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Case Report

ORAL AMEBIOSIS CAUSED BY *ENTAMOEBA GINGIVALIS,* MIMICKING CANINE CHRONIC ULCERATIVE STOMATITIS IN A FOUR-YEAR-OLD BASENJI DOG

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Abstract

Protozoan diseases within the oral cavity of companion animals are quite rare, and such cases are not common in veterinary cytology. In this case report, we describe a case of oral cavity amebiasis in a four-year-old Basenji dog with signs of severe generalized stomatitis and bilateral mandibular lymph node enlargement. In touch imprint cytologic samples, among inflammatory and epithelial cells, protozoa with morphologic features characteristic of *Entamoeba gingivalis* were found. After diagnosis the patient was successfully treated with a combination of local and systemic metronidazole two times per day for three weeks. Therefore, in cases of generalized gingivostomatitis in dogs, there is reason to suspect the presence of protozoal agents and pay more attention to evaluating touch imprint smears from the oral cavity.

Keywords: amebiosis, cytology, dog, protozoa, stomatitis

INTRODUCTION

Canine chronic ulcerative stomatitis is a pathologic condition associated with inflammation, pain and mucosal necrosis. The etiology of this condition is uncertain but a common hypothesis is that it is related to the persistence of dental plaques, which cause chronic inflammation and subsequent immune-mediated damage. The diagnosis

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is established comprehensively through comparisons of clinical signs, blood analysis, histopathology, and immunohistochemistry (Anderson, J. G. et al., 2017, 2020). The treatment can include retraction of the tooth next to an ulcer or medical management with cyclosporine and metronidazole (Anderson, J. G. et al., 2017, Ford, K.R., 2023 et al.). In addition, there are a number of pathological conditions, mainly of autoimmune and neoplastic genesis, that must be differentiated from the canine chronic ulcerative stomatitis (Anderson, J. G. et al., 2017). Notably, assessment of dental canine plaques as probable causes of chronic inflammation in the oral cavity have focused mainly on the bacterial microbiome (Ruparell, A. et al., 2020, Santibáñez, R. et al., 2021) and have rarely mentioned protozoal residents (Kačírová, J. et al., 2020). However, in human medicine, the possibility of the presence and pathogenic influence of protozoans in the oral cavity has been considered in many studies (Bonner, M. et al., 2018, Bao X. et al., 2020, Jiao, J. et al., 2022, Yaseen, A. et al., 2021, Fadhil Ali Malaa, S. et al, 2022, Becker C. et al., 2023, Miranda, B.P. et al., 2025).

CASE PRESENTATION

A four-year-old male Basenji dog was admitted to the veterinary clinic with complaints of halitosis and salivation which had been observed for approximately two months. The patient's general condition was good and no signs of weight loss were observed.

During the clinical examination of oral cavity pain, generalized gingivitis, multifocal ulcers on the gingival and buccal mucous membranes and an ulcer on the lateral tongue surface were observed (Fig. 1).

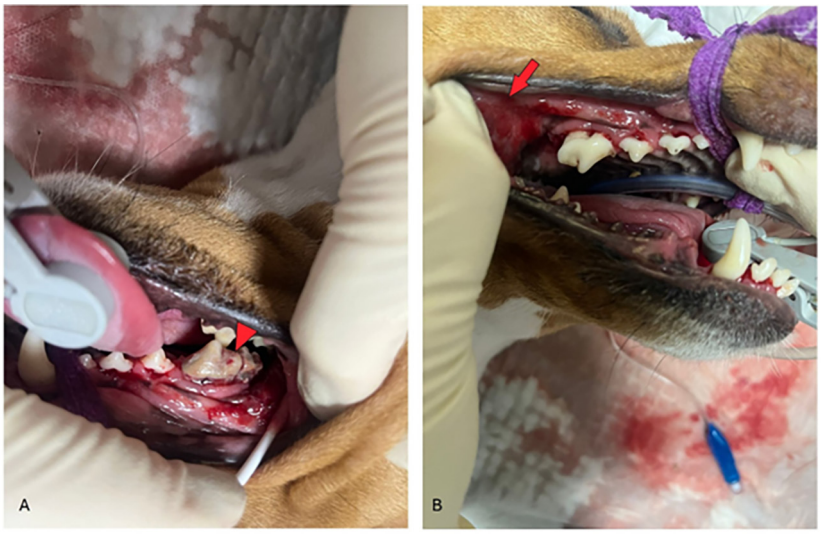

Fig. 1. Lesions of oral mucosa and bacterial plaques. **A.** Bacterial plaques are seen on the molar's surface (red arrowhead). **B.** Teeth after sanitation. Marked inflammatory regions are seen in the buccal mucosa (red arrow).

There was also an excessive accumulation of dental plaque. A preliminary clinical diagnosis of canine chronic ulcerative stomatitis was made.

First, touch imprint smears were prepared for the assessment of inflammation type and cytological compounds and then general sanitation with ultrasonic tooth cleaning was performed.

Four cytological touch imprint smears were made in the veterinary clinic and sent to the veterinary laboratory Science Vet Lab where the smears were stained with May-Grünwald-Giemsa stain and assessed under an oil-immersion objective at 900× magnification.

In all four smears, degenerative neutrophils with a few macrophages, erythrocytes and many microorganisms were observed. One cytologic smear, among the described cell types, also revealed large round protozoan cells with pale blue, often vacuolated cytoplasm and a relatively small round nucleus with a single light nucleolus (Fig. 2). Many of these cells had unusual small vacuoles near their nucleus and dark round inclusions in the cytoplasm. On the basis of a set of morphological characteristics, these protozoans were identified as *Entamoeba*, suggesting *E. gingivalis*, a unique Entamoeba that can reside in the oral cavity (Bonner, M. et al., 2018). In general, the observed smears were characterized by neutrophilic inflammation with a significant amount of bacterial cells and a moderate amount of partially lytic erythrocytes.

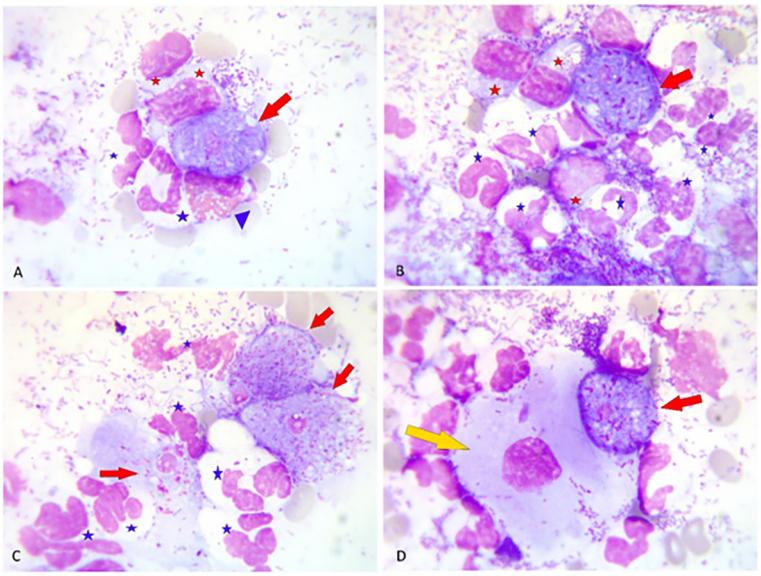

Fig. 2. Cytologic smears stained with May-Grünwald-Giemsa. **A.** *Entamoeba gingivalis* trophozoite (red arrow) surrounded by neutrophils (blue stars), macrophage (red stars) and eosinophil (blue arrowhead). **B.** Single *Entamoeba gingivalis* trophozoite (red arrow) surrounded by numerous neutrophils (blue stars) and macrophages (red stars). **C.** Three *Entamoeba gingivalis* trophozoites (red arrows) among neutrophils (blue stars). **D.** *Entamoeba gingivalis* trophozoite (red arrow) near a squamous epithelial cell (yellow arrow). All photomicrographs show high numbers of microorganisms on the background.

Before the cytology results were obtained, subcutaneous injections of meloxicam at a dosage of 0.05 mL/kg (total 0.6 mL) were given once, and Synulox in a 500 mg tablet form (PO, 1/3 of a tablet with food, twice a day for one week) had no significant effect (Fig. 3).

Fig. 3. Absence of significant effect of Synulox course. **A.** Inflammation of buccal mucosa (red arrow). **B.** Ulcer on the mucosa surface (red arrowhead). **C.** Erosions on the lateral side of the tongue (blue arrow). All clinical signs and marked inflammation were observed after Synulox course.

After *Entamoeba* determination, the treatment was corrected. To reduce inflammation, subcutaneous injections of meloxicam at a dosage of 0.05 mL/kg (total 0.6 mL) were repeatedly administered once a day for three days. Metronidazole in tablets (PO, 25 mg/kg twice a day, for three weeks). An everyday oral irrigation procedure was also added for the maintenance of local hygiene. After three weeks of treatment, there were no signs of inflammation in the oral mucosa (Fig. 4). The control cytologic smears at that stage did not contain any protozoal or microbial agents and only a few epithelial cells were observed.

Fig. 4. Significant effect of metronidazole course. **A-C.** Oral cavity after the three-week course of metronidazole and irrigations with saline isotonic solution. The color of mucosa is pink (red arrowhead), no ulcers are observed (red arrow), and tongue erosions are cured (blue arrow).

DISCUSSION

There is very scarce information about the inflammatory process in the oral and nasal cavities caused by protozoa in animals (Patel, N. et al., 2017, Randolph, N.K. et al., 2022). However, the pathogenic role of *E. gingivalis*, is well defined in human medicine, and it has been determined that the persistence of this resident is strongly associated with periodontitis, although it can be found in healthy individuals (Bonner, M. et al., 2018, Bao X. et al., 2020, Jiao, J. et al., 2022, Yaseen, A. et al., 2021, Becker C. et al., 2023).

Currently, *E. gingivalis* is considered the only pathogenic amoeba species of the oral cavity (Bonner, M. et al., 2018). Interestingly, when this pathogen was first discovered in dogs, a series of experiments were conducted and revealed that canine – and human-associated *Entamoeba* spp. require different conditions and, thus, belong to different species (Simitch, T. 1938). Researchers have concluded that humans and dogs share one pathogenic *Entamoeba* – *E. gingivalis* (Kačírová, J. et al., 2020, Patel, N. et al., 2017). Unlike other pathogenic amoeba species (e.g., *E. histolytica*), *E. gingivalis* cannot form cysts (Bonner, M. et al., 2018, Becker C. et al., 2023) and lives only as a trophozoite. As mentioned earlier, *E. gingivalis* in humans is associated with periodontitis and is found in gingival pockets, usually mixed with local microorganisms. *E. gingivalis* can attack host cells via phagocytosis and trogocytosis and can attract neutrophils to support inflammation on its own (Bonner, M. et al., 2018, Bao X. et al., 2020). Moreover, *E. gingivalis* can inhibit the proliferation of epithelial cells and cause their apoptosis (Bao X. et al., 2020).

CONCLUSION

E. gingivalis undoubtedly plays an important role in oral inflammatory processes. This case report highlights the involvement of *E. gingivalis* in the oral inflammatory process in a dog, which was successfully treated with antiprotozoal drugs. Therefore, in cases of canine ulcerative stomatitis, diagnostic measures, including cytology, aimed at the detection of oral protozoa may be useful.

Authors' contributions

All the authors took part in this study. E.S. Makarenko undertook the cytology staining procedure and acquired images of the cytologic smears. E.S. Makarenko and V.S. Ivanov evaluated the cytologic smears and defined the diagnosis. V.S. Ivanov reviewed this manuscript for structure and intellectual content. Yu. Ya. Okuneva guided the patient, prescribed the treatment and provided the dog's photo. All the authors contributed to the article and approved the submitted version.

Ethical Statement

The owner of the animal provided informed written consent for the use of data related to their pet for the purpose of publishing professional and scientific papers.

Competing Interests statement

The authors declare that they have no competing interests.

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ORALNA AMEBIJAZA IZAZVANA VRSTOM *ENTAMOEBA GINGIVALIS*, KOJA OPONAŠA HRONIČNI ULCERATIVNI STOMATITIS PASA KOD ČETVOROGODIŠNJEG PSA RASE BASENJI

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Kratak sadržaj

Protozoarne bolesti usne duplje kućnih ljubimaca relativno su retke, a takvi slučajevi nisu česti ni u veterinarskoj citologiji. U ovom prikazu slučaja opisana je amebijaza usne duplje kod četvorogodišnjeg psa rase basenji, sa znacima teškog generalizovanog stomatitisa i bilateralnim uvećanjem mandibularnih limfnih čvorova. U citološkim preparatima dobijenim metodom otiska, među inflamatornim i epitelnim ćelijama, utvrđeno je prisustvo protozoa sa morfološkim karakteristikama tipičnim za *Entamoeba gingivalis*. Nakon postavljanja dijagnoze, pacijent je uspešno lečen kombinacijom lokalne i sistemske primene metronidazola, dva puta dnevno, tokom tri nedelje. Stoga, u slučajevima generalizovanog gingivostomatitisa pasa, opravdano je posumnjati i na prisustvo protozoarnih agenasa, uz posebnu pažnju pri proceni citoloških preparata otiska iz usne duplje.

Ključne reči: amebijaza, citologija, pas, protozoe, stomatitis