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Manuscripts

1   **Case Report.**

2   **Oropharyngeal Botryomycosis in a Geriatric Mare.**

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21   **Summary**

22   Botryomycosis is an uncommon chronic bacterial infection that can have cutaneous and visceral

23   involvement. This report describes an 18-year-old mixed-breed mare presented with dysphagia,

24   dyspnea, and an upper respiratory noise that developed secondary to oropharyngeal botryomycosis.

25   Histological examination of the mass showed a granulomatous formation with Splendore-Hoeppli

26   phenomenon surrounding gram-positive bacteria. This report describes clinical signs, approach and

27   management of an oropharyngeal *Staphylococcus aureus* granuloma in a geriatric mare.

28   **Introduction**

29   Cutaneous and visceral botryomycosis has been described in equids, ruminants, companion

30   animals, guinea pigs, elephant and humans for over a century. (Bollinger 1870, Bostrom *et al* 1969,

31   Sheikh-Omar and Abdullah 1985, Bonifaz and Carrasco 1996, Pandey *et al* 1997, Margaret *et al*

32   2001, Miller *et al* 2001, Sleeman, J *et al* 2003, Scott 2007). Botryomycosis is an uncommon

33   condition, characterized by formation of a slow growing chronic granuloma caused by non-

34   filamentous aerobic and anaerobic bacteria such as *Staphylococcus aureus*, *Pseudomona* spp. and

35   *Bacteroides*. *S. aureus* is the most common agent encountered.

36   Diagnosis is based on histological identification of granulomatous inflammation and isolation of the

37   microorganism. In the case described herein, a subepiglotic oropharyngeal granuloma caused by *S.*

38   *aureus* was diagnosed based on the characteristic histologic findings and positive culture.

39   Histologically, botryomycosis is characterized by the presence of radiating, star-like asteroid or

40   club-shaped eosinophilic material, Splendor-Hoeppli (SH) reaction around the infectious agent;

41   which represents a localized immunological host reaction to antigens (Hussein 2008). Fungal

42   granulomas, bacterial abscessation, foreign body, amyloidosis, and neoplasia should be included in

43   its differential diagnosis. (Miller and Campbell 1982, Shaw *et al.* 1987, Kiper *et al.* 1992, Jones

44   1994)

45   This report describes the clinical manifestations of a granuloma caused by *S. aureus* in the

46   oropharynx of a geriatric mare outlining a diagnostic and surgical approach as well as management

47   of the case. To the authors' knowledge this is the first case of botryomycosis in the oropharynx

48   described in equine.

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49   **Case history**

50   An 18-year-old mixed breed mare weighing approximately 450 kg was presented to *Fundació*

51   *Hospital Clínic Veterinari* of the *Universitat Autònoma de Barcelona*, with a 3-year history of a

52   mass located in the submandibular area and a second mass in the pharyngeal region. Over the

53   month prior to admission, the mare was occasionally pyrexic and presented intermittent dysphagia.

54   The mare also had episodes of dyspnea, upper respiratory noise, and bilateral mucoid nasal

55   discharge, especially when exercised.

56

57   **Clinical findings**

58   On presentation, the mare was quiet, alert and responsive. Physical exam was within normal limits.

59   Bilateral mucoid nasal discharge was evident. Two firm, round shaped and smooth masses were

60   palpable in the submandibular and pharyngeal areas to the right side of midline, 5 and 3 cm in

61   diameter respectively. The skin overlying the masses was normal and there was no evidence of pain

62   or pruritus.

63   Ultrasonography of the submandibular mass revealed soft tissue density with multiple hypoechoic

64   compartments. The pharyngeal mass was encapsulated with the presence of punctiform hyperechoic

65   structures and mineral opacities as well as hypoechoic areas similar to the other mass. (Fig 1) No

66   communication between the two masses was identified. An ultrasound guided fine-needle aspirate

67   was obtained under local anesthesia with mepivacaine from both masses. Cytology revealed mixed

68   neutrophilic and mononuclear inflammatory cells with presence of gram-positive cocci and

69   bacterial culture was negative.

70   Skull radiography revealed a well circumscribed, round soft tissue radio-opacity in the oropharynx

71   over the base of the tongue that obscured the epiglottis and hyoid apparatus over which it was

72   superimposed.

73   Endoscopy of the upper respiratory tract (URT) revealed the soft palate protruding dorsally into the

74   nasopharynx as though being pushed upwards. The soft palate was displaced dorsal to the epiglottis

75   throughout the endoscopic examination. Oral endoscopy identified a 3 cm diameter, ulcerated pink

76   mass at the base of the tongue slightly offset to the right of midline that occluded the end of the

77   oropharynx **(Fig.2)**. This mass was attached to the base of the tongue and ventral aspect of the

78   epiglottis. This finding explained the dysphagia and dyspnea.

79   **Treatment**

80   Surgical resection under general anesthesia was performed in two different procedures. Pre-

81   operatively, total protein and fibrinogen were elevated at 9.8 g/dl and 800 mg/dl respectively.

82   Rebreathing exam and thoracic ultrasonography were normal. Complete blood count, serum

83   biochemistry and electrocardiogram were within normal limits.

84   The patient received pre-operative sodium penicillin[1] (22.000 IU/kg bwt i.v.), gentamicin[2] (6.6

85   mg/kg bwt i.v.), phenylbutazone[3] (4.4 mg/kg bwt i.v.) and morphine[2] (0.1 mg/kg bwt i.m.).

86   The mare was sedated with romifidine[4] (0.06 mg/kg bwt i.v.) and butorphanol[3] (0.03 mg/kg bwt

87   i.v.), induced with ketamine[5] (2 mg/kg bwt i.v.) and diazepam[6] (0.05 mg/kg bwt i.v.), placed in

88   dorsal recumbency and maintained with isoflurane[7] in 100% oxygen through a mid cervical

89   tracheotomy performed prior to induction.

90   Initial intraoral examination under general anesthesia revealed a fibrous mass, approximately 1.5 x

91   5 x 3 cm, from the base of the tongue to the oropharynx and slightly offset to the right of midline,

92   involving the mucosa of the oropharynx.

93   The ventral aspect of the head and neck were clipped and prepared aseptically. Surgery was

94   performed through a submandibular approach and continued by pharyngotomy as well as intraoral

95   manipulation. The submandibular mass extended to the oropharynx involving contiguous structures

96   such as the hyoid apparatus. Because of the location of the mass, surgical excision was unsuccessful

97   in achieving clean margins. The submandibular area was sutured in four layers: mucosa, muscle,

98   subcutaneous tissue and skin; and the pharyngotomy was left to heal by second intention. The

99   oropharyngeal mass excised surgically was fixed in 10% formalin and processed routinely for

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

101   sections.

102   Two days later, under intravenous anesthesia with xylazine, ketamine, and guafenesin, the second

103   surgery was performed in sternal recumbency. Through an intraoral approach, the remaining part of

104   the mass adhered to the right aryepiglottic fold was removed (**Fig.3**). Laparoscopic instruments

105   were used for the procedure guided by nasal endoscopy. Additionally, an adhesion between the

106   subepiglottic fold and epiglottis was excised.

107   **Diagnosis**

108   The different sections of the samples evaluated showed a similar histopaholagic image: the normal

109   architecture of the tissue was extensively obliterated by irregularly sized pyogranulomas constituted

110   by collections of degenerate neutrophils with variable numbers of surrounding macrophages,

111   epithelioid cells and foreign body multinucleated cells. A dense fibrovascular granulation tissue

112   infiltrated mainly by lymphocytes and plasma cells was seen between pyogranulomas. Within the

113   pyogranuloma there were numerous cocoid Gram positive stained bacterial colonies imbedded in an

114   amorphous eosinophilic material deeply eosinophilic, Splendore-Hoeppli phenomenon (Fig. 4). The

115   histological diagnosis was focally extensive severe pyogranulama with intralesional Gram positive

116   bacteria, compatible with botryomycosis.

117   Culture of the mass isolated coagulase positive *Staphylococcus aureus* and antimicrobial

118   susceptibility testing confirmed *in vitro* sensitivity to amikacin, cephotaxime, ceftiofur, ceftriaxone,

119   doxycycline, enrofloxacin, erythromycin, gentamicin, rifampin and trimethoprim-sulfamethoxazole.

120   **Therapeutic plan and follow up**

121   Following the first surgery the patient received systemic sodium penicillin (22,000 IU/kg bwt i.v. q.

122   6 h) and gentamicin (6.6 mg/kg bwt i.v. q. 24 h) for 4 days, which was changed to doxycycline[8] (10

123   mg/kg bwt *per os* q. 24 h) when the susceptibility testing was available. Initially, anti-inflammatory

124   treatment consisted of phenylbutazone (4.4 mg/kg bwt i.v. q. 12 h) for 3 days, followed by

125   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.)

126   was administered post-operatively.

127   Fluid therapy with isotonic solution, Ringer Lactate[2] (10 L), supplemented with calcium

128   borogluconate[2] 23% (4 mEq/L) was administered overnight. Then enteral fluids with water

129   supplemented with sodium chloride[9] (135 mEq/L), potassium chloride[9] (5 mEq/L) and honey, as a

130   source of glucose, (15 ml/L) at a rate of 2 ml/kg/h for 7 days, was administered through a 5 mm

131   diameter nasogastric tube left in place sutured to the alar fold.

132   The mouth was flushed with water after doxycycline administration and diluted clorhexidine[10] as an

133   oral antiseptic. Ten milliliters of throat spray consisting of nitrofurazone[11] (100 g), dexamethasone[12]

134   (6 ml, 2 mg/ml) and dimethyl sulfoxide[9] (33 ml) was administered twice a day. On day 4, the mare

135   was gradually fed, which she tolerated well. During hospitalization, the mare gradually improved,

136   nasal discharge and secretion from the tracheotomy site decreased. The tracheostomy tube was

137   removed 3 days after the second surgery and a wound dressing gauze was applied over the defect.

138   The tracheostomy, pharyngotomy, and submandibular incisions healed well by second intention.

139   On day 9 post-operatively, endoscopy revealed mild swelling of the soft palate and epiglottis. No

140   adhesions were observed between the epiglottis and subepiglottic fold. The patient was discharged

141   on day 10. Doxycycline was continued for 3 weeks as well as oral flushing with diluted

142   clorhexidine after doxycycline administration. The mare was treated with suxibuzone and throat

143   spray for 1 week. Pharyngotomy and tracheotomy incisions were cleaned as needed.

144   On re-examination 3 weeks later, incisions healed uneventfully and plasma fibrinogen

145   concentration had decreased to 400 mg/dl. On upper airway endoscopy at that time the soft palate

146   was still dorsally displaced. Intermittent movement of the tip of the epiglottis over the soft palate

147   and lateralization was recognized. Food material was present in the nasal cavity and mucous

148   secretions in the trachea. Doxycycline was continued for an additional 2 weeks.

149   On a recheck two months later, total protein and fibrinogen had increased to 9 g/dl and 500 mg/dl

150   respectively. Surgical incisions and the tracheotomy site were not visible. On external palpation of

151   the pharynx, a 5 cm mass was evident on the right side of the tongue. Dorsal displacement of the

152   soft palate was still seen on endoscopy. Delineation of the mass by intraoral endoscopy was not

153   possible, but mild mucosal ulcerations on the surface of the mass were still observed. Therapy with

154   doxycycline was discontinued as no significant improvement was observed; trimethoprim-

155   sulfamethoxazole[13] (30 mg/kg bwt *per os* q. 12 h) and potassium iodide[9] (30 mg/kg bwt *per os* q24

156   h) was initiated for one week followed by potassium iodide at 20 mg/kg *per os* q24h for 3 weeks.

157   Iodides were chosen as these have been used for treatment of chronic or encapsulated bacterial

158   infections. Potassium iodide was to be discontinued if epiphora or skin exfoliation were observed.

159   The owners were informed this condition could progress and the aim after the surgical resection

160   was to prevent regrowth of the remaining oropharyngeal mass. The mare was discharged with

161   instructions to reexamine her in our hospital in 3-4 weeks, and phone call check-ups every two

162   weeks.

163   **Outcome**

164   The initial response to this treatment was satisfactory; the mare did not lose weight and had a good

165   quality of life. However, approximately 18 months after discharge from the hospital the mare was

166   euthanized due to progressive weight loss, progressive dysphagia and secondary aspiration

167   pneumonia. Postmortem examination could not be performed at the farm.

168   **Discussion**

169   Botryomycosis is a pyogranulomatous inflammation associated with eosinophilic granules that have

170   peripheral club formation, Splendor-Hoeppli phenomenon, and gram-positive cocci or gram-

171   negative bacilli (Speirs *et al.* 1971). *S. aureus* is the most common isolated bacteria. Occasionally,

172   other species such as *E. coli*, *Proteus, Streptococcus, Pseudomona* spp., *Bacteroides* and

173   *Micrococcus* spp. have been implicated (Winslow 1959, Akiyama 1996, Bonifaz and Carrasco

174   1996). It has also been reported under different names such as staphylococcal actinophytosis,

175   granular bacteriosis, actinobacillosis, bacterial pseudomycosis, bacterial granuloma and

176   staphylococcal pseudomycetoma (Saadat *et al.* 2007, Scott 2007, Smiet *et al* 2012).

177   The occurrence of botryomycosis is rare in horses. It can be divided into cutaneous and visceral

178   forms. (Winslow 1959, Smiet *et al* 2012). The most common presentation is as a staphylococcal

179   wound infection (Miller *et al.* 2001) especially after castration (Knottenbelt 2009) and occasionally

180   in tissues and skin of the mammary gland (Smiet *et al*. 2012). Diagnosis is made on the basis of

181   clinical signs and by histologic characteristics. One of the histological characteristics in

182   botryomycosis is the identification of the Splendor-Hoeppli (SH) phenomenon (Schlossberg *et al.*

183   1998, Snowden *et al.* 2003, Hussein 2008). It usually appears as strongly eosinophilic amorphous

184   material with star-like or club-shaped configurations surrounding or adjacent to the causative agent.

185   The exact nature of the Splendore-Hoeppli reaction is not well understood yet. It represents a

186   localized immunological host reaction to antigens, in our case to *Staphylococcus aureus* (Saadat *et*

187   *al.* 2007, Hussein 2008, Rath *et al.* 2012). The extensive fibrotic and granulomatous lesion that

188   characterizes botryomycosis hinders diffusion of antimicrobials into the site of infection, and the

189   Splendore-Hoeppli material probably prevents phagocytosis and intracellular killing of the insulting

190   agent resulting in chronicity of the infection (Hussein 2008).

191   It is important to consider that sometimes the typical histological characteristics of botryomycosis

192   may be not identified. Atypical presentations with uncharacteristic lesions resembling other

193   conditions have been identified in humans. SH phenomenon was not identified in botryomycosis

194   patients with concurrent acquired immunodeficiency disease (Brunken *et al.* 1983, Patterson *et al.*

195   1987, Coulibaly *et al* 2008). Therefore, culture plays a significant role in the definitive diagnosis

196   and management of the patient.

197   Computed tomography and magnetic resonance imaging would have been key to define the extent

198   of the lesion and involvement of surrounding structures. Both diagnostic imaging techniques were

199   offered to the owner, but these were only available at a distant referral hospital and the owner

200   refused to perform them.

201   In the differential diagnosis of an oro-pharyngeal mass the conditions that should be considered are

202   fungal granulomas, bacterial abscess, foreign body penetration, amyloidosis and neoplasia. Most of

203   the granulomas described in previous reports affecting the nasal passages, nasopharynx and

204   oropharynx are associated with fungus (Hodgin and Conaway 1984). The conditions identified are

205   phycomycosis (Miller and Campbell 1982, Zamos *et al.* 1996), cryptococcosis (Watt 1970, Carrig

206   1968 Roberts *et al.* 1981, Malik *et al.* 1997, Stewart *et al.* 2009, Cruz *et al.* 2009), and granulomas

207   by *Pseudallescheria* spp. (Brearley *et al.* 1986), or *Cocciodes* spp. (Hodgin and Conaway 1984)

208   In a study of clinical observations of equine phycomycosis, the horses usually presented with a

209   large granulomatous nasal mass, nasal discharge, and dyspnea due to mechanical blockage (Miller

210   and Campbell 1982). In the case described in this report, the mass in the oropharynx was

211   responsible for the loss of normal anatomy in the nasopharynx. In addition, the mass occluded the

212   end of the oropharynx, which resulted in dysphagia, dyspnea and nasal discharge. Likewise, the

213   case reports of cryptococcal granulomas in the nasal cavity with involvement of the nasopharynx

214   presented with anorexia, fever, inspiratory and expiratory difficulties, dyspnea, and malodorous

215   sanguineous or mucopurulent nasal discharge. On endoscopy, cryptococcal granulomas can be

216   identified as pale yellow, mucous-covered masses (Watt 1970, Carrig 1968 Roberts *et al.* 1981,

217   Malik *et al.* 1997, Stewart *et al.* 2009, Cruz *et al.* 2009). Considering the importance of climate

218   factors, such as warmth and humidity (Roberts *et al.* 1981), Spain does not provide the ideal

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219   environment for development of these mycosis and we considered these very unlikely as a possible

220   diagnosis in this mare.

221   Neoplasia of the larynx and pharynx in horses can include squamous cell carcinoma (Jones 1994),

222   lymphosarcoma (Burba *et al.* 1991), fibroma (Madewell 1976, Cotchin 1977) and melanoma (Dorn

223   and Priester 1976). Squamous cell carcinoma represents the most frequent neoplasm of the upper

224   respiratory and gastrointestinal tracts (Jones 1994).

225   Botryomycosis has a slow progression. The pathogenesis of botryomycosis has been discussed and

226   is not clearly understood. Host factors such as altered immune factors, debilitating illnesses, or

227   concurrent infections are prerequisites to the development of the disease (Brunken *et al.* 1983).

228   Botryomycosis has been reported in patients with lowered resistance, immune deficiency or

229   immunosuppression. In the case described, the mare was healthy but aged. For cryptococcosis,

230   damaged tissue that predisposes to infection has been suggested as a contributing factor (Roberts *et*

231   *al.* 1981, Cruz *et al.* 2009). For example, passage of a nasogastric tube or floating teeth could allow

232   the development of a scaffold for the organism. Because of the development of the mass in the

233   oropharynx, we hypothesize that an insult in the area could have been the beginning of the process.

234   However, this case had no history of lingual and oro-pharyngeal trauma or associated periodontal

235   disease, therefore the source of infection could not be established.

236   Treatment of botryomycosis includes surgical excision combined with systemic antimicrobial

237   therapy. Effective treatment of botryomycosis depends on the nature of the inciting organism, the

238   site of lesion, and the horse's immune status (Saadat *et al.* 2007). In the case described, because of

239   the poor accessibility of the mass, its hard and fibrotic consistency and the infiltrative nature of its

240   attachment to underlying tissues the resection was not completely achieved. Endoscopically-guided

241   diode laser excision combined with an oral approach may have resulted in a higher degree of

242   success. Surgical approach to lesions at the base of the tongue is somewhat limited unless

243   aggressive midline submandibular approaches are used. Intraoral approaches provide limited

244   exposure to subepiglotic and aboral tongue structures. Similarly, a ventral midline pharyngotomy

245 only provides limited access to structures below the soft palate and epiglottis. In addition the mass

246 was located far too orally in order to be accessed by a laringotomy approach.

247 Antimicrobial therapy is impaired in cases of botryomycosis by the Splendore-Hoeppli reaction that

248 appears to prevent achieving bactericidal antibiotic concentrations inside the granuloma. It may also

249 prevent phagocytosis and intracellular killing of the bacteria and thus influence treatment (Saadat *et*

250 *al.* 2007, Smiet *et al* 2012). In the case described, the choice of antibiotic therapy was based on

251 culture and susceptibility results.

252 Potassium iodide has been the treatment of choice for Sporotrichosis (Rosser *et al.* 1981, Werner

253 and Werner 1994) and is commonly used in treatment of fungal infections in horses (Zamos *et al.*

254 1996, Davis *et al.* 2000, Schwarz *et al.* 2009, Crothers *et al.* 2009). Review of the existing literature

255 revealed that potassium iodide (KI) has not been reported previously as a therapeutic option for

256 botryomycosis. We introduced it following the recommendation of a veterinary colleague

257 (Knottenbelt 2013). Iodides have traditionally been used in selected cases for treatment of chronic

258 or well encapsulated bacterial or fungal infections. Iodides are anti-inflammatory agents by virtue

259 of their ability to quench toxic oxygen metabolites and inhibit neutrophil chemotaxis.(Sterling and

260 Heyman 2000). Side effects during treatment may include epiphora, ocular discharge, cough, and

261 dry seborrhea of the skin and coat (Davis *et al.* 2000, Knottenbelt 2002). No adverse effects were

262 noted in the case described here.

263 Research into *S. aureus* abscess formation and persistence in host tissue has demonstrated that

264 different surface proteins involved in disease pathogenesis may be useful for vaccine development

265 in the future and provide a new line of treatment (Cheng *et al.* 2009).

266 We have described the first report of chronic botryomycosis in equine presented as an

267 oropharyngeal mass, which is comparable with similar masses in other parts of the body. The slow

268 onset of clinical signs and difficulty in visualization and/or recognizing this abnormality may allow

269 significant enlargement and extensive tissue invasion to occur prior to presentation for examination

270 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.

278   **Manufacturers' addresses**   15

279   [1]Laboratorios ERN S.A., Barcelona, Spain.

280   [2]B. Braun Vet Care, Rubi, Barcelona, Spain.

281   [3]Zoetis, Alcobendas, Madrid, Spain.

282   [4]Boehringer Ingelheim, Sant Cugat del Vallès, Barcelona, Spain.

283   [5]Merial Laboratorios, Barcelona, Spain.

284   [6]Roche, Sant Cugat del Vallès, Barcelona, Spain.

285   [7]Laboratorios Dr. Esteve, Martorelles, Barcelona, Spain.

286   [8]Laboratorios Syva S.A.U., León, Spain.

287   [9]Fragon Iberica S.A.U., Terrasa, Barcelona, Spain.

288   [10]AGB, Ajalvir, Madrid, Spain.

289   [11]Laboratorios Seid, Lliça de Vall, Barcelona, Spain.

290   [12]Schering-Plough Animal Health, San Agustín de Guadalix, Madrid, Spain.

291   [13]Fatro Ibérica, Sant Just Desvern, Barcelona, Spain.

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396

397 **Figure Legends**

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

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

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

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

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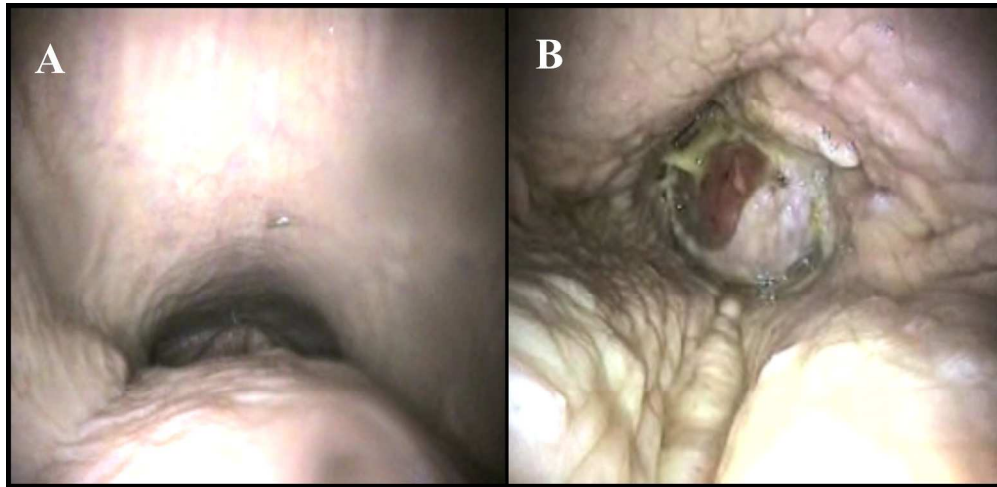
266x200mm (72 x 72 DPI)

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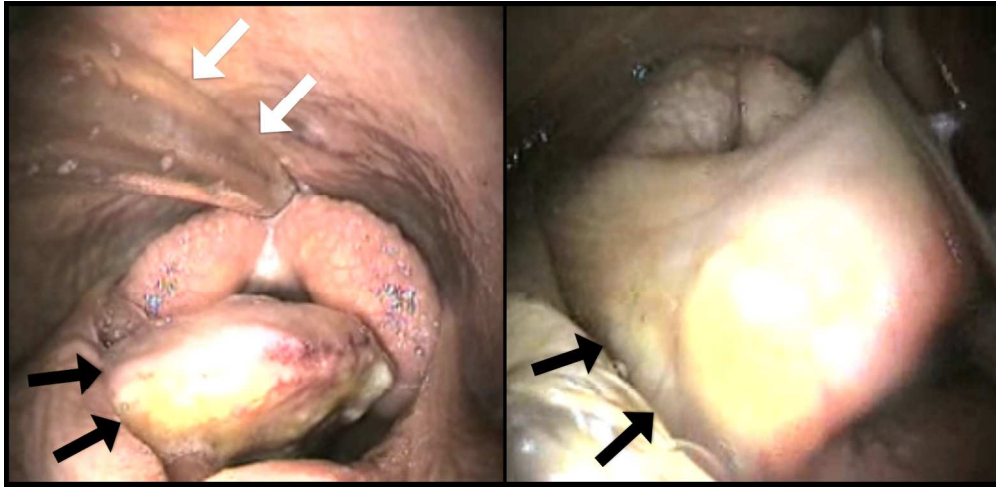
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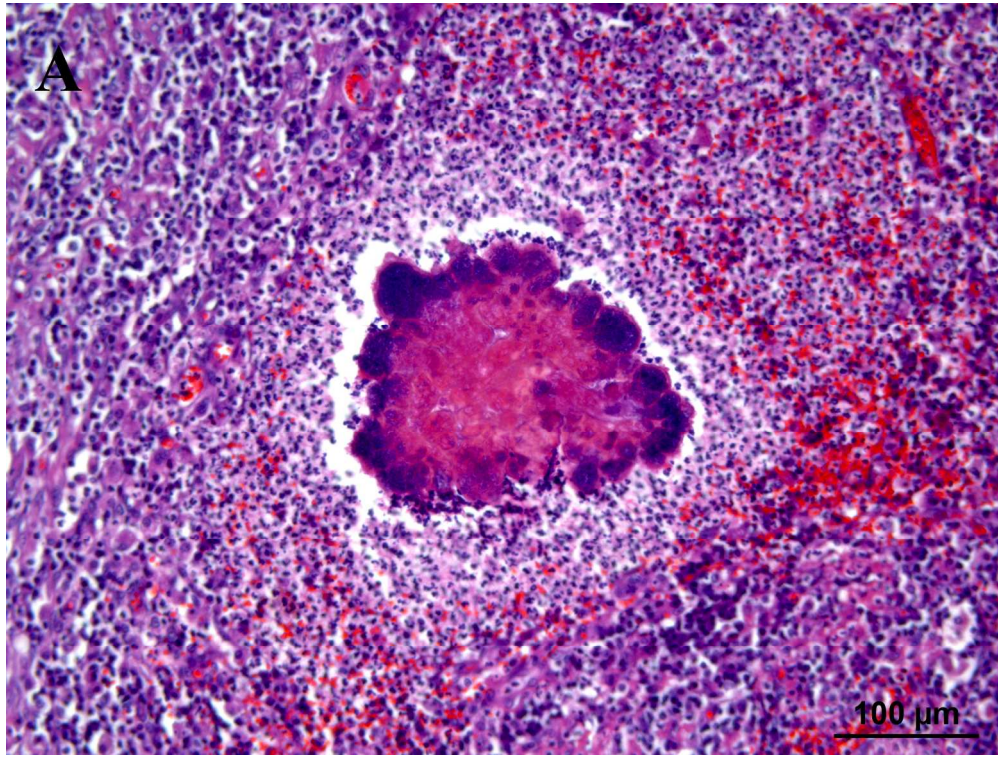
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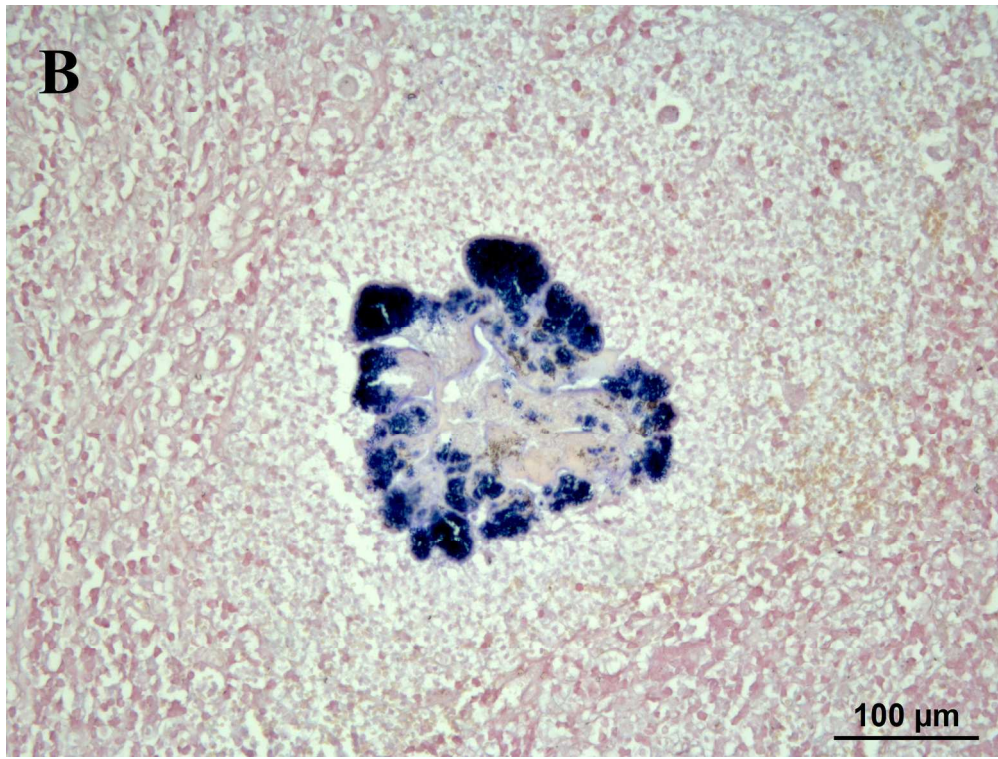
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