

Atypical actinomycotic pyogranuloma of the frontal and parietal region in a roe deer (*Capreolus capreolus*) - a case report

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ABSTRACT

Actinomycosis is a rare infectious bacterial disease that affects domestic and wild ruminants, horses, small carnivores, monkeys, rabbits, birds and humans. *Actinomyces* spp. are gram-positive, acid-fast-negative filamentous bacteria which can grow anaerobically and cause abscesses and pyogranulomatous inflammation. In this report, a case of an atypical actinomycotic pyogranuloma due to *Actinomyces* sp. of the frontal and parietal region of the head in a roe deer (*Capreolus capreolus*) is described. Based on the gross and histopathological findings and bacterial culture results, a diagnosis of extrafacial actinomycosis affecting the neurocranial part of the skull and soft tissues of the frontal and parietal regions was made.

Key words: Actinomycosis; roe deer; *Capreolus capreolus*; histopathology; bacteriology

Introduction

Actinomycosis is a rare, infectious, non-contagious bacterial disease, caused by *Actinomyces* species, occurring in both animals and humans. The *Actinomyces* genus is part of the family Actinomycetaceae, and it currently comprises 50 species and two subspecies that have been characterised by phenotypic and molecular methods, from which 21 species are known to be the cause of a wide spectrum of diseases in animals (LPSN, 2019). *Actinomyces* spp. are gram-positive, acid-fast-negative filamentous bacteria which can grow anaerobically (VALOUR et al., 2014) and cause abscesses and pyogranulomatous inflammation.

Actinomyces spp. are generally considered as a normal microbiota of mucous membranes, primarily in the oral cavity, nasopharynx, gastrointestinal tract and urogenital tract (COUTO et al., 2000; KAYIKCIOGLU et al., 2005; VOS, 2007). However, many *Actinomyces* sp. are opportunistic pathogens of humans and other mammals, particularly in the oral cavity (YEUNG, 1999; MARDIS and MANY, 2001) and they become pathogenic only under certain conditions (REICHENBACH et al., 2009). The disease seems to occur through direct extension of the infection from the gums, apparently following trauma, infection, or as a complication of periodontitis of other causes (BERTONE and REBHUM, 1984).

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Although cervicofacial actinomycosis is the most frequent form of actinomycosis with bone involvement, *Actinomyces* spp. may also be involved in extrafacial bone and joint infection (VALOUR et al., 2014). Such actinomycosis can also be further classified into distinct clinical forms according to the anatomical site infected: orocervicofacial, thoracic, abdominopelvic, central nervous system, musculoskeletal, and disseminated (WONG et al., 2011). Once *Actinomyces* spp. have invaded tissues, they develop a chronic suppurative and granulomatous inflammation, and formation of multiple abscesses and sinus tracts that may discharge tiny clumps (sulphur granules) due to their yellow colour (DE MONTREVILLE et al., 1999).

The most prevalent species of the genus *Actinomyces*, isolated from ruminant infections and found in most clinical forms of actinomycosis, is *A. bovis*, but many other species have also been described and are associated with pathogenic conditions. Among wild ungulates in Europe, suppurative lesions caused by bacteria of the *Actinomyces* genus have been reported in Alpine ibex (*Capra ibex*) (FAGAN et al., 2005), chamois (*Rupicapra rupicapra*) (RADAELLI et al., 2007) and roe deer (*Capreolus capreolus*) (PEWSNER et al., 2017).

To the best of our knowledge, all previously published cases represent the cervicofacial form of actinomycosis, while the extrafacial form has not been previously described in wild ungulates. This is the first case report of extrafacial actinomycosis affecting the neurocranial part of the skull and soft tissues of the frontal and parietal region in a roe deer (*C. capreolus*), caused by *Actinomyces* sp.

Materials and methods

A juvenile female roe deer was found in agony and harvested in March 2018 by a local hunter at the Polzela hunting area, northwest of the Lower Savinja Valley in east Slovenia and submitted for necropsy, histopathological, and bacteriological examination to the Veterinary Faculty (University of Ljubljana, Ljubljana, Slovenia). The animal was in poor body condition with an enlarged head.

Tissue and swab samples of the lesion were taken for bacteriology, and tissue samples were taken for histopathology. Samples for histopathology were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned and stained with H&E, Periodic acid-Schiff, Gram and Ziehl-Neelsen stains, according to the standard protocols. For bacteriology, culture from tissue samples was performed on Columbia Agar (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with 5% sheep blood, and incubated aerobically and anaerobically at 37 °C for 72 h. The isolate was analysed with matrix-assisted, laser desorption ionization-time of flight, mass spectrometry MALDI-TOF-MS (Bruker Daltronik GmbH, Bremen, Germany) according to the manufacturer's instruction.

Results

The pathoanatomical examination of the head revealed a well circumscribed, exophytic, firm lesion, measuring 11 × 9 × 6 centimetres, located in the frontal and parietal region (Fig. 1A). The skin above the lesion was intact, but there was a fistula on the aboral side of the lesion connected to the centre of the lesion, and it drained thick, brownish, mucopurulent exudate. Examination of the cut surface of the skull revealed a well demarcated, oval-shaped, non-encapsulated, greyish, gelatinous lesion that involved the skin and subcutis of the frontal and parietal region, frontal sinuses, ethmoid bone, frontal and parietal bone, and the latter were lysed (Fig. 1B). No pathology was noted on gross examination of the oral cavity, hard palate and other organs.

Histopathological examination of the samples revealed a pyogranulomatous inflammation with an extended area of lytic necrosis in the deep dermis; subcutis and adjacent bone (Fig. 1C). Between the inflammatory cells, there were numerous clusters of filamentous, gram-positive, acid-fast-negative bacteria, surrounded by eosinophilic Splendore-Hoeppli material, which stained PAS positive (Fig. 1D). The microscopic appearance was highly suggestive of infection by *Actinomyces* spp.

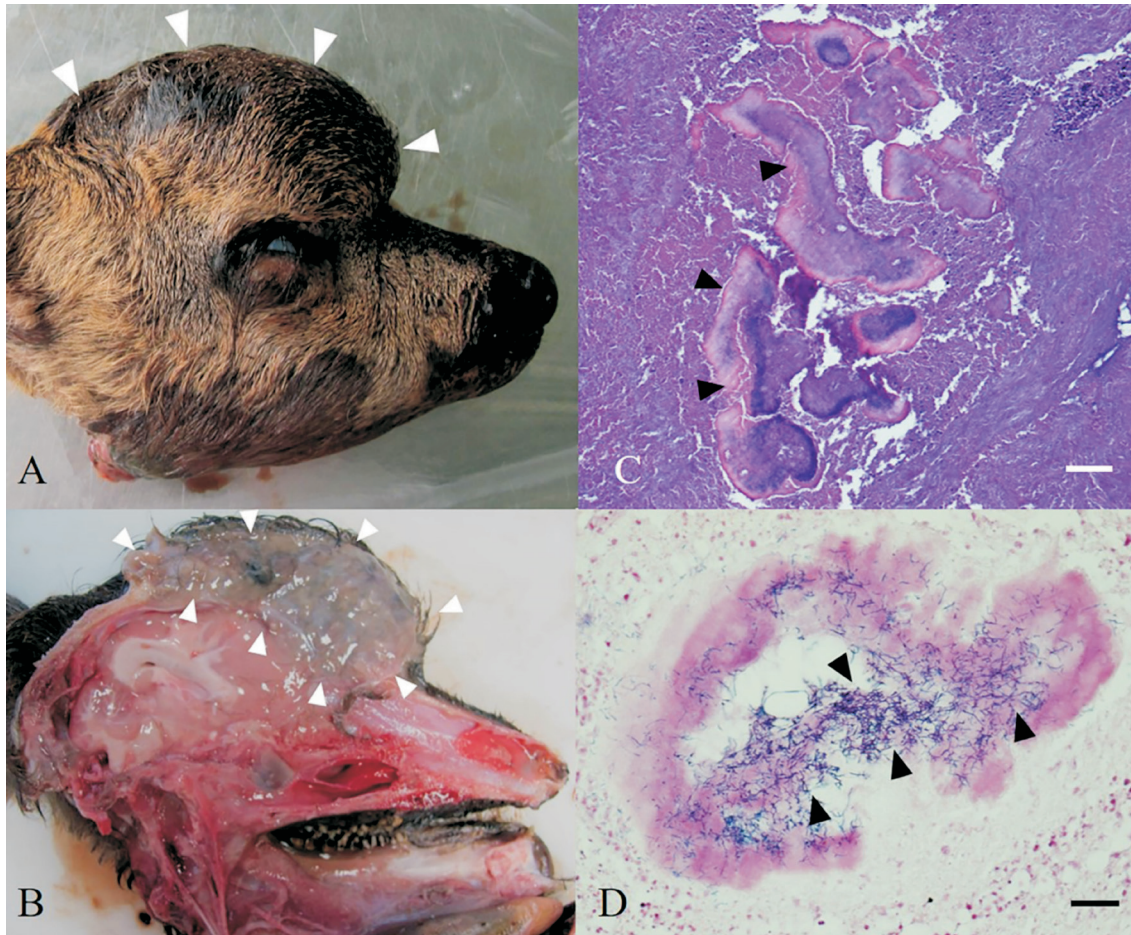


Fig. 1. A-D. Actinomycotic pyogranuloma of the frontal and parietal region, roe deer. 1A - Macroscopic presentation. A well-circumscribed, exophytic, firm lesion located in the frontal and parietal region. The edges of the lesion are marked with arrowheads; 1B - Macroscopic presentation. The cut surface of the skull with the lesion that involved the skin and subcutis of the frontal and parietal region, frontal sinuses, ethmoid bone, and frontal and parietal bones. The edges of the lesion are marked with arrowheads; 1C - Microscopic presentation. Pyogranulomatous inflammation with extended area of lytic necrosis and bacterial colonies (arrows). H&E, $\times 100$. Scale bar = 100 μm . 1D - Microscopic presentation. Numerous clusters of filamentous, gram-positive bacteria (arrows). Gram staining; scale bar = 50 μm ; $\times 400$.

Using the conventional bacteriological culture method, the colonies that developed within 48 hours of incubation were non-hemolytic, white and adhered to the surface of the medium. The growth was slightly better on the plates that were incubated anaerobically. The identification with MALDI TOF MS was reliable to the genus level, but not

reliable to the species level. Therefore, the result was concluded as *Actinomyces* sp.

On the basis of the gross and histopathological findings, and bacterial culture results, a diagnosis of extrafacial actinomycosis affecting the neurocranial part of the skull and soft tissues of the frontal and parietal regions was made.

Discussion

In the current study, a severe form of the disease manifested with pyogranulomatous inflammation of the soft tissues of the frontal and parietal region, osteolysis of the neurocranial part of the skull, and

draining fistula in the parietal region. The gross and histopathological features presented in this case are similar to lesions described by other authors, and are characteristic of actinomycosis (MOHAMED

et al., 2011; CRAIG et al., 2016); however, the location of the lesion is not typical.

Actinomyces spp. are mainly opportunistic pathogens of humans and other mammals, that cause lesions, particularly in the oral cavity (YEUNG, 1999; MARDIS and MANY, 2001). Horizontal transmission of the infection usually occurs via bite wounds, following a penetrating injury of the oral mucosa (KONJEVIĆ et al., 2011; MAULDIN and PETERS-KENNEDY, 2016). In dogs, infection of the subcutaneous tissue usually represents extension of a cervicofacial, thoracic or retroperitoneal disease, whereas in cats it is caused by bite wounds (SYKES, 2012). The source of the infection in the present case could not be determined. In the oral cavity and hard palate we did not find any pathological lesions; therefore, we believe that infection extension to the parietal and frontal region is less likely. A possible entry point for bacteria in our case can be injured skin of the parietal or frontal region through which the agent could enter during a bite or licking a bite, or licking (SYKES, 2012). From this point we can only speculate that the entry point of the bacteria could have coincided with a fistula. Actinomycosis is a slowly progressing disease and it can take months or even years to develop a grossly visible lesion (MAULDIN and PETERS-KENNEDY, 2016).

The confirmation of actinomycosis is rarely carried out because bacteria belonging to the *Actinomyces* genus are fastidious and difficult to culture (MABEZA and MACFARLANE, 2003). In our case, *Actinomyces* sp. was bacteriologically confirmed. The growth was in pure culture and developed within 24 hours. However, the culture could not be identified to the species level since MALDI-TOF MS, which is widely used in clinical laboratories, has some limitations. A number of *Actinomyces* spp. cannot be identified to the species level using current databases because of the genus diversity (LYNCH et al., 2016).

The typical histological lesions and the specific staining properties of microorganism make histopathology the most reliable and rapid diagnostic tool and, thus, the gold standard for definitive diagnosis of actinomycotic infection (THUKRAL et al., 2017). Upon histopathological

assessment and bacteriological confirmation, a diagnosis of actinomycotic pyogranulomatous osteomyelitis of the frontal skull and extensive soft-tissue inflammation was made.

Roe deer (*C. capreolus*) are one of the most abundant and widely distributed small ruminant mammal species in Slovenia. Several diseases of roe deer have been recorded within the framework of a passive wildlife health surveillance program in Slovenia, including actinomycosis. General wildlife health surveillance is a valuable source of information on the causes of mortality, disease susceptibility and pathology of the investigated hosts (PEWSNER et al., 2017). In Slovenia, necropsies of 617 free ranging roe deer were performed within the framework of the passive wildlife health surveillance program, in the period from 2000 to 2018. Mandibular actinomycosis was diagnosed in two cases, and extrafacial actinomycosis in one case. The 19 years prevalence of actinomycosis in roe deer wildlife health surveillance program in Slovenia highlighted the very low prevalence of 0.48%. Similar findings were reported in Switzerland (0.13%) (PEWSNER et al., 2017) and Sweden (0.2%) (AGUIRRE et al., 1999). Characteristic mandibular actinomycotic osteomyelitis of roe deer (without detection of the causative agents), collected in Slovenia in 2007, revealed 4.2% prevalence after examination of 41,854 mandibular samples (KONJEVIĆ et al., 2011). In a healthy, wild, ruminant population, actinomycotic lesions in the mandibles occur at low frequencies (HOEFS and BUNCH, 2001), while stress, poor diet, and high animal population have been reported to contribute to the disease in wild animals (AZORIT et al., 2012).

Although the prevalence of actinomycosis due to *Actinomyces* spp. in roe deer is very low, we believe that additional cases will continue to occur sporadically.

Conclusions

This case report describes a case of extrafacial actinomycosis affecting the neurocranial part of the skull and soft tissues of the frontal and parietal regions in a roe deer. Histopathology and bacteriology are the most reliable diagnostic tools for the definitive

diagnosis of actinomycotic infection. Species-level identification using MALDI-TOF MS and current databases has some limitations because of the genus diversity. The prevalence of actinomycosis in roe deer (in Slovenia) is very low; however, additional cases will probably occur sporadically. Several infections of wildlife have been recorded within the framework of a passive wildlife health surveillance program in Slovenia, and it relies greatly on good cooperation with hunters who are in everyday contact with wildlife and report sick or dead animals.

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SAŽETAK

Aktinomikoza je rijetka zarazna bakterijska bolest koja zahvaća domaće i divlje preživače, konje, male mesojede, majmune, kuniće, ptice i ljude. Bakterije *Actinomyces* spp. jesu gram-pozitivne, filamentne bakterije koje se boje Ziehl-Neelsenovim bojenjem, a mogu rasti anaerobno te uzrokovati apscese i piogranulomatoznu upalu. U ovom se radu opisuje slučaj atipičnog aktinomikotičnog piogranuloma uzrokovanog bakterijom *Actinomyces* sp. u frontalnoj i parijetalnoj regiji glave srne (*Capreolus capreolus*). Na temelju makroskopskog i histopatološkog nalaza te bakteriološke pretrage ustanovljena je dijagnoza ekstrapacijalne aktinomikoze koja je zahvatila neurokranijalni dio lubanje i meka tkiva frontalne i parijetalne regije.

Ključne riječi: aktinomikoza; srna; *Capreolus capreolus*; histopatologija; bakteriologija
