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Oropharyngeal Botryomycosis in a Geriatric Mare

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Abstract:	Botryomycosis is an uncommon chronic bacterial infection that can have cutaneous and visceral involvement. This report describes an 18-year-old mixed-breed mare presented with dysphagia, dyspnea, and an upper respiratory noise that developed secondary to oropharyngeal botryomycosis. Histological examination of the mass showed a granulomatous formation with Splendore-Hoeppli phenomenon surrounding gram-positive bacteria. This report describes clinical signs, approach and management of an oropharyngeal Staphylococcus aureus granuloma in a geriatric mare.

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Case Report.

Oropharyngeal Botryomycosis in a Geriatric Mare.

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Summary

Botryomycosis is an uncommon chronic bacterial infection that can have cutaneous and visceral involvement. This report describes an 18-year-old mixed-breed mare presented with dysphagia, dyspnea, and an upper respiratory noise that developed secondary to oropharyngeal botryomycosis. Histological examination of the mass showed a granulomatous formation with Splendore-Hoeppli phenomenon surrounding gram-positive bacteria. This report describes clinical signs, approach and management of an oropharyngeal *Staphylococcus aureus* granuloma in a geriatric mare.

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Introduction

Cutaneous and visceral botryomycosis has been described in equids, ruminants, companion animals, guinea pigs, elephant and humans for over a century. (Bollinger 1870, Bostrom *et al* 1969, Sheikh-Omar and Abdullah 1985, Bonifaz and Carrasco 1996, Pandey *et al* 1997, Margaret *et al* 2001, Miller *et al* 2001, Sleeman, J *et al* 2003, Scott 2007). Botryomycosis is an uncommon condition, characterized by formation of a slow growing chronic granuloma caused by non-filamentous aerobic and anaerobic bacteria such as *Staphylococcus aureus*, *Pseudomona* spp. and *Bacteroides*. *S. aureus* is the most common agent encountered.

Diagnosis is based on histological identification of granulomatous inflammation and isolation of the microorganism. In the case described herein, a subepiglotic oropharyngeal granuloma caused by *S. aureus* was diagnosed based on the characteristic histologic findings and positive culture. Histologically, botryomycosis is characterized by the presence of radiating, star-like asteroid or club-shaped eosinophilic material, Splendor-Hoeppli (SH) reaction around the infectious agent; which represents a localized immunological host reaction to antigens (Hussein 2008). Fungal granulomas, bacterial abscessation, foreign body, amyloidosis, and neoplasia should be included in its differential diagnosis. (Miller and Campbell 1982, Shaw *et al.* 1987, Kiper *et al.* 1992*,* Jones 1994)

This report describes the clinical manifestations of a granuloma caused by *S. aureus* in the oropharynx of a geriatric mare outlining a diagnostic and surgical approach as well as management of the case. To the authors' knowledge this is the first case of botryomycosis in the oropharynx described in equine.

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Case history

An 18-year-old mixed breed mare weighing approximately 450 kg was presented to *Fundació Hospital Clínic Veterinari* of the *Universitat Autònoma de Barcelona*, with a 3-year history of a mass located in the submandibular area and a second mass in the pharyngeal region. Over the month prior to admission, the mare was occasionally pyrexic and presented intermittent dysphagia. The mare also had episodes of dyspnea, upper respiratory noise, and bilateral mucoid nasal discharge, especially when exercised.

Clinical findings

On presentation, the mare was quiet, alert and responsive. Physical exam was within normal limits. Bilateral mucoid nasal discharge was evident. Two firm, round shaped and smooth masses were palpable in the submandibular and pharyngeal areas to the right side of midline, 5 and 3 cm in diameter respectively. The skin overlying the masses was normal and there was no evidence of pain or pruritus.

Ultrasonography of the submandibular mass revealed soft tissue density with multiple hypoechoic compartments. The pharyngeal mass was encapsulated with the presence of punctiform hyperechoic structures and mineral opacities as well as hypoechoic areas similar to the other mass. (Fig 1) No communication between the two masses was identified. An ultrasound guided fine-needle aspirate was obtained under local anesthesia with mepivacaine from both masses. Cytology revealed mixed neutrophilic and mononuclear inflammatory cells with presence of gram-positive cocci and bacterial culture was negative.

Skull radiography revealed a well circumscribed, round soft tissue radio-opacity in the oropharynx over the base of the tongue that obscured the epiglottis and hyoid apparatus over which it was superimposed.

Endoscopy of the upper respiratory tract (URT) revealed the soft palate protruding dorsally into the nasopharynx as though being pushed upwards. The soft palate was displaced dorsal to the epiglottis

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throughout the endoscopic examination. Oral endoscopy identified a 3 cm diameter, ulcerated pink mass at the base of the tongue slightly offset to the right of midline that occluded the end of the oropharynx **(Fig.2)**. This mass was attached to the base of the tongue and ventral aspect of the epiglottis. This finding explained the dysphagia and dyspnea.

Treatment

Surgical resection under general anesthesia was performed in two different procedures. Pre-operatively, total protein and fibrinogen were elevated at 9.8 g/dl and 800 mg/dl respectively. Rebreathing exam and thoracic ultrasonography were normal. Complete blood count, serum biochemistry and electrocardiogram were within normal limits.

The patient received pre-operative sodium penicillin[1] (22.000 IU/kg bwt i.v.), gentamicin[2] (6.6 mg/kg bwt i.v.), phenylbutazone[3] (4.4 mg/kg bwt i.v.) and morphine[2] (0.1 mg/kg bwt i.m.).

The mare was sedated with romifidine[4] (0.06 mg/kg bwt i.v.) and butorphanol[3] (0.03 mg/kg bwt i.v.), induced with ketamine[5] (2 mg/kg bwt i.v.) and diazepam[6] (0.05 mg/kg bwt i.v.), placed in dorsal recumbency and maintained with isoflurane[7] in 100% oxygen through a mid cervical tracheotomy performed prior to induction.

Initial intraoral examination under general anesthesia revealed a fibrous mass, approximately 1.5 x 5 x 3 cm, from the base of the tongue to the oropharynx and slightly offset to the right of midline, involving the mucosa of the oropharynx.

The ventral aspect of the head and neck were clipped and prepared aseptically. Surgery was performed through a submandibular approach and continued by pharyngotomy as well as intraoral manipulation. The submandibular mass extended to the oropharynx involving contiguous structures such as the hyoid apparatus. Because of the location of the mass, surgical excision was unsuccessful in achieving clean margins. The submandibular area was sutured in four layers: mucosa, muscle, subcutaneous tissue and skin; and the pharyngotomy was left to heal by second intention. The oropharyngeal mass excised surgically was fixed in 10% formalin and processed routinely for

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histopathological studies. Hematoxilin-eosin and Gram staining were performed on the different sections.

Two days later, under intravenous anesthesia with xylazine, ketamine, and guafenesin, the second surgery was performed in sternal recumbency. Through an intraoral approach, the remaining part of the mass adhered to the right aryepiglottic fold was removed (**Fig.3**). Laparoscopic instruments were used for the procedure guided by nasal endoscopy. Additionally, an adhesion between the subepiglottic fold and epiglottis was excised.

Diagnosis

The different sections of the samples evaluated showed a similar histopahologic image: the normal architecture of the tissue was extensively obliterated by irregularly sized pyogranulomas constituted by collections of degenerate neutrophils with variable numbers of surrounding macrophages, epithelioid cells and foreign body multinucleated cells. A dense fibrovascular granulation tissue infiltrated mainly by lymphocytes and plasma cells was seen between pyogranulomas. Within the pyogranuloma there were numerous cocoid Gram positive stained bacterial colonies imbedded in an amorphous eosinophilic material deeply eosinophilic, Splendore-Hoeppli phenomenon (Fig. 4). The histological diagnosis was focally extensive severe pyogranulama with intralesional Gram positive bacteria, compatible with botryomycosis.

Culture of the mass isolated coagulase positive *Staphylococcus aureus* and antimicrobial susceptibility testing confirmed *in vitro* sensitivity to amikacin, cephotaxime, ceftiofur, ceftriaxone, doxycycline, enrofloxacin, erythromycin, gentamicin, rifampin and trimethoprim-sulfamethoxazole.

Therapeutic plan and follow up

Following the first surgery the patient received systemic sodium penicillin (22,000 IU/kg bwt i.v. q. 6 h) and gentamicin (6.6 mg/kg bwt i.v. q. 24 h) for 4 days, which was changed to doxycycline[8] (10 mg/kg bwt *per os* q. 24 h) when the susceptibility testing was available. Initially, anti-inflammatory treatment consisted of phenylbutazone (4.4 mg/kg bwt i.v. q. 12 h) for 3 days, followed by

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suxibuzone[7] (3.3 mg/kg btw i.v. q. 12 h) for 8 days. Another dose of morphine (0.1 mg/kg bwt i.m.) was administered post-operatively.

Fluid therapy with isotonic solution, Ringer Lactate[2] (10 L), supplemented with calcium borogluconate[2] 23% (4 mEq/L) was administered overnight. Then enteral fluids with water supplemented with sodium chloride[9] (135 mEq/L), potassium chloride[9] (5 mEq/L) and honey, as a source of glucose, (15 ml/L) at a rate of 2 ml/kg/h for 7 days, was administered through a 5 mm diameter nasogastric tube left in place sutured to the alar fold.

The mouth was flushed with water after doxycycline administration and diluted clorhexidine[10] as an oral antiseptic. Ten milliliters of throat spray consisting of nitrofurazone[11] (100 g), dexamethasone[12] (6 ml, 2 mg/ml) and dimethyl sulfoxide[9] (33 ml) was administered twice a day. On day 4, the mare was gradually fed, which she tolerated well. During hospitalization, the mare gradually improved, nasal discharge and secretion from the tracheotomy site decreased. The tracheostomy tube was removed 3 days after the second surgery and a wound dressing gauze was applied over the defect. The tracheostomy, pharyngotomy, and submandibular incisions healed well by second intention.

On day 9 post-operatively, endoscopy revealed mild swelling of the soft palate and epiglottis. No adhesions were observed between the epiglottis and subepiglottic fold. The patient was discharged on day 10. Doxycycline was continued for 3 weeks as well as oral flushing with diluted clorhexidine after doxycycline administration. The mare was treated with suxibuzone and throat spray for 1 week. Pharyngotomy and tracheotomy incisions were cleaned as needed.

On re-examination 3 weeks later, incisions healed uneventfully and plasma fibrinogen concentration had decreased to 400 mg/dl. On upper airway endoscopy at that time the soft palate was still dorsally displaced. Intermittent movement of the tip of the epiglottis over the soft palate and lateralization was recognized. Food material was present in the nasal cavity and mucous secretions in the trachea. Doxycycline was continued for an additional 2 weeks.

On a recheck two months later, total protein and fibrinogen had increased to 9 g/dl and 500 mg/dl respectively. Surgical incisions and the tracheotomy site were not visible. On external palpation of

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the pharynx, a 5 cm mass was evident on the right side of the tongue. Dorsal displacement of the soft palate was still seen on endoscopy. Delineation of the mass by intraoral endoscopy was not possible, but mild mucosal ulcerations on the surface of the mass were still observed. Therapy with doxycycline was discontinued as no significant improvement was observed; trimethoprim-sulfamethoxazole[13] (30 mg/kg bwt *per os* q. 12 h) and potassium iodide[9] (30 mg/kg bwt *per os* q24 h) was initiated for one week followed by potassium iodide at 20 mg/kg *per os* q24h for 3 weeks. Iodides were chosen as these have been used for treatment of chronic or encapsulated bacterial infections. Potassium iodide was to be discontinued if epiphora or skin exfoliation were observed. The owners were informed this condition could progress and the aim after the surgical resection was to prevent regrowth of the remaining oropharyngeal mass. The mare was discharged with instructions to reexamine her in our hospital in 3-4 weeks, and phone call check-ups every two weeks.

Outcome

The initial response to this treatment was satisfactory; the mare did not lose weight and had a good quality of life. However, approximately 18 months after discharge from the hospital the mare was euthanized due to progressive weight loss, progressive dysphagia and secondary aspiration pneumonia. Postmortem examination could not be performed at the farm.

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Discussion

Botryomycosis is a pyogranulomatous inflammation associated with eosinophilic granules that have peripheral club formation, Splendor-Hoeppli phenomenon, and gram-positive cocci or gram-negative bacilli (Speirs *et al.* 1971). *S. aureus* is the most common isolated bacteria. Occasionally, other species such as *E. coli*, *Proteus, Streptococcus, Pseudomona* spp., *Bacteroides* and *Micrococcus* spp. have been implicated (Winslow 1959, Akiyama 1996, Bonifaz and Carrasco 1996). It has also been reported under different names such as staphylococcal actinophytosis, granular bacteriosis, actinobacillosis, bacterial pseudomycosis, bacterial granuloma and staphylococcal pseudomycetoma (Saadat *et al.* 2007, Scott 2007, Smiet *et al* 2012).

The occurrence of botryomycosis is rare in horses. It can be divided into cutaneous and visceral forms. (Winslow 1959, Smiet *et al* 2012). The most common presentation is as a staphylococcal wound infection (Miller *et al.* 2001) especially after castration (Knottenbelt 2009) and occasionally in tissues and skin of the mammary gland (Smiet *et al*. 2012). Diagnosis is made on the basis of clinical signs and by histologic characteristics. One of the histological characteristics in botryomycosis is the identification of the Splendor-Hoeppli (SH) phenomenon (Schlossberg *et al.* 1998, Snowden *et al.* 2003, Hussein 2008). It usually appears as strongly eosinophilic amorphous material with star-like or club-shaped configurations surrounding or adjacent to the causative agent. The exact nature of the Splendore-Hoeppli reaction is not well understood yet. It represents a localized immunological host reaction to antigens, in our case to *Staphylococcus aureus* (Saadat *et al.* 2007, Hussein 2008, Rath *et al.* 2012). The extensive fibrotic and granulomatous lesion that characterizes botryomycosis hinders diffusion of antimicrobials into the site of infection, and the Splendore-Hoeppli material probably prevents phagocytosis and intracellular killing of the insulting agent resulting in chronicity of the infection (Hussein 2008).

It is important to consider that sometimes the typical histological characteristics of botryomycosis may be not identified. Atypical presentations with uncharacteristic lesions resembling other conditions have been identified in humans. SH phenomenon was not identified in botryomycosis

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patients with concurrent acquired immunodeficiency disease (Brunken *et al.* 1983, Patterson *et al.* 1987, Coulibaly *et al* 2008). Therefore, culture plays a significant role in the definitive diagnosis and management of the patient.

Computed tomography and magnetic resonance imaging would have been key to define the extent of the lesion and involvement of surrounding structures. Both diagnostic imaging techniques were offered to the owner, but these were only available at a distant referral hospital and the owner refused to perform them.

In the differential diagnosis of an oro-pharyngeal mass the conditions that should be considered are fungal granulomas, bacterial abscess, foreign body penetration, amyloidosis and neoplasia. Most of the granulomas described in previous reports affecting the nasal passages, nasopharynx and oropharynx are associated with fungus (Hodgin and Conaway 1984). The conditions identified are phycomycosis (Miller and Campbell 1982, Zamos *et al.* 1996), cryptococcosis (Watt 1970, Carrig 1968 Roberts *et al.* 1981, Malik *et al.* 1997, Stewart *et al.* 2009, Cruz *et al.* 2009), and granulomas by *Pseudallescheria* spp. (Brearley *et al.* 1986), or *Cocciodes* spp. (Hodgin and Conaway 1984)

In a study of clinical observations of equine phycomycosis, the horses usually presented with a large granulomatous nasal mass, nasal discharge, and dyspnea due to mechanical blockage (Miller and Campbell 1982). In the case described in this report, the mass in the oropharynx was responsible for the loss of normal anatomy in the nasopharynx. In addition, the mass occluded the end of the oropharynx, which resulted in dysphagia, dyspnea and nasal discharge. Likewise, the case reports of cryptococcal granulomas in the nasal cavity with involvement of the nasopharynx presented with anorexia, fever, inspiratory and expiratory difficulties, dyspnea, and malodorous sanguineous or mucopurulent nasal discharge. On endoscopy, cryptococcal granulomas can be identified as pale yellow, mucous-covered masses (Watt 1970, Carrig 1968 Roberts *et al.* 1981, Malik *et al.* 1997, Stewart *et al.* 2009, Cruz *et al.* 2009). Considering the importance of climate factors, such as warmth and humidity (Roberts *et al.* 1981), Spain does not provide the ideal

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environment for development of these mycosis and we considered these very unlikely as a possible diagnosis in this mare.

Neoplasia of the larynx and pharynx in horses can include squamous cell carcinoma (Jones 1994), lymphosarcoma (Burba *et al.* 1991), fibroma (Madewell 1976, Cotchin 1977) and melanoma (Dorn and Priester 1976). Squamous cell carcinoma represents the most frequent neoplasm of the upper respiratory and gastrointestinal tracts (Jones 1994).

Botryomycosis has a slow progression. The pathogenesis of botryomycosis has been discussed and is not clearly understood. Host factors such as altered immune factors, debilitating illnesses, or concurrent infections are prerequisites to the development of the disease (Brunken *et al.* 1983). Botryomycosis has been reported in patients with lowered resistance, immune deficiency or immunosuppression. In the case described, the mare was healthy but aged. For cryptococcosis, damaged tissue that predisposes to infection has been suggested as a contributing factor (Roberts *et al.* 1981, Cruz *et al.* 2009). For example, passage of a nasogastric tube or floating teeth could allow the development of a scaffold for the organism. Because of the development of the mass in the oropharynx, we hypothesize that an insult in the area could have been the beginning of the process. However, this case had no history of lingual and oro-pharyngeal trauma or associated periodontal disease, therefore the source of infection could not be established.

Treatment of botryomycosis includes surgical excision combined with systemic antimicrobial therapy. Effective treatment of botryomycosis depends on the nature of the inciting organism, the site of lesion, and the horse's immune status (Saadat *et al.* 2007). In the case described, because of the poor accessibility of the mass, its hard and fibrotic consistency and the infiltrative nature of its attachment to underlying tissues the resection was not completely achieved. Endoscopically-guided diode laser excision combined with an oral approach may have resulted in a higher degree of success. Surgical approach to lesions at the base of the tongue is somewhat limited unless aggressive midline submandibular approaches are used. Intraoral approaches provide limited exposure to subepiglotic and aboral tongue structures. Similarly, a ventral midline pharyngotomy

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only provides limited access to structures below the soft palate and epiglottis. In addition the mass was located far too orally in order to be accessed by a laringotomy approach.

Antimicrobial therapy is impaired in cases of botryomycosis by the Splendore-Hoeppli reaction that appears to prevent achieving bactericidal antibiotic concentrations inside the granuloma. It may also prevent phagocytosis and intracellular killing of the bacteria and thus influence treatment (Saadat *et al.* 2007, Smiet *et al* 2012). In the case described, the choice of antibiotic therapy was based on culture and susceptibility results.

Potassium iodide has been the treatment of choice for Sporotrichosis (Rosser *et al.* 1981, Werner and Werner 1994) and is commonly used in treatment of fungal infections in horses (Zamos *et al*. 1996, Davis *et al*. 2000, Schwarz *et al*. 2009, Crothers *et al*. 2009). Review of the existing literature revealed that potassium iodide (KI) has not been reported previously as a therapeutic option for botryomycosis. We introduced it following the recommendation of a veterinary colleague (Knottenbelt 2013). Iodides have traditionally been used in selected cases for treatment of chronic or well encapsulated bacterial or fungal infections. Iodides are anti-inflammatory agents by virtue of their ability to quench toxic oxygen metabolites and inhibit neutrophil chemotaxis.(Sterling and Heyman 2000). Side effects during treatment may include epiphora, ocular discharge, cough, and dry seborrhea of the skin and coat (Davis *et al.* 2000, Knottenbelt 2002). No adverse effects were noted in the case described here.

Research into *S. aureus* abscess formation and persistence in host tissue has demonstrated that different surface proteins involved in disease pathogenesis may be useful for vaccine development in the future and provide a new line of treatment (Cheng *et al.* 2009).

We have described the first report of chronic botryomycosis in equine presented as an oropharyngeal mass, which is comparable with similar masses in other parts of the body. The slow onset of clinical signs and difficulty in visualization and/or recognizing this abnormality may allow significant enlargement and extensive tissue invasion to occur prior to presentation for examination and treatment. In conclusion, oropharyngeal botryomycosis should be distinguished from other

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271 conditions in the horse, which are characterized, by mucopurulent nasal discharge, airway
272 obstruction, respiratory noise, dyspnea, and dysphagia.

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Authors' declaration of interest

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Figure Legends

Fig 1: Ultrasonographic examination of the oropharyngeal mass to the right of midline. A well-encapsulated mass with puntiform hyperechoic structures and mineral opacities with interspersed hypoechoic areas is observed. The mass was approximately 2 × 2.6 cm.

Fig 2: Endoscopy of the nasopharynx; dorsal displacement of the soft palate is observed **(A)** due to swelling underneath the soft palate that obliterates the view of the larynx. Endoscopy of the oropharynx, an ulcerated subepiglotic mass is identified partially obscuring the epiglottis **(B)**.

Fig 3: Endoscopy of the nasopharynx; reminiscence of the mass attached to the aryepiglottic fold (black arrows) obstructing the entrance to the trachea and persistent dorsal displacement of the soft palate. Nasogastric tube for enteral fluid therapy (white arrows) (A). A modified esophageal grasping forceps with a horizontal jaw was used to grip the mass (B).

Fig 4: Staphylococcal pyogranuloma The pyogranuloma is constituted by a deeply eosinophilic material (Splendore-Hoeppli phenomenon) and numerous cocoid bacterial colonies surrounded by collections of degenerate neutrophils with variable numbers of surrounding macrophages and epithelioid cells. HE staining **(A)**. In a serial section it can be observed the Gram positive bacterial colonies showing a deep blue staining. Gram stain **(B)**.

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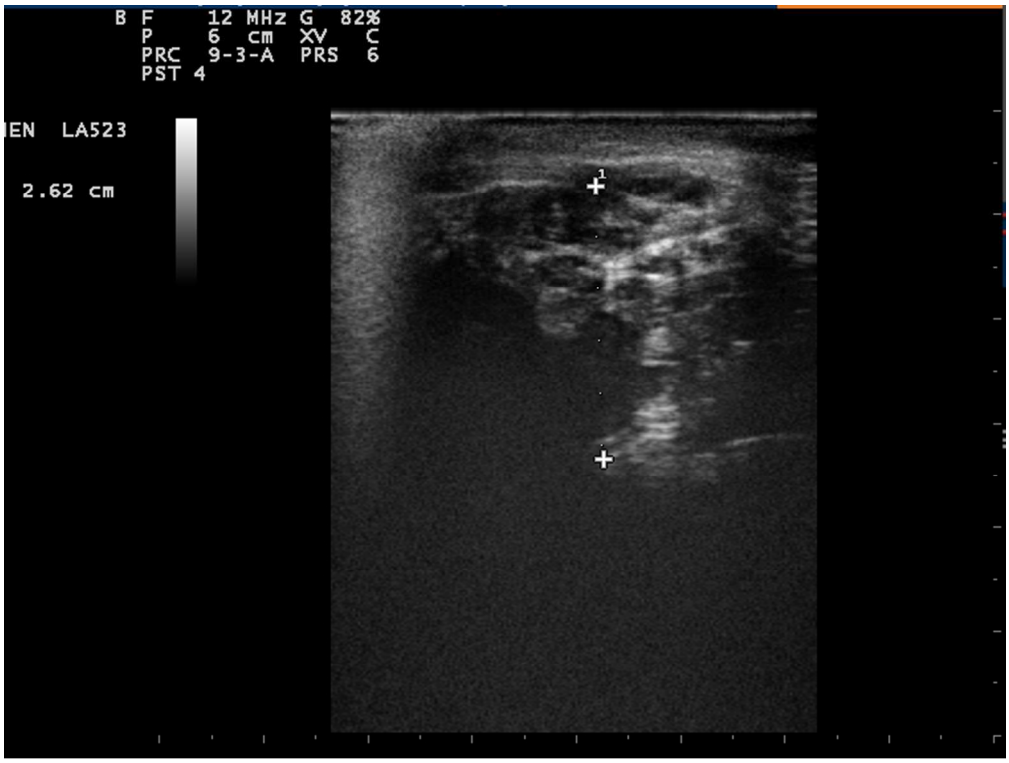

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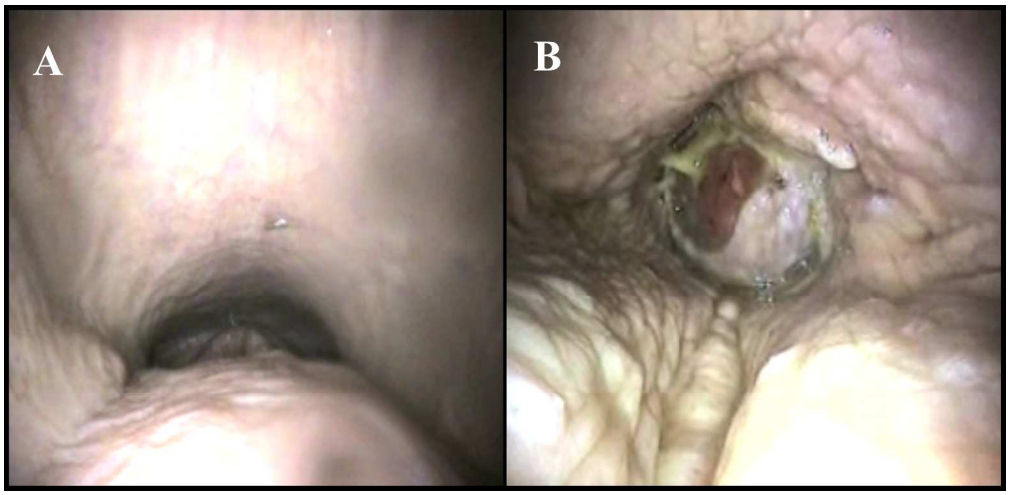

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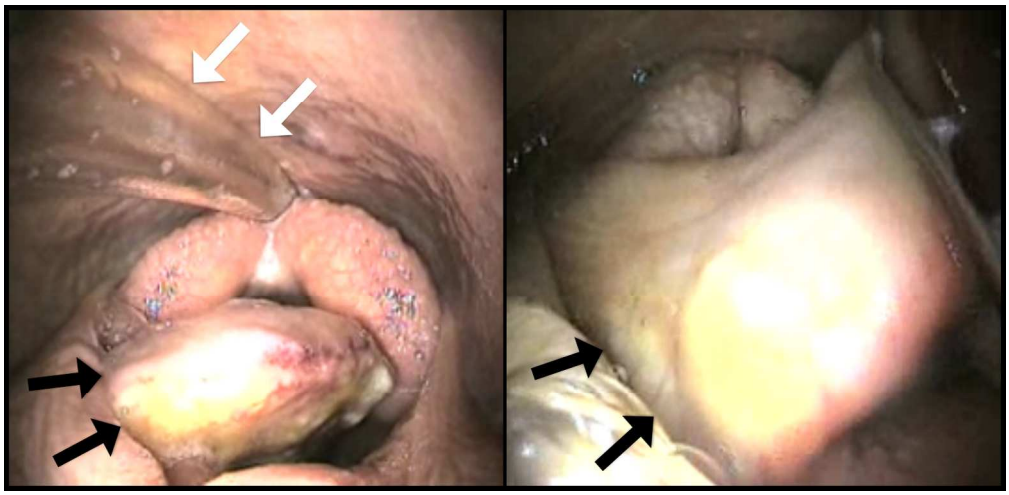

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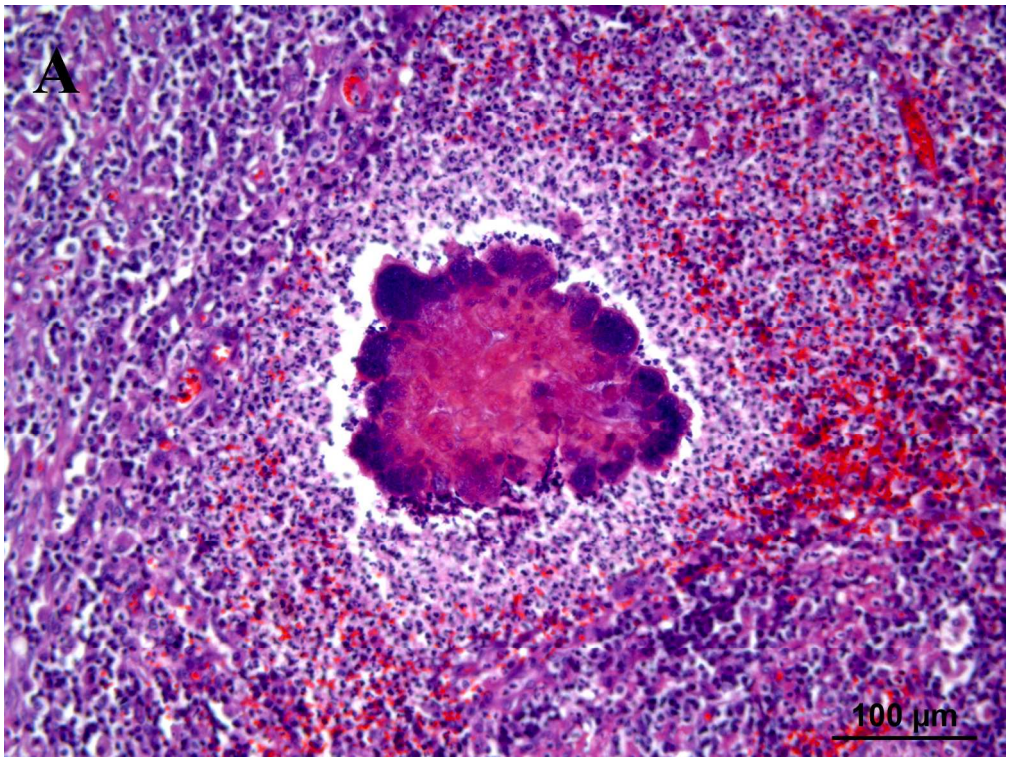

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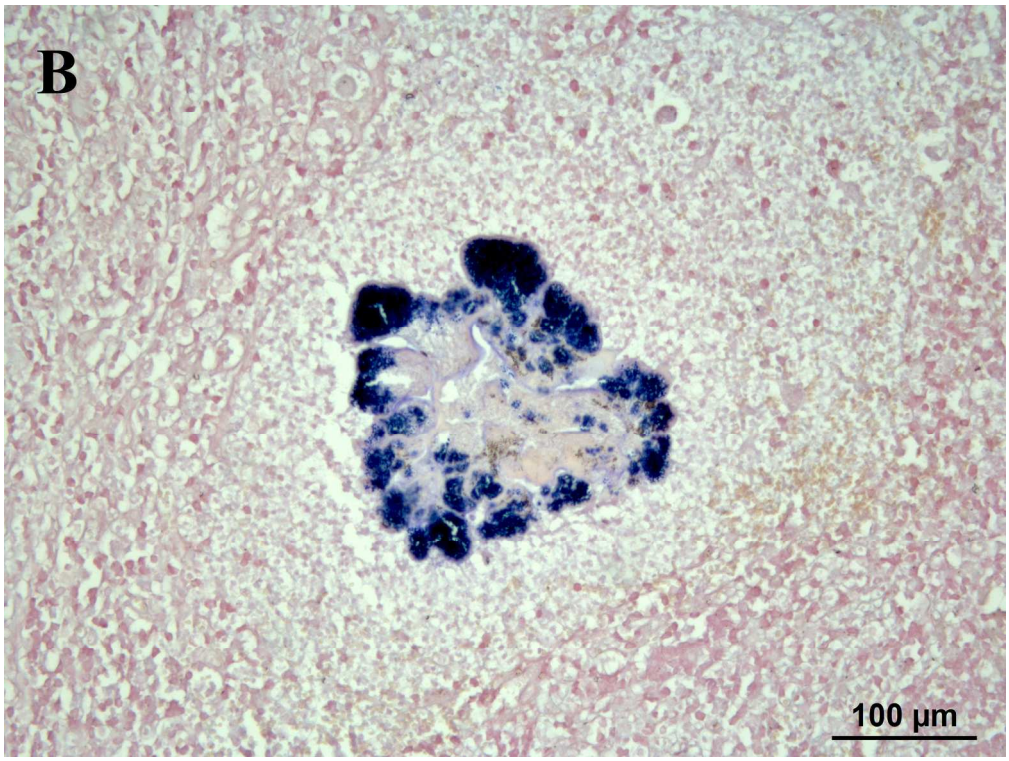

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