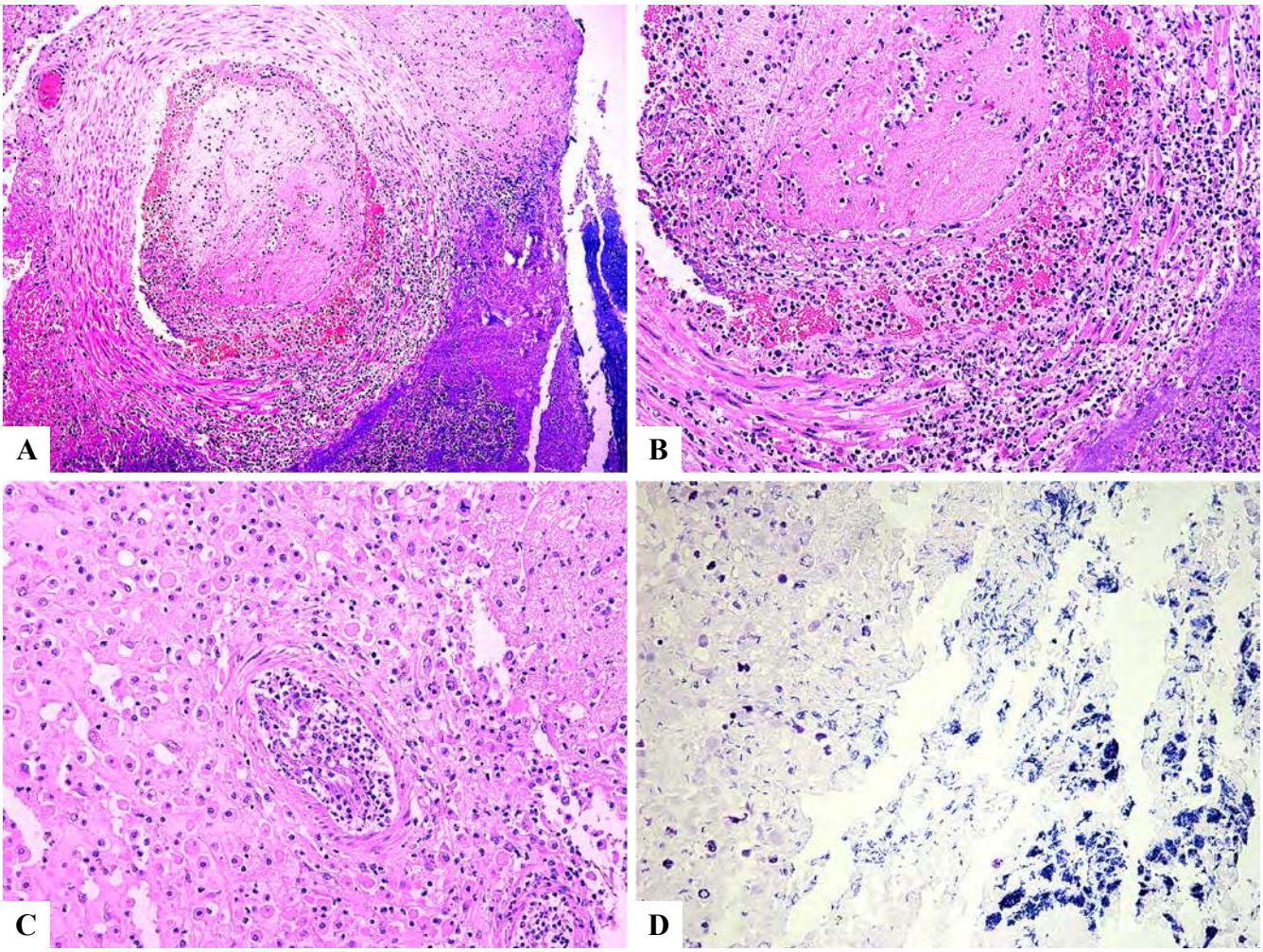

Figure 4. *Truerella pyogenes*-induced suppurative meningoencephalitis in an adult gray brocket deer. **A.** Blood vessel in the meninges, severe vasculitis, with endothelial necrosis and thrombosis. Hematoxylin and eosin, 100X. **B.** Detail of dilated blood vessel with vasculitis and thrombosis. Hematoxylin and eosin, 200X. **C.** Brainstem with perivascular cuff of lymphocyte and macrophages, and large number of Gitter cells. Hematoxylin and eosin, 400X. **D.** Abundant monomorphic Gram-positive rod-shaped intralesional bacterial population. Gram stain, 400x.

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microbiologic and molecular techniques. To the best of our knowledge, this is the first report of *T. pyogenes*-induced abscess and meningitis in a neotropical deer. Intracranial abscesses caused by bacteria in deer are frequent, with *T. pyogenes* being commonly isolated of these lesions in North America (2; 3, 6, 7, 14, 21). Intracranial abscess and meningoencephalitis in deer caused by *T. pyogenes* are important cause of morbidity and mortality in white-tailed deer (*Odocoileis virginianus*) in North America (14, 21). This condition usually affects males during the breeding season due to sexual competition, when pyogenic bacteria opportunistically invade cuts, abrasions, or injured tissues as a consequence of horn inflicted lesions (2, 6, 3, 7, 14, 24).

In this case, there were no skin lesions adjacent to the abscess. In contrast, there was a fistula through the oral mucosa. Therefore, it is reasonable to hypothesize that an oral wound may have been the source of infection that led to abscess formation. However, we cannot rule out the possibility that bacterial infection could have been the result of an older healed skin lesion that allowed tissue invasion and colonization by *T. pyogenes*. The pathogenesis of *T. pyogenes* causing intracranial abscesses in white-tailed deer is associated with its ability to successfully penetrated tissues and adhere to host cells (13). The route of *T. pyogenes* infections is frequently difficult to establish. Usually, bacterial penetration is associated with traumatic injuries to the skin or mucous membranes. (24, 25, 27, 29). *T. pyogenes*, as well as other actinomycetes, invade host tissues and cause suppurative infections leading to abscesses, empyema, or pyogranulomas (16, 24). *T. pyogenes* is pathogenic for a variety of animal species, causing purulent or necrotizing lesions (16, 17, 25, 28).

Although, *T. pyogenes* is recognized as a relevant cause of abortion in cattle, sheep, and pigs, in this case the fetus and placenta were not affected, where there were no lesions attributable to *T. pyogenes* (12, 29, 30).

Bacterial identification in this case was based on isolation followed by identification using MALDI-ToF, which is increasingly used in clinical microbiology with reliable results for bacterial identification at the species levels. Protein extracts from lysates are employed for generating a protein spectrum that is compared to standard spectra from the database (17, 20, 22). In addition, the etiologic agent in this case was further confirmed by amplification of *T. pyogenes* genomic DNA from tissue samples with lesions.

T. pyogenes is considered an opportunist pathogen. It is a component of microbiota in the skin, mucous membranes, upper respiratory and urogenital tracts of animals (23, 26). However, *T. pyogenes* may occasionally invade host tissues causing suppurative lesions as demonstrated in this case, in which intralesional *T. pyogenes* was confirmed by microbiologic and molecular techniques (19, 28). Therefore, this report described a case of *T. pyogenes*-associated abscess with a fistulous tract extending the infection to the orbit, resulting in suppurative meningitis in a gray brocket deer. This report supports the notion that *T. pyogenes* has a pathogenic potential in gray brocket deer that is likely similar to other ruminant species. Thus, *T. pyogenes* should be considered in the differential diagnosis of suppurative lesions in neotropical free-living deer.

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