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Millena de Oliveira Firmino

Patologias micóticas e neoplásicas da cavidade nasal de
pequenos ruminantes e equino

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Millena de Oliveira Firmino

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pequenos ruminantes e equino

Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Doutora em Ciência e Saúde Animal.

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MILLENA DE OLIVEIRA FIRMINO

PATOLOGIAS MICÓTICAS E NEOPLÁSICAS DA CAVIDADE NASAL DE PEQUENOS RUMINANTES E EQUINO

Tese apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutor em Ciência e Saúde Animal.

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Dedico...

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"Os rios não bebem sua própria água; as árvores não comem seus próprios frutos.
O sol não brilha para si mesmo; e as flores não espalham sua fragrância para si.
Viver para os outros é uma regra da natureza (...).
A vida é boa quando você está feliz;
Mas a vida é muito melhor quando os outros estão felizes por sua causa".

Papa Francisco

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RESUMO

Esta tese foi constituída por quatro capítulos que resultaram em quatro artigos científicos relacionados a doenças da cavidade nasal em pequenos ruminantes e equino diagnosticados no Laboratório de Patologia Animal, do Hospital Veterinário Universitário Prof. Dr. Ivon Macedo Tabosa, da Universidade Federal de Campina Grande, Patos, Paraíba. No primeiro capítulo descreve-se os achados epidemiológicos, clínicos e anatomopatológicos de meningoencefalite secundária a rinite por *Pythium insidiosum* em ovino que apresentava discreta dispneia e sinais neurológicos. Na cavidade nasal havia massa vermelho-enebecida, irregular e friável que comprometia bilateralmente o septo nasal e porção rostral das conchas nasais. No encéfalo observou-se espessamento difuso das leptomeninges do cerebelo e porção ventral do tronco encefálico por material granular amarelado associado à hiperemia dos vasos. No assoalho do quarto ventrículo havia deposição de material amarelado, irregular e levemente granular que se projetava em direção ao óbex e deslocava dorso-lateralmente o cerebelo. Microscopicamente verificou-se rinite piogranulomatosa, eosinofílica e necrosante, e meningoencefalite fibrinossupurativa, eosinofílica e necrosante, ambas associadas a trombos, vasculite e imagens negativas de hifas intralesionais. As hifas foram impregnadas pela prata e imunopositivas para o anticorpo anti-*Pythium insidiosum*. O segundo capítulo é referente as características clínico-patológicas da rinite por *Exserohilum rostratum* em caprino no qual apresentava anorexia, dispneia e deformidade da face há dois meses. Na cavidade nasal havia área focalmente extensa amarelo-acastanhada, irregular, ulcerada e friável que drenava secreção purulenta afetando o vestíbulo nasal até a porção caudal da concha dorsal. A lesão foi caracterizada como rinite e osteomielite piogranulomatosa e necrosante, associada a estruturas fúngicas. Morfologicamente, as hifas caracterizavam-se por paredes delgadas, levemente tortuosas e raramente, múltiplas cadeias de conídios individuais ou em grupos e clamiconídeos pigmentados. Foram positivas nas colorações histoquímicas especiais de Fontana masson, metenamina nitrato de prata de Grocott e ácido periódico de Schiff. O agente foi determinado através do isolamento microbiológico associado a identificação molecular. No terceiro artigo relata-se linfoma de células T nasal de caprino, que apresentou dispneia e ruído respiratório. No corte sagital da cabeça observou-se massa rósea-acinzentada, superfície lisa, multilobulada, macia, fortemente aderida a porção rostral da concha dorsal e ocluindo o meato nasal dorsal nas cavidades nasais direita e esquerda. No exame histopatológico revelou uma neoplasia de origem linfocítica, confirmado linfoma de células T por imuno-histoquímica. No quarto capítulo apresenta um caso de leiomiossarcoma primário de cavidade nasal em equino que apresentava diminuição do rendimento esportivo e secreção purulenta nasal bilateral com aproximadamente seis meses de evolução. Na cavidade nasal, observou-se massa de superfície irregular, amarelada, brilhante, fibroelástica, obstruindo a narina esquerda. No exame histopatológico foi observado células neoplásicas fusiformes, que coraram em vermelho na coloração de Tricômico de Masson e houve imunomarcagem positiva para os anticorpos 1A4, HHF35, Desmina e S100. O diagnóstico de leiomiossarcoma foi baseado nos aspectos morfotintoriais das células e confirmado através da imuno-histoquímica. Dentre tais novas condições anatomopatológicas diagnosticadas no LPA/HVU/UFCG nos animais de produção verificou-se que as infecciosas e as neoplasia ocorrem esporadicamente e são responsáveis por morte/eutanásia dos pacientes. Cursam com sinais clínicos semelhantes e inespecíficos, o que dificulta o diagnóstico clínico e macroscópico. Sendo necessário a utilização de exames histopatológicos, imuno-histoquímicos, microbiológicos e moleculares para confirmação da etiologia de cada condição.

PALAVRAS-CHAVE: dispneia; obstrução nasal; rinite fúngica; neoplasia mesenquimal; deformidade facial.

ABSTRACT

This thesis was constituted by four chapters that resulted in four scientific articles related to diseases of the nasal cavity in small ruminants and horses diagnosed at the Animal Pathology Laboratory, of the Veterinary University Hospital Prof. Dr. Ivon Macedo Tabosa, from the Federal University of Campina Grande, Patos, Paraiba. The first chapter describes the epidemiological, clinical and anatomopathological findings of meningoencephalitis secondary to *Pythium insidiosum* rhinitis in sheep that presented mild dyspnea and neurological signs. In the nasal cavity there was a red-black, irregular and friable mass that bilaterally compromised the nasal septum and the rostral portion of the nasal turbinates. In the brain, diffuse thickening of the leptomeninges of the cerebellum and ventral portion of the brain stem was observed by yellowish granular material associated with hyperemia of the vessels. On the floor of the fourth ventricle there was deposition of yellowish, irregular and slightly granular material that protruded towards the obex and displaced the cerebellum dorsolaterally. Microscopically, there was pyogranulomatous, eosinophilic and necrotizing rhinitis, and fibrinossuppurative, eosinophilic and necrotizing meningoencephalitis, both associated with thrombi, vasculitis and negative images of intralesional hyphae. The hyphae were impregnated with silver and immunopositive for the anti-*Pythium insidiosum* antibody. The second chapter refers to the clinical and pathological characteristics of rhinitis caused by *Exserohilum rostratum* in goats in which he had anorexia, dyspnoea and deformity of the face for two months. In the nasal cavity, there was a focal area, extensive yellow-brown, irregular, ulcerated and friable, which drained purulent secretion affecting the nasal vestibule up to the caudal portion of the dorsal concha. The lesion was characterized as rhinitis and pyangranulomatous and necrotizing osteomyelitis, associated with fungal structures. Morphologically, hyphae were characterized by thin walls, slightly tortuous and rarely, multiple chains of individual conidia or in groups and pigmented clamiconides. They were positive in the special histochemical stains of Fontana masson, methenamine nitrate of silver of Grocott and periodic acid of Schiff. The agent was determined through microbiological isolation associated with molecular identification. The third article reports on goat nasal T-cell lymphoma, which showed dyspnea and respiratory noise. In the sagittal section of the head, a pinkish-gray mass, smooth, multilobulated, smooth surface, strongly adhered to the rostral portion of the dorsal concha and occluding the dorsal nasal meatus in the right and left nasal cavities was observed. Histopathological examination revealed a neoplasm of lymphocytic origin, confirmed T-cell lymphoma by immunohistochemistry. In the fourth chapter, he presents a case of primary leiomyosarcoma of the nasal cavity in horses that presented a decrease in sports performance and bilateral purulent nasal secretion with approximately six months of evolution. In the nasal cavity, an irregular, yellowish, shiny, fibroelastic mass was observed, obstructing the left nostril. In the histopathological examination, spindle-shaped neoplastic cells were observed, which stained red in the Masson Trichomic stain and there was positive immunostaining for antibodies 1A4, HHF35, Desmina and S100. The diagnosis of leiomyosarcoma was based on the morphotintorial aspects of the cells and confirmed through immunohistochemistry. Among such new anatomopathological conditions diagnosed in LPA/HVU/UFCG in production animals, it was found that infectious and neoplasia occur sporadically and are responsible for the death / euthanasia of patients. They attend with similar and nonspecific clinical signs, which makes the clinical and macroscopic diagnosis difficult. It is necessary to use histopathological, immunohistochemical, microbiological and molecular exams to confirm the etiology of each condition.

KEY-WORDS: dyspnoea; nasal obstruction; fungal rhinitis; mesenchymal neoplasm; facial deformity.

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INTRODUÇÃO GERAL

As doenças do sistema respiratório são atribuídas como uma importante causa de morbidade e mortalidade nos animais e uma das principais fontes de perdas econômicas (LÓPEZ, 2017). A alta frequência de tais enfermidades deve-se ao fato de que o sistema respiratório está sob constante agressão por diversos agentes potencialmente prejudiciais, principalmente por via aerógena, que podem ser desencadeadas por microrganismos patogênicos, agentes tóxicos, químicos e gases poluentes (CASWELL & WILLIAMS, 2007).

Dentre as doenças das vias respiratórias superiores, as patologias da cavidade nasal são as mais frequentes e mais importantes, e são classificadas em: origens infecciosas (rinites virais/bacterianas/fúngicas/oomicóticas/algas/parasitárias), neoplásicas, degenerativas, idiopáticas e raramente as malformações (CASWELL & WILLIAMS, 2007).

A ocorrência de rinite por agentes infecciosos resulta do desequilíbrio dos mecanismos protetores da mucosa como bactérias e fungos comensais, secreção de muco e imunoglobulinas. Fatores como estresse e, conseqüente, imunossupressão também influenciam no aparecimento das enfermidades (LÓPEZ, 2017). Nos animais de produção, as rinites são um grupo heterogêneo de doenças (PORTELA et al., 2010) e, geralmente, cursam com quadro clínico semelhante e inespecífico. São classificadas quanto à evolução em aguda, crônica ou crônico-ativa, e ao tipo de exsudato, em seroso, catarral, purulento, hemorrágico, fibrinoso ou granulomatoso (LÓPEZ, 2017; SANTOS & GUEDES, 2016). Além disso, pode ser classificada de acordo com a severidade, tipo de agente etiológico envolvido e a localização (LÓPEZ, 2017).

As doenças da cavidade nasal tem sido diagnosticada em diferentes laboratórios de diagnóstico do país (MUSTAFA et al., 2015), incluindo o Laboratório de Patologia Animal do HVU/UFMG, destacando-se para ocorrência de conidiobolomicose e pitiose em ovinos (RIET-CORREA et al., 2008; PORTELA et al., 2010; AGUIAR et al., 2014), e casos

esporádicos de pitiose nasal (SOUTO et al., 2016), amiloidose nasal em equinos (PORTELA et al., 2012), aspergilose (CARMO et al., 2014) e prototecose em caprino (CAMBOIM et al., 2010).

Entretanto, chama a atenção da ocorrência de agentes desconhecidos como causa de rinite, bem como formas de apresentação da doença, pouco comuns ou ainda não relatadas causadas por *Pythium insidiosum*, e ainda a ocorrência de lesões tumoriformes de origem neoplásica em equino que são pouco frequentes e/ou raras.

Desse modo, faz-se necessário a descrição para conhecimento e inclusão nos diagnósticos diferenciais, servindo desta forma como subsídios para adoção de medidas profiláticas com o intuito de reduzir o percentual de óbitos e contribuir para a melhoria da qualidade de vida dos animais, frente à determinadas manifestações clínicas ou padrão anatomopatológico das espécies estudadas. Portanto, a tese objetiva-se descrever os principais aspectos clínicos e anatomopatológicos dos casos de meningoencefalite por *Pythium insidiosum* secundário a rinite em ovino (Artigo 1), rinite por *Exserohilum rostratum* em caprino (Artigo 2), linfoma de células T nasal em caprino (Artigo 3) e leiomiossarcoma nasal em equino (Artigo 4).

REFERÊNCIAS

- AGUIAR, G. M. N.; SIMÕES, S. V. D.; SANTOS, S. A.; MARQUES, A. L. A.; SILVA, T. R.; DANTAS, A. F. M.; RIET-CORREA, F. Epidemiological aspects of conidiobolomycosis in sheep in the Northeastern Brazilian semiarid region. **Ciência Rural**. v. 44, n. 12, p. 2210-2216, 2014.
- CAMBOIM E.K.A., NEVES P.B., GARINO JR F., MEDEIROS J.M. & RIET-CORREA, F. Prototecose: uma doença emergente. **Pesquisa Veterinária Brasileira**, v. 30, n. 1, p. 94-101, 2010.
- CARMO, P.M.S.; PORTELA, R.A.; DE OLIVEIRA-FILHO, J.C.; DANTAS, A.F.M.; SIMÕES, S.V.D.; GARINO JR., F.; RIET-CORREA, F. Nasal and Cutaneous Aspergillosis in a Goat. **Journal of Comparative Pathology**, v. 150, n. 1, p. 4-7, 2014.
- CASWELL, J. L.; WILLIAMS, K. J. Respiratory system. In: MAXIE, M. G. **Jubb, Kennedy and Palmer's Pathology of Domestic Animals**. 5th. Saunders Elsevier: Philadelphia. 2007. p. 523-653.
- LOPEZ, A.; MARTINSON, S.A. Respiratory System, Mediastinum, and Pleurae. In: Zachary J.F. (Eds). **Pathologic Basis of Veterinary Disease**. 6th. St. Louis Missouri: Elsevier. 2017. p. 471-560.
- MUSTAFA, V. S.; GUEDES, K. M. R.; LIMA, E. M. M.; BORGES, J. R. J.; CASTRO, M. B. Doenças da cavidade nasal em pequenos ruminantes no Distrito Federal e no Estado de Goiás. **Pesquisa Veterinária Brasileira**. v.35, n.7, p. 627-636, 2015.
- PORTELA, R. A.; RIET-CORREA, F.; GARINO, J. F.; DANTAS, A. F. M.; SIMÕES, S. V. D.; SILVA, S. M. S. Doenças da cavidade nasal em ruminantes no Brasil. **Pesquisa Veterinária Brasileira**. v. 30, n. 10, p. 844-854, 2010.
- PORTELA, R. A.; DANTAS, A. F. M.; MELO, D. B.; MARINHO, J. M.; NÓBREGA NETO, P. I.; RIET-CORREA, F. Nasal amyloidosis in a horse. **Brazilian Journal Veterinary Pathology**. v. 5, n. 2, p. 86-88, 2012.

RIET-CORREA, F.; DANTAS, A. F. M.; AZEVEDO, E. O.; SIMÕES, S. D. V.; SILVA, S. M. S.; VILELA, R.; MENDONZA, L. Outbreaks of rhinofacial and rhinopharyngeal zygomycosis in sheep in Paraíba, Northeastern Brazil. **Pesquisa Veterinária Brasileira**. v. 28, n. 1, p. 29-35, 2008.

SANTOS, R. L.; GUEDES, R. M. C. Sistema Respiratório In: Santos R. L. & Alessi A. C. (Eds), **Patologia Veterinária**. 2ª Ed. Roca: Rio de Janeiro. 2016. p. 1-50.

SOUTO, E. P. F.; MAIA, L. A.; OLINDA, R. G.; GALIZA, G. J. N.; KOMMERS, G. D.; MIRANDA-NETO, E. G.; RIET-CORREA, F. Pythiosis in the Nasal Cavity of Horses. **Journal of Comparative Pathology**. v. 155, n. 2, p.126-129, 2016.

CAPÍTULO I:**Meningoencephalitis secondary to rhinitis caused by *Pythium insidiosum* in sheep**

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(Qualis A2)

Meningoencephalitis secondary to rhinitis caused by *Pythium insidiosum* in sheep

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ABSTRACT

This study reports a case of meningoencephalitis caused by *Pythium insidiosum* secondary to rhinitis in a three-year-old, crossbred, female sheep. The animal presented mild dyspnea, blindness, mydriasis, opisthotonos, nystagmus, incoordination, decreased mandibular tone, and spasticity of the pelvic limbs. Macroscopic examination of the nasal cavity showed a blackish-red, irregular, friable mass that bilaterally compromised the nasal septum and the rostral portion of the nasal turbinates. In the brain, there was diffuse thickening of the leptomeninges of the cerebellum and ventral portion of the brainstem characterized by yellowish, granular material associated with vessel hyperemia. On the floor of the fourth ventricle, there was deposition of yellowish, irregular, slightly granular material that protruded towards the obex and displaced the cerebellum dorsolaterally. Microscopically, there were pyogranulomatous, eosinophilic, necrotizing rhinitis and fibrinosuppurative, eosinophilic, necrotizing meningoencephalitis, both associated with thrombosis, vasculitis, and intralesional hyphae. The hyphae were impregnated with silver and presented thin, parallel walls, rarely septate and branched. At immunohistochemistry, the hyphae were immunostained with polyclonal anti-*P. insidiosum* antibody in fragments of the cerebellum and nasal cavity. The findings evidenced that *P. insidiosum* affects the nervous system of sheep, causing nonspecific neurological symptoms.

Keywords: hematogenic dissemination, vasculitis, pythiosis, oomycete.

Pythiosis is a chronic pyogranulomatous/granulomatous disease caused by the aquatic oomycete *Pythium insidiosum*, belonging to phylum *Oomycota* and family Pythiaceae (Leal, 1999). The equine species is the most affected (Santurio *et al*, 2006) by this disease, followed by the canine species (Trost *et al*, 2009). Infection occurs when the animal comes into contact

with water contaminated with mobile zoospores (Santurio *et al*, 2006) or ingests water contaminated with the infectious form (Pessoa *et al*, 2012).

In Brazil, sheep are frequently infected with *P. insidiosum*, with cases described in the Northeast (Tabosa *et al*, 2004; Riet-Correa *et al*, 2008; Portela *et al*, 2010; Carrera *et al*, 2013), Midwest (Santurio *et al*, 2008; Ubiali *et al*, 2013; Mustafa *et al*, 2015) and South (Bernardo *et al*, 2015) regions of the country. The clinical forms reported in sheep include nasal (Santurio *et al*, 2008; Portela *et al*, 2010; Bernardo *et al*, 2015; Mustafa *et al*, 2015), cutaneous (Tabosa *et al*, 2004; Riet-Correa *et al*, 2008) and digestive (Pessoa *et al*, 2012) infections.

Cases of meningoencephalitis associated with rhinopharyngeal rhinitis have been reported in sheep as a consequence of infection by *Conidiobolus* sp., and involvement of the nervous system usually occurs by direct extension of the nasal cavity lesions (Portela *et al*, 2010; Ubiali *et al*, 2013; Mustafa *et al*, 2015). A case of meningoencephalitis caused by *P. insidiosum* in a sheep was recently described (Mori *et al*, 2017), but without lesions in other tissues, which hindered identification of the agent pathway of entry in the central nervous system (CNS). Therefore, clinical and pathological characterization of pythiosis with brain involvement is necessary to understand the pathogenesis of infection and the spread of *P. insidiosum* in animals. In this context, this study aimed to describe the epidemiological, clinical and anatomopathological aspects of nasal pythiosis with brain involvement in a sheep.

The case of a three-year-old, crossbred, female sheep that presented imbalance, falls, and head tilting to the left side for one day is reported. The animal was dewormed, raised extensively, with access to a weir. On physical examination, it presented regular body score, dyspnea, blindness, mydriasis, opisthotonos, nystagmus, incoordination, decreased mandibular tone, and spasticity of the pelvic limbs. In view of the clinical signs, polioencephalomalacia was suspected, and a therapeutic protocol with dexamethasone and

vitamin B1 was introduced. The next day, the sheep died and was sent for necropsy. Organ fragments were collected, fixed in 10% buffered formaldehyde, processed routinely for histopathology, and stained with hematoxylin and eosin (HE). Histological sections of the lesions in the nasal cavity and cerebellum were submitted to Grocott methenamine silver nitrate (GMS) and periodic acid-Schiff (PAS) staining. Immunohistochemistry (IHC) was performed according to the protocol of (Gabriel *et al*, 2008) modified by (Martins *et al*, 2012) using the primary (non-commercial) polyclonal anti-*P. insidiosum* antibody followed by streptavidin-biotin-alkaline phosphatase with Liquid Permanent Red (LPR) chromogen. Histological sections of a confirmed case of equine pythiosis were used as positive control, and the same sections with replacement of the primary antibody with phosphate-buffered saline (PBS) and polysorbate 20 were used as negative control.

Macroscopic examination showed lesions restricted to the nasal cavity and the brain. After a sagittal cut of the head, a blackish-red, irregular, friable mass measuring approximately 7x5.5 cm that bilaterally compromised the nasal septum and rostral portion of the nasal turbinates was observed in the nasal cavity (Fig. 1A).

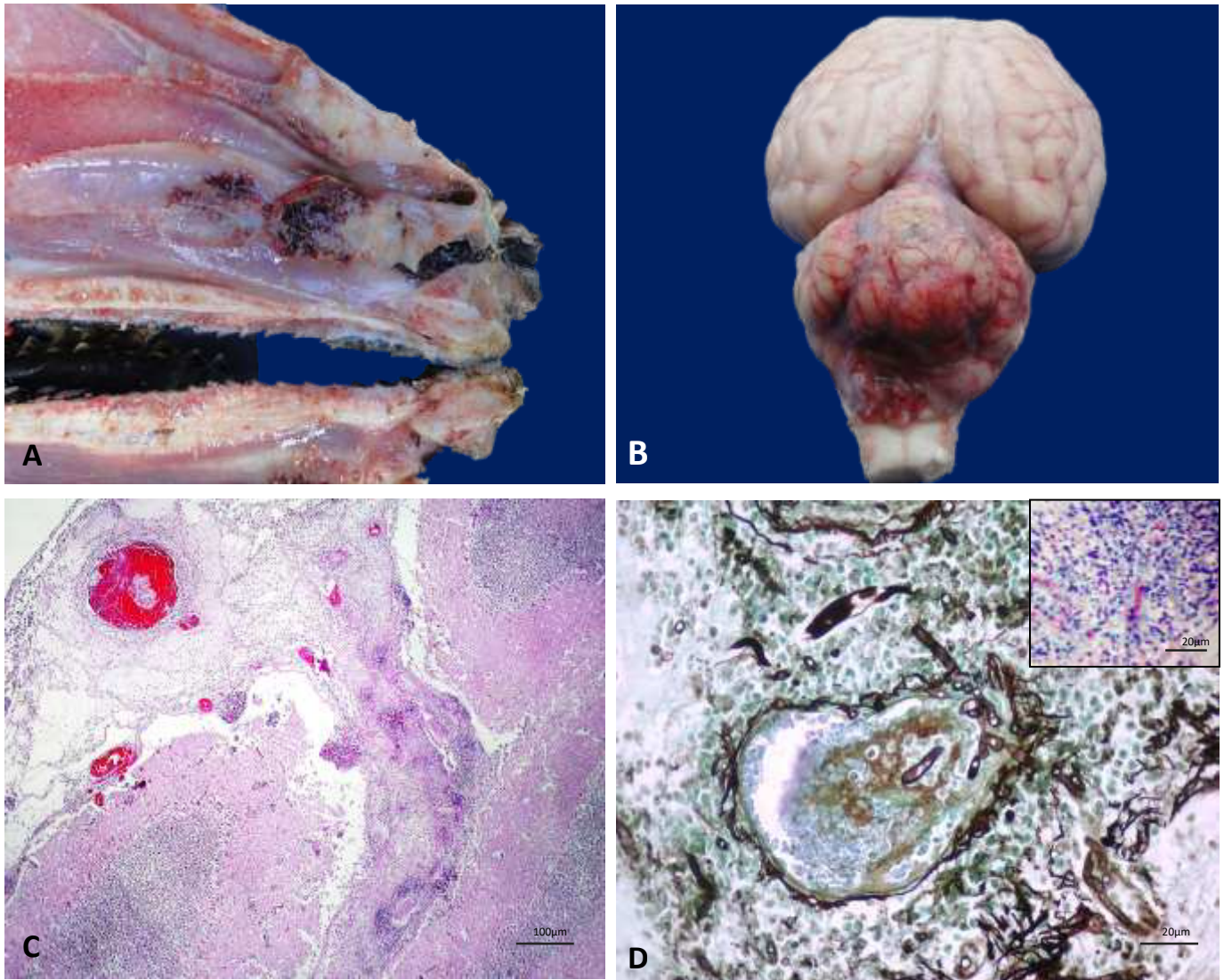


Figure 1 - Meningoencephalitis secondary to rhinitis caused by *Pythium insidiosum* in sheep. A) Nasal cavity. A blackish, irregular, friable mass in the nasal septum and rostral portion of the nasal turbinates. B) Brain. Thickening of the leptomeninges of the cerebellum characterized by granular and yellowish material associated with hyperemia of blood vessels. C) Cerebellum. Fibrinosuppurative, eosinophilic meningoencephalitis associated with thrombosis and vasculitis. HE. Bar = 100 μ m. D) Cerebellum. Hyphae with strong silver impregnation characterized by thin, parallel walls, rarely septate, in the lumen and wall of a blood vessel. GMS. Bar = 20 μ m. Inset: Cerebellum. Hyphae red-immunostained with polyclone anti-*Pythium insidiosum* antibody. IHQ. Alkaline phosphatase. Bar = 20 μ m.

Upon opening of the cranial box, there was diffuse thickening of the leptomeninges of the cerebellum and ventral region of the brainstem characterized by granular, yellowish material associated with vessel hyperemia (Fig. 1B). On the floor of the fourth ventricle, there was a deposition of yellowish, irregular, slightly granular material that protruded toward the obex and displaced the cerebellum dorsolaterally.

Histopathological examination of the nasal cavity revealed pyogranulomatous, eosinophilic, necrotizing rhinitis associated with vasculitis and thrombosis, characterized by thickening of the own blade and marked, diffuse inflammatory infiltrate located mainly around blood vessels and often distending the vascular wall, as well as by eosinophils and fewer neutrophils, macrophages, and multinucleated giant cells associated with multifocal areas of necrosis and negative images of hyphae in longitudinal and transverse sections, sometimes surrounded by amorphous, granular, hypereosinophilic material similar to the *Splendore-Hoeppli* phenomenon. There was marked congestion and fibrin thrombi occluding the vascular lumen, sometimes associated with hyphae. The nasal mucosa was diffusely ulcerated.

In the cerebellum, brainstem, and parietal cortex there was fibrinosuppurative, eosinophilic meningoencephalitis associated with vasculitis and thrombosis (Fig. 1C), characterized by multifocal and coalescent areas of marked thickening of the leptomeninges, with inflammatory infiltrate located mainly around the blood vessels and distending the vascular wall, consisting of eosinophils and fewer neutrophils and macrophages associated with fibrin and negative images of hyphae amid a reaction similar to the *Splendore-Hoeppli* phenomenon and in the lumen of blood vessels.

There was marked congestion and fibrin thrombi obstructing the lumen of most blood vessels. In the adjacent gray and white matter, a similar inflammatory infiltrate was observed, mainly around the blood vessels, associated with thrombosis and hyphae in the vascular lumen and in the neuropile. The choroid plexus presented mild inflammatory infiltrate consisting predominantly of macrophages, eosinophils, and rare neutrophils and lymphocytes. In the parietal cortex, adjacent to the foci of inflammation, slight neuronal necrosis was observed.

At GMS, the hyphae were heavily impregnated by silver, with thin, parallel walls rarely septate and branched, measuring 4-25µm (Fig. 1D). The hyphae did not stain in PAS. At IHC, the hyphae were strongly immunostained with polyclonal anti-*P. insidiosum* antibody in fragments of the cerebellum and nasal cavity (Fig. 1D, Inset).

The diagnosis of meningoencephalitis secondary to rhinitis caused by *P. insidiosum* was established based on anatomopathological findings and confirmed through IHC.

Generally, animals infected with oomycetes have access to dams (Riet-Correa *et al*, 2008), where grazing occurs in swampy areas with low vegetation, most often associated with the large amount of decomposing organic matter, with an ideal temperature for proliferation of the agent (Santurio *et al*, 2006). It is believed that, in this case, access to the reservoir favored the animal's contact with the infective form of *P. insidiosum*.

Meningoencephalitis associated with rhinopharyngeal rhinitis has been frequently described in sheep as a consequence of infection by *Conidiobolus* sp. (Riet-Correa *et al*, 2008; Portela *et al*, 2010; Mustafa *et al*, 2015). Involvement of the CNS in conidiobolomycosis occurs by direct extension of proliferative and destructive granulomatous lesions that extend from the middle third of the nasal cavity to the cribriform plaque, olfactory bulb and, more often, to the frontal cortex (Riet-Correa *et al*, 2008; Portela *et al*, 2010; Ubiali *et al*, 2013; Mustafa *et al*, 2015). In the present case, the lesions were located in the rostral portion of the nasal cavity, presenting macroscopic aspects similar to the rhinofacial pythiosis described by other authors (Santurio *et al*, 2008; Portela *et al*, 2010; Carrera *et al*, 2013; Bernardo *et al*, 2015; Mustafa *et al*, 2015). However, the histopathological observation of vasculitis and thrombosis associated with *P. insidiosum* hyphae in the lumen of vessels suggests that the involvement of the CNS occurred through hematogenesis.

In this case report, the absence of lesions in the nasal conchae and cribriform plaque, which could possibly facilitate the agent's ascension by direct extension of the lesion in the

nasal cavity, reinforces the possibility of the agent having reached the CNS by the hematogenous route.

The absence of craniofacial asymmetry drew attention in this sheep. This clinical aspect has been frequently described in cases of rhinofacial pythiosis (Santurio *et al*, 2008; Portela *et al*, 2010; Bernardo *et al*, 2015; Mustafa *et al*, 2015) and popularly characterizes the disease as ‘bull snout’. It is believed that the impairment of the CNS and the evolution of the neurological clinical condition and, consequently, the death of the animal did not allow evolution of the nasal cavity lesions and appearance of craniofacial asymmetry.

Although polioencephalomalacia was considered a presumptive diagnosis, the animal did not respond to therapeutic treatment with dexamethasone and vitamin B1 and died the following day. The anatomopathological findings in the brain stem and cerebellum associated with *P. insidiosum* were serious and justify the neurological condition.

Considering the macroscopic and microscopic findings observed in this case, which corroborate those described in the literature for a case of *P. insidiosum* meningoencephalitis with absence of lesions in other organs (Mori *et al*, 2017), it is proposed that infection by *P. insidiosum* be included in the list of differential diagnoses of meningoencephalitis associated with rhinitis, or of diseases that affect the CNS of sheep in isolation. The main diseases that can also cause injuries in the places identified in this case are rabies (Guedes *et al*, 2007; Borges *et al*, 2016), nervous form of listeriosis (Guedes *et al*, 2007), and bacterial meningitis (Guedes *et al*, 2007)

Conclusion

In conclusion, *P. insidiosum* spreads via the hematogenous route and causes meningoencephalitis in sheep with nasal pythiosis, which can trigger varied, nonspecific neurological symptoms. This characteristic has not yet been described in the literature. In

addition, pythiosis with brain involvement should be included in the differential diagnosis of diseases that affect the central nervous system of sheep.

Conflict of interest statement

The authors declare that there are no conflicts of interest in relation to the publication of this manuscript.

References

- Bernardo FD, Conhizak C, Ambrosini, F, Jesus FP, Santurio JM et al. (2015) Pythiosis in sheep from Paraná, southern Brazil. *Pesquisa Veterinária Brasileira*, **35**, 513-517.
- Borges IL, Firmino MO, Rocha EF, Junior MLS, Lima TS et al. (2016) Surto de raiva em ovinos na Paraíba. *Pesquisa Veterinária Brasileira*, **36(Supl.2)**, 193-194.
- Carrera MV, Peixoto RM, Gouveia GV, Pessoa CR, Jesus FP et al. (2013) Pitiose em ovinos nos estados de Pernambuco e Bahia. *Pesquisa Veterinária Brasileira*, **33**, 476-482.
- Gabriel, AL. et al. Surto de pitiose cutânea em bovinos. *Pesquisa Veterinária Brasileira*, **28**, p.583-587, 2008.
- Guedes KMR, Riet-Correa F, Dantas AFM, Simões SVD, Miranda Neto EG et al. (2007) Doenças do sistema nervoso central em caprinos e ovinos no semiárido. *Pesquisa Veterinária Brasileira*, **27**, 29-38.
- Leal, AT. *Pythium insidiosum: Caracterização antigênica preliminar e avaliação de adjuvantes na indução de resposta sorológica em coelhos*. (1999) Dissertação (Mestrado em Medicina Veterinária) – Universidade Federal de Santa Maria.
- Lima EF, Riet-Correa F, Tabosa IM, Dantas AFM, Medeiros JM, et al. (2005) Polioencefalomalacia em caprinos e ovinos na região semiárida do Nordeste do Brasil. *Pesquisa Veterinária Brasileira*, **25**, 9-14.

- Martins TB, Kommers GD, Trost ME, Inkelmann MA, Figuera RA et al. (2012) A Comparative Study of the Histopathology and Immunohistochemistry of Pythiosis in Horses, Dogs and Cattle. *Journal of Comparative Pathology*, **46**, 122-131.
- Mori AP, Lorenzetti MP, Bandinelli MB, Henker LC, Soares FS et al. (2017) Meningoencefalite por *Pythium insidiosum* em ovino. *Pesquisa Veterinária Brasileira*, **37(Supl.)**, 45-46.
- Mustafa VS, Guedes KM, Lima EM, Borges JR, Castro MB. (2015) Doenças da cavidade nasal em pequenos ruminantes no Distrito Federal e no Estado de Goiás. *Pesquisa Veterinária Brasileira*, **35**, 627-636.
- Pessoa CR, Riet-Correa F, Pimentel LA, Garino F, Dantas AFM et al. (2012) Pythiosis of digestive tract in sheep. *Journal of Veterinary Diagnostic Investigation*, **24**, 1133-1136.
- Portela RDA, Riet-Correa F, Garino F, Dantas AFM, Simões SVD et al. (2010) Doenças da cavidade nasal em ruminantes no Brasil. *Pesquisa Veterinária Brasileira*, **30**, 844-854.
- Riet-Correa F (2007) Conidiobolomicose em ovinos. In: *Doenças dos ruminantes e equinos*, 3th Edit., F Riet-Correa, AL Schild, RAA Lemos, JRJ Borges Eds., Palloti, Santa Maria, p.448-450.
- Riet-Correa F, Dantas AFM, Azevedo EO, Simões SVD, Silva SM et al. (2008) Outbreak of rhinopharyngeal zygomycosis in sheep in Paraíba, Northeastern Brazil. *Pesquisa Veterinária Brasileira*, **28**, 29-35.
- Santurio JM, Argenta JS, Schwendler SE, Cavalheiro AS, Pereira DIB (2008). Granulomatous rhinitis associated with *Pythium insidiosum* infection in sheep. *Veterinary Record*, **163**, 276-277.
- Santurio JM, Alves SH, Pereira DB, Argenta JS (2006). Pitiose: uma micose emergente. *Acta Scientiae Veterinariae*, **34**, 1-14.

- Silva SM, Castro RS, Costa FA, Vasconcelos AC, Batista MC et al. (2007) Conidiobolomycosis in Sheep in Brazil. *Veterinary Pathology*, **44**, 314–319.
- Souto EPF, Maia LA, Olinda RG, Galiza GJN, Kommers GD et al. (2016) Pythiosis in the Nasal Cavity of Horses. *Journal of Comparative Pathology*. **155**, p.126-129.
- Tabosa IM, Riet-Correa F, Nobre VMT, Azevedo EO, Reis-Junior JL (2004) Outbreaks of pythiosis in two flocks of sheep in northeastern Brazil. *Veterinary Pathology*, **41**, 412-415.
- Trost ME, Gabriel AL, Masuda EK, Figuera RA, Irigoyen LF et al. (2009) Aspectos clínicos, morfológicos e imuno-histoquímicos da pitiose gastrointestinal canina. *Pesquisa Veterinária Brasileira*, **29**, 673-678.
- Ubiali DG, Cruz RAS, De Paula DAJ, Silva MC, Mendonça FS et al. (2013) Pathology of nasal infection caused by *Conidiobolus lamprauges* and *Pythium insidiosum* in sheep. *Journal of Comparative Pathology*, **149**, 137-145.

CAPÍTULO II:

Rhinitis by *Exserohilum rostratum* (*Setosphaeria rostrata*) in goats

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Rhinitis by *Exserohilum rostratum* (*Setosphaeria rostrata*) in goats

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ABSTRACT

We describe a rhinitis case caused by *Exserohilum rostratum* in goats, mixed breed, female, three years old, with decreased appetite, dyspnea and face deformity, with the evolution of approximately two months. In the nasal cavity, there was a focal area, extensive brownish yellow, irregular, ulcerated and friable, which drained purulent secretion affecting the nasal vestibule and extending the caudal portion of the dorsal concha, associated with nasal meatus obstruction, destruction of the nasal septum and nasal bone palatine and vomer. Histologically, the lesion was characterized as rhinitis and pyogranulomatous and necrotizing osteomyelitis, multifocal to coalescent, sharp, chronic, associated with myriad fungal structures. Morphologically, the hyphae were characterized by thin, slightly tortuous and rarely septate walls measuring 2 to 6 μm in diameter, multiple chains of individual conidia or in groups measuring 8 to 10 μm in diameter and pigmented chlamydoconidia measuring from 7.5 to 15 μm in diameter. They were positive in the special histochemistry of Fontana Masson, methenamine silver nitrate by Grocott and periodic acid by Schiff. The agent was determined through microbiological isolation associated with molecular identification. Pyrogranulomatous rhinitis caused by *E. rostratum* in goats is unprecedented, being the first isolation of the agent in animals in Brazil and the first case of nasal phaeohyphomycosis in the goat species.

Keywords: nasal cavity, dyspnoea, dematiaceous fungi, phaeohyphomycosis.

Exserohilum spp. (*Setosphaeria rostrata*) are saprophytic and dematiaceous fungi considered important plant pathogens (Poltronieri *et al*, 2007; Gauthier *et al*, 2013; Katragkou *et al*, 2014) and rarely affect humans (Gauthier *et al*, 2013; Katragkou *et al*, 2014) and animals (Whitford *et al*, 1989; More *et al*, 2019). In humans, cases occur in immunosuppressed individuals, in different clinical forms, including systemic, cutaneous, subcutaneous and ocular (Adler *et al*, 2006; Gauthier *et al*, 2013; Katragkou *et al*, 2014).

Rare cases of *E. rostratum* rhinitis were described in equine (More *et al*, 2019) and humans (Adler *et al*, 2006), however, data on *E. rostratum* infection in domestic animals remain scarce, especially the involvement of the nasal cavity in the goat species. Thus, the objective is to describe a case of rhinitis by *E. rostratum* in a goat, highlighting the clinical and anatomopathological aspects of the infection.

A three-year-old female goat, mixed breed, from São José do Bonfim-PB, presented decreased appetite, dyspnea and increased volume on the face with an evolution of approximately two months. The animal was raised in a semi-extensive regime with commercial feed, native pasture and free access to a weir.

On clinical examination, the animal was cachectic in lateral decubitus, comatose, dehydrated, pale mucous membranes, mucopurulent bilateral nasal discharge and facial deformity characterized by an irregular, bilateral volume increase, located in the nasal sinuses and nasal cavity, in addition to dyspnea.

Radiographic examination of the face revealed loss of continuity in the nasal bone and increased radiopacity in the nasal cavity. The animal died and was necropsied.

Macroscopically on the head, deformity of the face was observed, characterized by an increase in bilateral, firm volume, including the nasal cavities. After the sagittal section of the head, in the nasal cavity, a focal area was extensive, yellowish-brown, irregular, ulcerated and friable, which drained purulent secretion affecting the nasal vestibule and extending the caudal portion of the dorsal concha, associated with nasal meatus obstruction, destruction of the nasal septum and nasal bone, palate and vomer (Fig.1A).

In the abomasum mucosa, there was a marked infestation by nematode parasites compatible with *Haemonchus* sp. Also, subcutaneous edema was observed in the submandibular region, pulmonary edema, hydropericardium and mild ascites.

Fragments of all organs were collected and fixed in 10% buffered formaldehyde, processed routinely and stained with hematoxylin-eosin (HE). Fragments of the nasal cavity lesion were subjected to special stains including Grocott's methenamine silver stain (GMS), Schiff's periodic acid (PAS) and Fontana Masson (FM) to demonstrate the morphological characteristics of the agent.

Fragments of the nasal cavity lesion were sent for cultivation and microbiological isolation, using Sabouraud agar as the culture medium (Fig. 1B). The colony was stored in mineral oil and sent for DNA extraction according to Del Poeta *et al.*, (1999). In determining the fungal species, the DNA was subjected to PCR with the oligonucleotides from the Internal Transcribed Spacer (ITS) ITS4 (5'-TCCTCCGCTTATTGATATGC) and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') regions, according to White *et al.* (1990). DNA analysis was performed using the CLC Genomics Workbench 4.9 software (CLC bio, Denmark) and the species was subsequently confirmed in the GenBank database, in the BLAST software, NCBI server (<https://www.ncbi.nlm.nih.gov/BLAST>).

Histologically, the lesions were characterized by rhinitis and pyogranulomatous and necrotizing osteomyelitis, multifocal to coalescent, severe, chronic, associated with myriad fungal structures. In the nasal cavity, multifocal and coalescent areas of marked thickening of the lamina propria by inflammatory infiltrate consisting of macrophages, epithelioid macrophages, multinucleated giant cells and neutrophils were observed, sometimes with central and extensive areas of necrosis associated with myriads of intralesional fungal structures, delimited by a moderate proliferation of fibrous connective tissue and lymphocytic infiltrate.

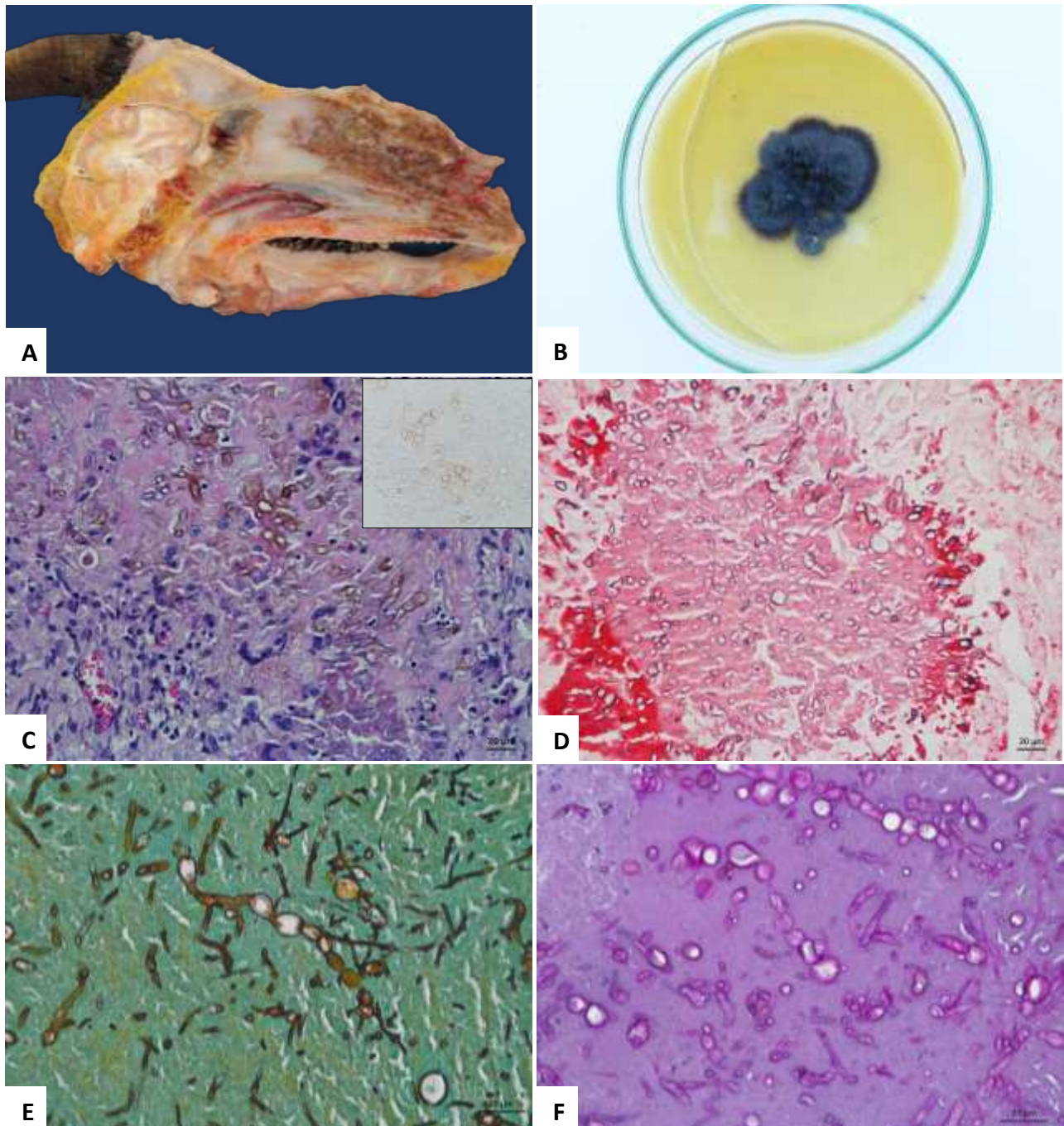


Figure 1 - *Exserohilum rostratum* rhinitis in goats. A) Sagittal section of the nasal cavity with enlarged volume characterized by a focal area which is yellowish brown, irregular, friable and ulcerated, affecting the nasal vestibule and extending the caudal portion of the dorsal concha associated with destruction of the nasal septum, nasal bone, palate and vomer. B) Microbiological cultivation. There is a blackish fungal colony with a cottony aspect. C) Nasal cavity. Numerous pigmented hyphae are observed in transverse and longitudinal sections with thin walls and rarely septated amid necrosis and in the cytoplasm of multinucleated giant cells. HE. Bar = 20 μ m. Inset: Hyphae with a pigmented wall, thin and rarely septate, evident in deparaffinized and unstained lamina. Bar = 20 μ m. D) Nasal cavity, there are numerous hyphae, chains of conidia strongly impregnated in black. FM. Bar = 20 μ m. E) Nasal cavity, there are numerous hyphae, chains of conidia and chlamydoconidia strongly impregnated in black. GMS. Bar = 20 μ m. F) Nasal cavity, multiple similar fungal structures are observed, stained intensely in pink. PAS. Bar = 20 μ m.

Destruction of the nasal septum and bone tissue adjacent to the inflammation was observed. Fungi were also frequently seen in the multinucleated giant cells cytoplasm. Slight congestion and occasional blood vessels with fibrin thrombus formation partially occluding the vascular lumen were also observed.

In the sections stained by HE, the fungi presented themselves as tubuliform structures in longitudinal and transverse sections with walls that varied between basophilic and brownish (Fig. 1C). In previously deparaffinized and unstained slides, it was possible to observe numerous fungal structures showing slightly brownish, septate and thin walls (Fig. 1C, Inset). Through GMS and FM, hyphae were heavily impregnated in black (Fig. 1D and 1E). Through PAS, hyphae were intensely rosy stained (Fig. 1F). Morphologically, hyphae were characterized by exhibiting thin, slightly tortuous and rarely septate walls measuring 2 to 6 μm in diameter, multiple individual conidial chains or in groups measuring 8 to 10 μm in diameter and pigmented chlamydoconidia measuring 7.5 to 15 μm in diameter.

The colonies had a black cottony aspect and on direct examination with cotton blue, septate and branched hyphae with dilations were observed, but with absent sporulation and without the formation of conidiophores. In the molecular test, a fragment of approximately 623bp was amplified, with 99.84% identity with the species *Exserohilum rostratum* (*Setosphaeria rostrata*).

The diagnosis of rhinitis by *E. rostratum* was made from microscopic lesions associated with the morphotintorial characteristics of the agent and confirmed through isolation and molecular testing. In the face of fungal infections, it is necessary to carry out microbiological isolation and molecular identification of the agent involved in each case, since in the histopathological examination the morphology of the agent can be indistinguishable from the numerous pathogenic fungal agents (Rai *et al*, 2021). Infection by *E. rostratum* likely occurred by inhaling spores present in the soil associated with a marked

amount of organic matter around the weir. The same epidemiological condition was reported in diseases of the nasal cavity of sheep in the region, including pythiosis and conidiobolomycosis in sheep (Portela *et al*, 2010).

Data related to the pathogenesis and behavior of *E. rostratum* in veterinary medicine are rare, with only cases of skin infections in cattle (Whitford *et al*, 1989) and recently a case of rhinitis in horses (More *et al*, 2019). Thus, it is necessary to describe this case, as it is the first isolation of the agent in animals in Brazil and the first case of nasal phaeohyphomycosis in the goat species.

In humans infected with *E. rostratum*, several clinical presentations are described, including systemic, cutaneous, subcutaneous and ocular forms (Adler *et al*, 2006; Katragkou *et al*, 2014), in addition to isolated central nervous system involvement in severe outbreaks (Katragkou *et al*, 2014). The fungus is considered opportunistic with a predominance of infections in people who have immunosuppression (Adler *et al*, 2006; Gauthier *et al*, 2013; Katragkou *et al*, 2014). In this case, it is believed that the severe condition of haemoncosis may have caused weakness of the animal and consequently triggered immunosuppression.

Lesions characterized by pyogranulomas with extensive areas of necrosis are typical of the infection (Whitford *et al*, 1989; More *et al*, 2019), as observed in the case of this report, and which can probably be related to the chronicity of the lesion. Few studies describe the *E. rostratum* morphology in a histopathological examination, however, it is known that there are several forms of presentation including hyphae, chlamydoconidia and conidial chains (More *et al*, 2019), all pigmented or with some degree of basophilia on their wall. Characteristics related to the morphology of the agent were similar in this case, where it was possible to visualize the different forms of presentation, located mainly in the necrosis areas. The degree of basophilic coloration of the hyphae was also described (Lyons *et al*, 2012) in a woman who had severe vasculitis and cerebral infarction associated with angioinvasive *Exserohilum*. In

this report, the agent was not observed inside vessels, however, there are vascular changes such as congestion and venous thrombi.

In the present report, the changes seen during necropsy including subcutaneous edema, pulmonary edema, hydropericardium and ascites were attributed to hypoproteinemia secondary to the high parasitic burden by *Haemonchus* sp., which supposedly culminated in the death of the animal. However, the lesions observed in the nasal cavity were severe and extensive, affecting approximately 80% of the nasal cavity, bilaterally, associated with the destruction of the nasal septum and adjacent bone structures, thus justifying the clinical signs of facial deformity and severe dyspnea. In cases where the lesions are extensive in the nasal cavity, therapeutic and surgical treatments become unfeasible and often inefficient, as observed in sheep with conidiobolomycosis and pythiosis (Portela *et al*, 2010).

Given the anatomopathological findings, a differential diagnosis should be made with another infectious rhinitis already described in goats such as aspergillosis (Portela *et al*, 2010) and protothecosis (Camboin *et al*, 2011). However, in a histopathological examination, it is possible to identify and differentiate morphologically such agents. Besides, it is necessary to include other fungal species that trigger phaeohyphomycosis (Rai *et al*, 2021), since they have pigmentation in fungal structures similar to that observed in this case.

Conclusion

Pyogranulomatous rhinitis caused by *E. rostratum* in goats is unprecedented, being the first isolation of the agent in animals in Brazil and the first case of nasal phaeohyphomycosis in the goat species. It should be considered as a differential diagnosis of chronic rhinitis and neoplasms. For the diagnosis of the disease, microbiological isolation and genetic sequencing of the agent is essential, to exclude other infections by fungi of the phaeohyphomycosis group, as well as other fungal agents, oomycetes and algae.

Reference

- Adler A, Yaniv I, Samra Z, Yacobovich J, Fisher S et al. (2006) Exserohilum: an emerging human pathogen. *European Journal of Clinical Microbiology and Infectious Diseases*, **25(4)**, 247-253.
- Camboim EK, Garino FJ, Dantas AFM, Simões SV, Melo MA et al. (2011) Prototecose por *Prototheca wickerhamii* em cabras. *Micoses*, **54 (4)**, e196-e200.
- Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J et al. (1999) Topoisomerase I is essential in *Cryptococcus neoformans*: role in pathobiology and as an antifungal target. *Genetics*, **152**, 167-78.
- Gauthier GM, Keller NP. (2013) Crossover fungal pathogens: the biology and pathogenesis of fungi capable of crossing kingdoms to infect plants and humans. *Fungal Genetics and Biology*, **61**, 146-157.
- Katragkou A, Pana ZD, Perlin DS, Kontoyiannis DP, Walsh TJ et al. (2014) *Exserohilum* infections: review of 48 cases before the 2012 United States outbreak. *Sabouraudia*, **52(4)**, 376-386.
- Lyons JL, Gireesh ED, Trivedi JB. et al (2012) Fatal *Exserohilum* Meningitis and Central Nervous System Vasculitis After Cervical Epidural Methylprednisolone Injection. *Ann Intern Med*, **157**, 835–836
- More SN, Hernandez O, Castleman WL. (2019) Mycotic Rhinitis and Sinusitis in Florida Horses. *Veterinary Pathology*, **56(4)**, 586–598.
- Poltronieri LS, Verzignassi JR, Benchimol RL, Freire FCO. (2008) Primeiro registro de *Exserohilum rostratum* (anamorfo de *Setosphaeria rostrata*) causando manchas foliares em açazeiro no Brasil. *Summa Phytopathol*, **34**, 195.

- Portela RA, Riet-correa F, Garino-junior F, Dantas AFM, Simões SVD, Silva SMS. (2010) Doenças da cavidade nasal em ruminantes no Brasil. *Pesquisa Veterinária Brasileira* **30(10)**, 844-854.
- Rai M, Ingle AP, Ingle P, Gupta I, Mobin M, Bonifaz A, Alves M. (2021) Recent advances on mycotic keratitis caused by dematiaceous hyphomycetes. *Journal of Applied Microbiology*.
- White T J, Bruns T, Lee S, Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. in PCR protocols: a guide to methods and applications. eds Innis M. A., Gelfand D. H., Sninsky J. J., White T. J. (Academic Press, Inc. New York, N.Y), 315–322
- Whitford HW, Schwartz WL, Richards H. (1989) *Exserohilum* dermal granulomas in a bovine. *Journal of Veterinary Diagnostic Investigation*, **1**, 78-81.

CAPÍTULO III:

Linfoma de células T na cavidade nasal de caprino

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Trabalho submetido à revista *Acta Scientiae Veterinariae*

(Qualis B1)

Linfoma de Células T na Cavidade Nasal de Caprino

T-cell Lymphoma in the Nasal Cavity in Goat

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ABSTRACT

Background: Lymphomas are considered uncommon in goats, being the multicentric form with the highest number of cases for the species. Primary intranasal lymphomas are often diagnosed in dogs, cats, and humans. In the literature, there is only a description of a multicentric case involving the frontal sinuses and mucosa of the nasal cavity in a goat; therefore, it is important to describe unusual cases of this disease for the inclusion of new clinical and pathological characteristics in the ruminant clinic medicine. The objective of this work is to describe a case of T-cell lymphoma in the nasal cavity of a young goat.

Case: The animal had dyspnea and respiratory noise for 15 days. Clinical examination showed nodulation in the right nasal cavity associated with serosanguinous secretion. Tracheostomy was performed; however, after 30 days the animal was euthanized. A sagittal

plane of the head showed a pinkish-gray mass in the right and left nasal cavity, with a smooth, multilobulated surface, smooth adhering to the rostral portion of the dorsal concha and occluding the dorsal nasal meatus. Submandibular lymph nodes were slightly enlarged. Microscopically, round cells proliferated in the submucosa and nasal mucosa. In submandibular lymph nodes, the neoplastic cells were located in the germinal centers. Immunohistochemistry showed positive immunostaining for Vimentin antibodies and CD3, and negative for pan CK and CD20.

Discussion: Lymphomas in the caprine species are rare in the Northeastern semi-arid, but that in the present diagnostic routine occasionally occurs, being important the first description of its nasal shape for its inclusion in the differential diagnoses of diseases that present with clinical obstruction and dyspnea for the species.

Keywords: Hematopoietic neoplasia; Immunophenotyping; Lymphocytes; Dyspnea.

INTRODUÇÃO

Linfomas são neoplasias mesenquimais malignas que se originam frequentemente em órgãos hematopoiéticos sólidos, como os linfonodos, baço ou tecido linfoide associado a mucosa [14,18]. Em caprinos são considerados pouco frequente [3,10,16], sendo a forma multicêntrica com maior número de casos [10,8]. Já os linfomas intranasais primários são frequentemente diagnosticados em cães [15], gatos [9] e humanos [6].

Na literatura, há descrição de um caso multicêntrico com envolvimento dos seios frontais e mucosa da cavidade nasal [8] em caprino, com isso torna-se importante a descrição de casos incomuns desta doença para a inclusão de novas características clínico-patológicas da enfermidade na clínica médica de ruminantes. Objetiva-se com este trabalho descrever um caso de linfoma de células T na cavidade nasal de um caprino jovem.

RELATO DE CASO

O caso ocorreu em um caprino, fêmea com um ano de idade da raça Anglo Nubiano que apresentou dispneia e ruído respiratório há aproximadamente 15 dias. Ao exame clínico, observou-se nodulação na cavidade nasal direita associada à secreção serossanguinolenta. O nódulo era avermelhado e macio com superfície irregular, medindo aproximadamente 1 x 1 cm. No exame radiográfico da cabeça não foi observada comprometimento do tecido ósseo. Por meio de excisão cirúrgica, fragmento do nódulo foi encaminhado para exame histopatológico. Amostra do nódulo foi fixada em formol a 10% tamponado, processado rotineiramente para histopatologia e coradas por Hematoxilina e Eosina (HE).

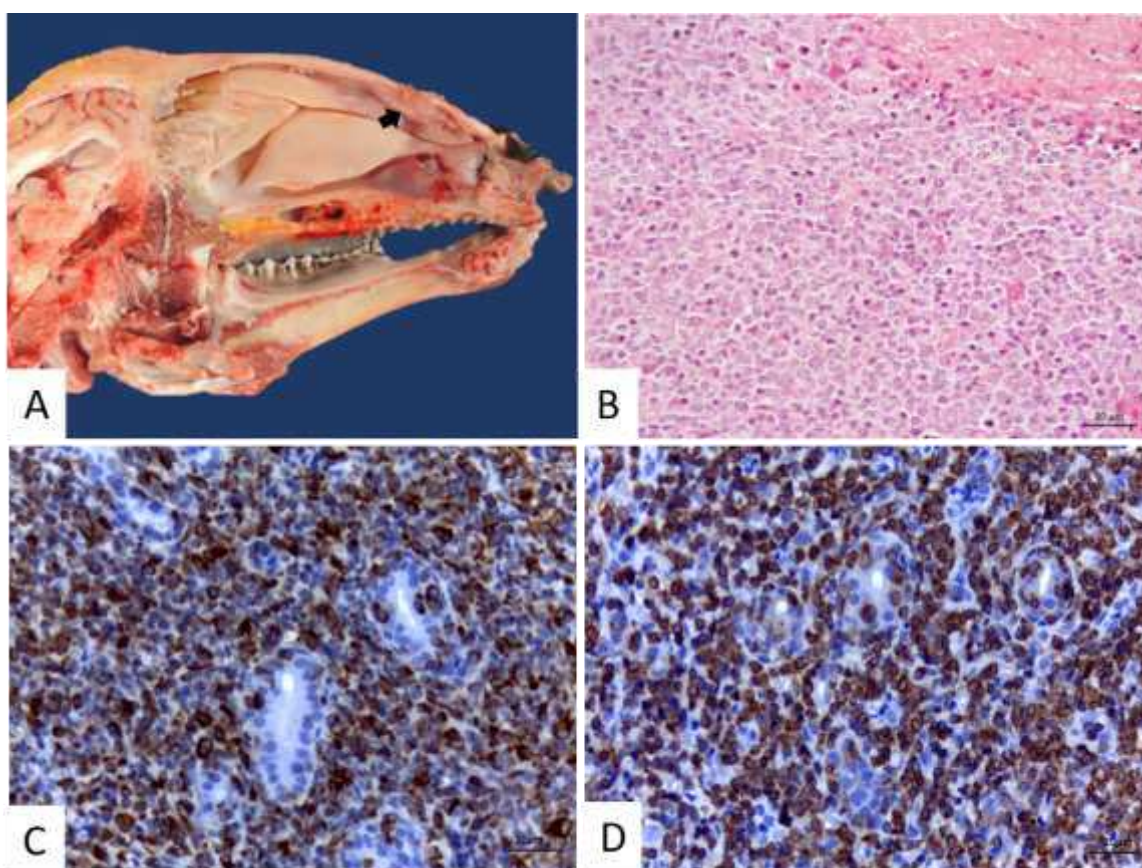


Figura 1 - Linfoma nasal em um caprino. A) Cavidade nasal esquerda, observa-se massa multilobulada, bem delimitada e rósea-acinzentada, localizada na porção rostral da concha nasal dorsal (seta). B) Cavidade nasal, observa-se proliferação de células redondas dispostas em manto compacto apoiadas em discreto estroma fibrovascular. O pleomorfismo é moderado as mitoses frequentes. No canto superior direito há área de necrose. HE. Bar=20µm. C) Cavidade nasal, imunomarcção positiva nas células neoplásicas utilizando o anticorpo Vimentina. IHC. DAB Bar=20µm. D) Cavidade nasal, imunomarcção positiva nas células neoplásicas utilizando o anticorpo CD3. IHC. DAB Bar=20µm.

No exame histopatológico da biópsia da cavidade nasal, observou-se massa tumoral não encapsulada, pouco delimitada e ulcerada composta por células redondas dispostas em manto apoiadas em discreto estroma fibrovascular distendendo a mucosa e lâmina própria. As células eram arredondas com citoplasma escasso, eosinofílico e pouco delimitado. Os núcleos variavam de redondos a alongados com cromatina condensada e nucléolos evidentes. Ocasionalmente visualizavam-se núcleos aberrantes, formato reniforme e células multinucleadas. O pleomorfismo era moderado caracterizado por anisocitose e anisocariose. As mitoses típicas e atípicas eram frequentes (0-4 por campo de maior aumento [400x]). Em meio a neoplasia observavam-se áreas multifocais de necrose e hemorragia associado a discreto infiltrado inflamatório linfocítico.

Após a biópsia o animal apresentou evolução da dispneia, sendo realizado procedimento de traqueostomia, no entanto após 30 dias de internamento, o quadro clínico piorou e foi eutanasiado.

Macroscopicamente, ao corte sagital da cabeça, observou-se massa multilobulada, rósea-acinzentada e macia com superfície lisa, medindo 5 x 4 x 2cm. A massa era fortemente aderida a porção rostral da concha dorsal e ocluindo o meato nasal dorsal nas cavidades nasais direita e esquerda (Fig. 1A). Microscopicamente a massa da cavidade nasal era semelhante a observada no nódulo da biópsia (Fig. 1B). Os linfonodos submandibulares estavam discretamente aumentados de volume e no exame histopatológico, observou-se células neoplásicas, semelhantes às descritas na cavidade nasal, localizadas principalmente nos centros germinativos.

Para confirmação do diagnóstico histopatológico, foi realizada a imuno-histoquímica de fragmento da massa da cavidade nasal. Foram utilizados os anticorpos Vimentina (clone v9), CK Pan (clone AE1AE3) e os policlonais CD3 e CD 20. Houve imunomarcagem positiva

nas células neoplásicas para os anticorpos Vimentina e CD3 (Fig 1C e 1D), foram negativos para os anticorpos CK Pan e CD20.

DISCUSSÃO

O diagnóstico de linfoma de células T nasal foi realizado com base nos achados histopatológicos e confirmado através da imuno-histoquímica. Os linfomas em caprinos são considerados incomuns [15] e geralmente acometem animais de meia idade ou idosos [12]. Entretanto, Kiser & Löhr [8], descreveu casos da neoplasia em animais jovens, conforme observado nesse caso, onde o animal apresentava um ano de idade.

Os linfomas apresentam sinais clínicos inespecíficos e variáveis, de acordo com a localização e o grau de comprometimento do órgão [8,15,17], sendo frequentemente diagnosticados em cães [14], gatos [9] e humanos [6]. No caso descrito, o quadro de dispneia grave foi justificado pela obstrução parcial da porção rostral de ambas as cavidades nasais.

Nos caprinos a forma frequente dos linfomas são os multicêntricos [4,8,15,17], em que geralmente são afetados múltiplos linfonodos superficiais e profundos, estando associado a quadros clínicos inespecíficos como exoftalmia e úlcera de córnea [16], envolvimento de ovário [4], pulmão [15] e comprometimento do canal medular [2,3].

Exames de imagem, como a radiografia, foi pouco esclarecedora, já que a neoplasia comprometia apenas o tecido mole (mucosa e lâmina própria), embora tenha demonstrado ausência de envolvimento ósseo. Nestes casos a utilização de tomografia e/ou ressonância são ferramentas de diagnóstico eficientes na identificação de alterações em tecidos moles [16] no entanto, ainda são técnicas de diagnóstico pouco disseminadas na medicina veterinária e com alto custo principalmente para animais de produção.

A imunofenotipagem de linfomas é pouco utilizada quando se trata de animais de produção, sendo poucos os estudos que utilizam esta técnica para o diagnóstico definitivo

dessas neoplasias para os pequenos ruminantes [1,8,15,16]. A utilização desta técnica deve ser considerada em cada caso, afim de determinar a patogenia, o diagnóstico acurado [7,17] e a origem dos linfócitos neoplásicos [17]. Nos caprinos os linfomas de células T são os mais diagnosticados [8,15], embora casos de linfomas de células B multicêntrico com envolvimento ocular tenham sido diagnosticados [16].

Diante do quadro clínico do caso descrito, deve ser incluído como diagnósticos diferenciais as rinites infecciosas já descritas em caprinos, como a aspergilose [5] e a prototecose [11]. Entretanto os achados anatomopatológicos facilitam o direcionamento do diagnóstico, já que as rinites infecciosas apresentam-se como nódulos/massas ulceradas ou áreas focalmente extensas de necrose associada à secreção purulenta e no exame histopatológico é possível observar os agentes etiológicos intralésionais. Além disso, deve ser incluído o tumor etmoidal enzoótico [18] já que cursa com sinais clínicos semelhantes e afeta animais jovens, porém são adenomas/adenocarcinomas que afetam as conchas nasais etmoidais induzidos por um retrovírus.

Conclui-se que os linfomas na cavidade nasal de caprinos são pouco frequentes e devem ser incluídos como diagnóstico diferenciais de enfermidades que cursam com quadro clínico de obstrução e dispneia para a espécie.

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REFERÊNCIAS

- 1 Anjos B.L., Trost M.E., Diefenbach A., Gressler L.T. & Irigoyen L.F. 2010** Linfossarcoma de células B multicêntrico em uma ovelha. *Acta Scientiae Veterinariae*. 38(3): 315-318.
- 2 Braun U., Tschuor A.C. & Sydler T. 2011.** Nachhandparese infolge eines malignen Lymphoms bei einer Ziege. *Schweizer Archiv für Tierheilkunde*, 153(3):123-126.
- 3 Carvalho F.K.L., Dantas A.F.M., Riet-Correa F., Andrade R.L.F.S., Nóbrega Neto P.I., Miranda Neto E.G., Simões S.V.D. & Azevedo S.S. 2014.** Estudo retrospectivo das neoplasias em ruminantes e equídeos no semiárido do Nordeste Brasileiro. *Pesquisa Veterinária Brasileira*. 34(3):211-216.
- 4 DiGrassie W.A., Wallace M.A. & Sponenberg D.P. 1997.** Multicentric lymphosarcoma with ovarian involvement in a Nubian goat. *The Canadian Veterinary Journal*. 38(6):383-384.
- 5 Do Carmo P.M.S., Portela R.A., de Oliveira-Filho J.C., Dantas A.F.M., Simões S.V.D., Garino Júnior F. & Riet-Correa F. 2014.** Nasal and cutaneous aspergillosis in a goat. *Journal of comparative pathology*. 150(1):4-7.
- 6 Fernandes M., Peixoto S., Da Silva N., Libório-Kimura T., De Lima L. & De Oliveira J. 2015.** Linfoma extranodal tipo nasal de células T/Natural Killer acometendo mucosa oral de paciente com histórico de leishmaniose. *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial*. 56: 251-5.
- 7 Jacobs R.M., Messick J.B. & Valli V.E. 2002.** Tumors of the hemolymphatic system. In: Meuten D.J. (Ed.). *Tumors in Domestic Animals*. 4th. Iowa State Press, Ames. pp.119-198.
- 8 Kiser P.K. & Löhr C.V. 2017.** Lymphoma Classification in Goats. *Veterinary Pathology*. 54(4):611-619.
- 9 Little L., Patel R. & Goldschmidt M. 2007.** Nasal and Nasopharyngeal Lymphoma in Cats: 50 Cases (1989–2005). *Veterinary Pathology*. 44(6): 885–892.

- 10 Löhr C.V. 2012.** One Hundred Two Tumors in 100 Goats (1987–2011). *Veterinary Pathology*. 50(4): 668-675.
- 11 Macêdo J.T., Riet-Corrêa F., Dantas A.F.M. & Simões S.V. 2008.** Cutaneous and Nasal Protothecosis in a Goat. *Veterinary Pathology*. 45(3): 352-354.
- 12 Mazaro R.D., Rizkallah I.P.J., Luz F.S., Lorensetti D.M., Cogliati B. & Fighera R.A. 2018.** Aspectos epidemiológicos, clínicos e anatomopatológicos do linfoma follicular em cães. *Pesquisa Veterinária Brasileira*. 38(9):1772-1780.
- 13 Panziera W., Bianchi R.M., Faccin T.C., Galiza G.J.N., Lopes E.M.B., Kommers G.D. & Fighera R.A. 2016.** Classificação de 86 casos de linfoma em bovinos de acordo com a *Working Formulation (WF) of Non-Hodgkin's Lymphomas for Clinical Usage e The Revised European-American Classification of Lymphoid Neoplasms (REAL)*. *Pesquisa Veterinária Brasileira*. 36(4):263- 271.
- 14 Rodrigues R.T.G.A., Medeiros V.B., Borges I.L., De Oliveira Z.C.R., Batista J. S. & Filgueira K.D. 2016.** Linfoma intranasal em cão: aspectos clínicopatológicos e imunoistoquímicos. *Acta Veterinaria Brasilica*. 10(1): 84-91.
- 15 Rozear L., Love N.E. & Van Camp S.L. 1998.** Radiographic diagnosis: Pulmonary lymphosarcoma in a goat. *Veterinary Radiology & Ultrasound*. 39:528–531.
- 16 Valentine B.A., Stieger-Vanegas S., Brown S.R., Tornquist S. J. & Young K. 2011.** Exophthalmos due to multicentric B-cell lymphoma in a goat. *Canadian veterinary journal = La revue veterinaire Canadienne*. 52(12):1350-1352.
- 17 Valli V.E.O., Kiupel M., Bienzle D. & Wood R.D. 2016.** Hematopoietic System. In: Maxie M.G. (Ed). *Jubb, Kennedy, and Palmer's pathology of domestic animals*. Vol. 3. **6Th**. St Louis, Missouri: Elsevier. pp. 102-268.
- 18 Wanderley G.G. 2009.** Processos etmoidais em ovinos. *Medicina Veterinária (UFRPE)*. 3(1):37-46

CAPÍTULO IV:

Nasal Leiomyosarcoma in Equine

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Trabalho submetido à revista Ciência Rural
(Qualis A4)

(Original Article)

Nasal Leiomyosarcoma in Equine

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ABSTRACT

We describe the clinical and anatomopathological findings observed in a case of nasal leiomyosarcoma in a Quarter Mile horse, a five-year-old male, whose main complaints were decreased sports performance and bilateral purulent nasal discharge. A mass was observed in the nasal cavity, obstructing the left nostril and associated with a purulent drainage. The mass was of an irregular, yellowish, shiny, fibroelastic surface, measuring 19.4 x 6.9 x 4.3 cm in size, with coalescent multifocal areas, brownish, friable, opaque and fetid. Histopathological examination showed spindle-shaped neoplastic cells, which stained red in Masson's Trichrome stain. A diagnosis of leiomyosarcoma was established based on the morphotintorial aspects of neoplastic cells and confirmed through immunohistochemistry, with positive immunostaining for antibodies 1A4, HHF35, Desmin and S100. Leiomyosarcoma affects primarily the nasal cavity of horses, and should be included in the differential diagnosis of diseases that affect the nasal cavity and cause nasal obstruction associated with dyspnea.

Key words: Nasal obstruction, dyspnea, desmin, soft tissue sarcoma.

RESUMO

Descreve-se os achados clínicos e anatomopatológicos observados em um caso de leiomiossarcoma nasal em um equino, Quarto de Milha, macho de cinco anos de idade, com queixa principal de diminuição do rendimento esportivo e secreção nasal purulenta bilateral. Na cavidade nasal, observou-se massa obstruindo a narina esquerda associada à secreção purulenta. A massa era de superfície irregular, amarelada, brilhante, fibroelástica, medindo 19,4 x 6,9 x 4,3cm de tamanho, com áreas multifocais a coalescente acastanhadas, friáveis, opacas e fétidas. No exame histopatológico foi observado células neoplásicas fusiformes, que coraram em vermelho na coloração de Tricrômico de Masson. O diagnóstico de leiomiossarcoma foi estabelecido com base nos aspectos morfotintoriais das células neoplásicas e confirmado através da imuno-histoquímica, no qual houve imunomarcagem positiva para os anticorpos 1A4, HHF35, Desmina e S100. O leiomiossarcoma pode afetar primariamente a cavidade nasal de equinos, devendo ser incluído no diagnóstico diferencial de doenças que afetam a cavidade nasal e que provocam quadros de obstrução nasal associado a dispneia.

Palavras-chave: Obstrução nasal, dispneia, desmina, sarcoma de tecidos moles.

INTRODUCTION

Tumors in the nasal cavity and paranasal sinuses of horses are uncommon (LÓPEZ & MARTINSON, 2017), and can originate from the epithelial, glandular, vascular, bone, cartilaginous and fibrous connective tissue (HEAD & DIXON, 1999). They generally have an insidious onset with slow growth and their main clinical signs are nasal discharge, epistaxis and dyspnea (NICKELS, 1993).

Neoplasms are described in sporadic cases, mainly squamous cell carcinomas (data not yet published), fusiform and osteogenic sarcomas, lymphosarcomas, poorly differentiated carcinomas, adenocarcinomas, fibromas, fibropapillomas, chondromas and osteomas

(NICKELS, 1993; KNOTTENBELT *et al.*, 2015; LÓPEZ & MARTINSON, 2017). The ethmoidal hematoma, the most common nasal proliferative lesion in horses, is not considered a true neoplasm, being a progressive syndrome which obstructs the nasal cavity in its caudal portion (WILSON, 2017).

Leiomyosarcoma is a neoplasm that originates from smooth muscle and commonly affects the gastrointestinal tract and genital system of dogs and humans (COOPER & VALENTINE, 2017). In horses, this neoplasm has been described as affecting the gastrointestinal tract (OREFF *et al.*, 2017), urogenital tract (HURCOMBE *et al.*, 2008), eyes (GROSÅS *et al.*, 2017), ovary (PINNA *et al.*, 2019), guttural pouch (DREW *et al.*, 2016), lungs (ROSSDALE *et al.*, 2004) and pelvic members (GRACCHI *et al.*, 2020), and also occurs in a multicentric way (KAWABATA *et al.*, 2016). However, there are no data on the primary involvement of the nasal cavity in the equine species. Thus, the objective of this work was to describe the clinical and pathological characteristics of the primary leiomyosarcoma of the nasal cavity in horses diagnosed in the hinterland of Paraiba, Northeastern Brazil.

MATERIAL AND METHODS

A horse with dyspnea was examined at the University Veterinary Hospital of the Federal University of Campina Grande. Radiographic examinations of the head and surgical resection were performed using mass trephination in the nasal cavity.

Tissue fragments were fixed in 10% buffered formalin, processed routinely and stained with hematoxylin and eosin (HE) for histological evaluation. Masson's Trichrome histochemical method was also used to demonstrate the morphotintorial characteristics of the cells.

Paraffin blocks were sent for immunohistochemistry (IHC) using primary antibodies 1A4, HHF35, desmin, S100, GFAP, MyoD1 and CD31. Histological sections were placed on

previously silanized slides. Antigenic recovery was performed by the method of moist heat under pressure for 20-30 min. The primary antibodies were incubated for 12 hours at 4°C. The polymer detection system (Advance Dako, Carpinteria, CA, USA) and chromogen 3,3'-diaminobenzidine (Liquid DAB + Substrate-Chromogen System, Dako Carpinteria, CA, USA) with Harris hematoxylin were used.

RESULTS

One horse, Quarter Horse, a five-year-old male, presented with the main complaint of decreased sports performance and bilateral purulent nasal discharge, more prominent in the left nostril, with approximately six months of evolution. Clinical examination revealed breathing difficulty and a slight increase in the volume of the left nostril. In the nasal cavity, a mass was observed obstructing the left nostril associated with purulent secretion. The radiography of the head showed no bone involvement.

During surgery, a mass adhered to the left nasal cavity was found, affecting the dorsal, ventral and ethmoidal shells, which was dissected and removed. The mass was sent for histopathological examination.

Macroscopically, the mass had an irregular, yellowish, shiny, fibroelastic surface, measuring 19.4 x 6.9 x 4.3 cm in size, with brownish, friable, opaque and fetid multifocal coalescent areas (Figure 1A). When cut, the surface was compact, grayish-white and smooth with yellow, friable, irregular multifocal areas, measuring 1-3.2 cm in diameter.

Upon histopathological examination, an ulcerated, infiltrative, little-delimited, non-encapsulated tumor mass was observed, composed of spindle-shaped mesenchymal cells arranged in compact bundles in several directions supported by a scarce stroma of fibrous connective tissue associated with rare blood vessels, distending the lamina propria and dissecting Bowman's glands. Neoplastic cells were elongated with eosinophilic cytoplasm,

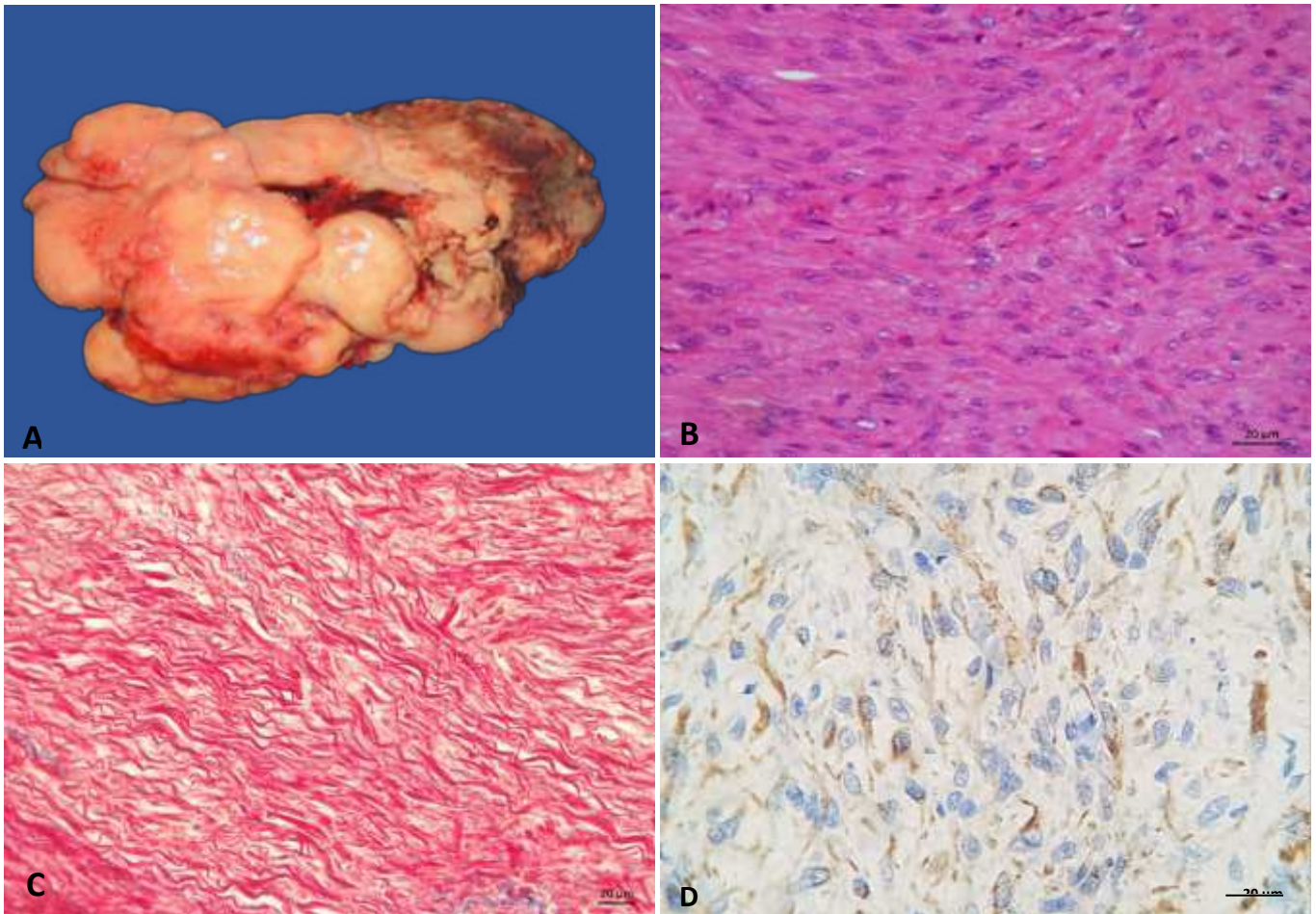


Figure 1 - Nasal leiomyosarcoma in horse. A) Biopsy of the nasal cavity, a mass with an irregular, yellowish, shiny, fibroelastic surface and multifocal brown coalescent areas, friable and opaque. B) Nasal cavity, elongated neoplastic cells arranged in bundles with eosinophilic cytoplasm, homogeneous and poorly delimited. The nuclei are elongated with finely granular to condensed chromatin and little evident nucleoli. HE. Bar = 20 μ m. C) Nasal cavity, neoplastic cells stained red are visualized. Masson's trichrome. Bar = 20 μ m. D) Nasal cavity, positive immunostaining using the Desmin antibody, on neoplastic cells. *IHQ*. Bar = 20 μ m.

homogeneous and poorly delimited. The nuclei were elongated, with finely granular to condensed chromatin and little evident nucleoli. Multinucleate cells were observed. The pleomorphism was accentuated and mitoses were moderate (1 to 3 per field of greatest increase [400x]) (Figure 1B). Amid the tumor mass, multifocal areas of discreet mononuclear inflammatory infiltrate were observed, consisting predominantly of lymphocytes and plasma cells, in addition to extensive areas of necrosis.

The neoplastic cells stained red in Masson's special trichrome stain (Figure 1C), and in the stroma there was a discreet and multifocal marking in blue of the collagen fibers.

Neoplastic cells expressed moderate and diffuse labeling for antibodies 1A4, HHF35 and Desmin (Figure 1D), and discrete and irregular labeling for S100. They were negative for GFAP, MyoD1 and CD31.

DISCUSSION

The diagnosis of leiomyosarcoma in the nasal cavity was made based on the morphotintorial characteristics of neoplastic cells and confirmed by IHC. In this report, observation of the morphological characteristics of the neoplastic cells together with the use of Masson's Trichrome technique made it possible to direct the diagnosis to a tumor of muscular origin (RAMOS et al., 2008), but such special staining should not be used as a reliable indicator for differentiating spindle tumors (WILSON, 2017), since poorly differentiated fibrosarcomas produce little collagen and may show little reactivity (GROSS et al., 2009). Thus, use of IHC is essential to determine cellular origin.

Immunohistochemistry is an important diagnostic tool to determine the cellular origin of neoplasms due to its practicality, high sensitivity and specificity when compared to other diagnostic methods (WILSON, 2017). In the evaluated sections, there was moderate and diffuse cytoplasmic reactivity in the neoplastic cells for the antibodies Desmin, 1A4, and HHF35, thus confirming the muscular origin and specifying the smooth muscle type using markers 1A4 and HHF35, since the latter markers are specific for alpha-smooth muscle actin (DREW et al., 2016; GRACCHI et al., 2020)

Simultaneous positive immunostaining of neoplastic cells for smooth muscle alpha desmin and actin is strongly suggestive of tumors of smooth muscle origin; although striated muscle cells often express positive desmin marking, they are rarely positive for alpha smooth muscle actin (COOPER & VALENTINE, 2017). In addition, absence of immunostaining of neoplastic cells for the MyoD1 antibody reinforces cellular origin as smooth muscle. The low

reactivity of neoplastic cells with the S100 antibody was unforeseen, as observed by other authors (GROSÅS et al., 2017).

Neoplasms of the nasal cavity are uncommon in domestic animals, with the canine species being the most affected (LÓPEZ & MARTINSON, 2017). Sporadic cases of mesenchymal tumors such as fibrosarcomas have been reported in the nasal cavity of horses (SCHMOTZER et al., 1987; HULTGREN et al., 1987; KNOTTENBELT et al., 2015; LÓPEZ & MARTINSON, 2017), besides hemangiosarcomas (LÓPEZ & MARTINSON 2017), mastocytomas, osteosarcomas, osteomas and myxomas (LÓPEZ & MARTINSON, 2017).

The main differential diagnoses include polyps of inflammatory origin (TROTTE, 2008), ethmoidal hematoma (TROTTE, 2008), amyloidosis (TROTTE, 2008; PORTELA et al., 2012), fibrous osteodystrophy, (LÓPEZ & MARTINSON, 2017) and rhinitis infectious diseases such as rhinosporidiosis (NICKELS, 1993; TROTTE 2008), cryptococcosis (CRUZ et al., 2017), aspergillosis (GREET, 1981) and pythiosis (SOUTO et al., 2016).

In this case, one must also differentiate leiomyosarcoma from other neoplasms that have similar cellular characteristics, such as fibrosarcomas, tumors of the peripheral nerve sheath, rhabdomyosarcomas, undifferentiated sarcomas (WILSON, 2017) and myofibroblastic sarcoma (SILVA et al., 2012) from those in which immunohistochemical techniques must be used to determine the cellular origin of neoplastic cells.

CONCLUSION

The conclusion is that leiomyosarcoma can affect the nasal cavity of horses, and should be included in the differential diagnosis of diseases that affect the nasal cavity and cause nasal obstruction associated with dyspnea.

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DECLARATION OF INTEREST

We have no conflict of interest to declare.

AUTHORS 'CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

REFERENCE

COOPER, B. J.; VALENTINE, B. A. Tumors of muscle. In: MEUTEN, D. J. **Tumors in Domestic Animals**. 5th. Ames, Iowa: John Wiley & Sons Inc.,2017. Cap. 11. pp. 425-466.

CRUZ, R. A. S. et al. Equine nasopharyngeal cryptococcoma due to *Cryptococcus gattii*. **Ciência Rural**, v. 47, n.10, Aug. 2017. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S010384782017001000551&lng=en&nrm=iso. Accessed: 23/03/2020. Doi: 10.1590/0103-8478cr20170151.

DREW, S. J. et al. Guttural pouch leiomyosarcoma causing nasopharyngeal compression in a pony. **Equine Veterinary Education**, v. 30, n. 2, p. 64-69. July 2018. Available from: https://beva.onlinelibrary.wiley.com/doi/full/10.1111/eve.12607?casa_token=2cqmCUN0ZuIAAAAA%3A_P0gv2bvpzJZxOMnI-iBVwrzoUdOjix1xbR4K1EBLRXdm1g5ANoxYCztbwSa8HfI1-grRYgvF0sxLMJJ Accessed: 12/03/2020. Doi: 10.1111/eve.12607.

GIACCHI, A. et al. An atypical presentation of leiomyosarcoma causing extremity compartment syndrome of the crural region in a Dutch Warmblood mare: a case report. **Journal of veterinary science**. v.21, n.1, Jan 2020. Available from: <https://synapse.koreamed.org/upload/SynapseData/PDFData/0118JVS/jvs-21-e3.pdf> Accessed: 06/04/2020. Doi: [10.4142/jvs.2020.21.e3](https://doi.org/10.4142/jvs.2020.21.e3).

GREET, T. R. Nasal aspergillosis in three horses. **The Veterinary Record**, v. 109, n. 22, p. 487-489, Out 1981. Available from: <https://europepmc.org/article/med/7034361> Accessed: 06/02/2021. Doi: 10.1136 / vr.109.22.487.

GROSÅS, S. et al. Uveal myxoid leiomyosarcoma in a horse. **Clinical case reports**, v. 5, n. 11, p. 1811, Set 2017. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5676260/> Accessed: 06/02/2021. Doi: 10.1002 / ccr3.1190.

GROSS, T.L. et al. Tumores fibrosos. In:_____. **Doenças da pele do cão e do gato: diagnóstico clínico e histopatológico**. 2 ed. São Paulo: Roca. 2009. Cap. 27. p. 694-717.

HEAD, K. W.; DIXON, P. M. Equine nasal and paranasal sinus tumours. Part 1: review of the literature and tumour classification. **The Veterinary Journal**, v.157, n. 3, p. 261-279, 1999. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1090023398903707> Accessed: 06/04/2020. Doi:

HULTGREN, B. D. et al. Nasal-maxillary fibrosarcoma in young horses: a light and electron microscopic study. **Veterinary pathology**, v. 24, n. 2, p.194-196, 1987. Available from: <https://journals.sagepub.com/doi/pdf/10.1177/030098588702400218> Accessed: 06/04/2020. Doi: 10.1177/030098588702400218.

HURCOMBE, S. D. A. et al. Poorly differentiated leiomyosarcoma of the urogenital tract in a horse. **Journal of the American Veterinary Medical Association**, v. 233, n.12, p.1908-1912, Nov 2008. Available from: <https://avmajournals.avma.org/doi/pdf/10.2460/javma.233.12.1908> Accessed: 12/03/2020. Doi: 10.2460/javma.233.12.1908.

KAWABATA, A. et al. Metastatic leiomyosarcoma causing ataxia in a horse. **Journal of Equine Veterinary Science**, v. 43, p. 23-27, Aug 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0737080616300594> Accessed: 29/03/2021. Doi Doi: 10.1016/j.jevs.2016.04.009.

KNOTTENBELT, D. C., et al. Smooth muscle and skeletal muscle neoplasms. In:_____. **Clinical equine oncology**. China:Elsevier. 2015. p.307-308.

LOPEZ, A. & MARTINSON, S.A. Respiratory System, Mediastinum, and Pleurae. In: Zachary J.F. (Eds). **Pathologic Basis of Veterinary Disease**. 6th. St. Louis Missouri: Elsevier. 2017. p. 471-560.

NICKEL, F.A. Diseases of the nasal cavity. **The Veterinary Clinics of North America. Equine Practice**. v.9, n.1, p.111- 121, Mar 1993. Available from: <https://europepmc.org/article/med/8472195> Accessed: 11/02/2020. Doi: 10.1016/s0749-0739(17)30418-2.

OREFF, G. L. et al. Successful removal of jejunal leiomyosarcoma in a Quarter Horse mare. **Equine Veterinary Education**, v. 30, n. 11, p. 458-462, June 2018. Available from: <https://beva.onlinelibrary.wiley.com/doi/abs/10.1111/eve.12769> Accessed: 12/03/2020. Doi: 10.1111/eve.12769.

PINNA, A. E. et al. Double ovarian tumour in the mare: Case report. **Reproduction in Domestic Animals**, v. 54, n. 6, p. 912-916, 2019. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/rda.13433> Accessed: 06/02/2021. Doi: 10.1111/rda.13433.

PORTELA, R. A. et al. Amiloidose nasal em um cavalo. **Brazilian journal veterinary pathology**, v. 5, p. 86-88, July 2012. Available from: https://www.researchgate.net/profile/Franklin_Riet-Correa/publication/268435867_Nasal_Amyloidosis_in_a_Horse/links/57043f1d08ae74a08e24621f.pdf Accessed: 06/02/2021. Doi:

RAMOS, A.T. et al. Tumores em animais de produção: aspectos comparativos. **Ciência Rural**, v.38, n.1, p.148-154, Feb 2008. Available from: https://www.scielo.br/scielo.php?pid=S0103-84782008000100024&script=sci_arttext&tlng=pt Accessed: 06/02/2021. Doi: 10.1590/S0103-84782008000100024.

ROSSDALE, P. D. et al. Pulmonary leiomyosarcoma in a 13-year-old Thoroughbred stallion presenting as a differential diagnosis to recurrent airway obstruction. **Equine Veterinary Education**, v.16, n.1, p. 21-28, 2004. Available from: <https://beva.onlinelibrary.wiley.com/doi/epdf/10.1111/j.2042-3292.2004.tb00263.x> Accessed: 06/02/2021. Doi: 10.1111/j.2042-3292.2004.tb00263.x.

SCHMOTZER, W. B. et al. Nasomaxillary fibrosarcomas in three young horses. **Journal of the American Veterinary Medical Association**, v. 191, n. 4, p. 437-439, July 1987. Available from: <https://europepmc.org/article/med/3654318> Accessed: 06/02/2021. Doi:

SILVA, J. F. et al. Myofibroblastic Sarcoma in the Limb of a Horse. **Journal of Equine Veterinary Science**, v. 32, n. 4, p. 197-200, April 2012. Available from: <https://www.sciencedirect.com/science/article/pii/S0737080611005089> Accessed: 17/03/2021. Doi: 10.1016/j.jevs.2011.08.019

SOUTO, E. P. F. et al. Pythiosis in the nasal cavity of horses. **Journal of comparative pathology**, v. 155, n. 2-3, p. 126-129, Out 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0021997516300603?via%3Dihub> Accessed: 29/03/2021. Doi: 10.1016/j.jcpa.2016.06.005.

TROTTE, M. N. S. et al. Histopatologia de lesões tumoriformes presentes na cavidade nasal de equídeos do Brasil. **Ciência Rural**, v. 38, n. 9, p. 2535-2539, 2008. Available

from:https://www.scielo.br/scielo.php?pid=S0103-84782008000900019&script=sci_arttext
Accessed: 29/03/2021. Doi: 10.1590/S0103-84782008005000004.

WILSON, D. W. Tumors of the Respiratory Tract. In: MEUTEN, D. J. **Tumors in Domestic Animals**. 5th . Ames, Iowa: John Wiley & Sons Inc.,2017. Cap. 12. pp. 467-498.

CONSIDERAÇÕES FINAIS

O Laboratório de Patologia Animal, do Hospital Veterinário Universitário Prof. Dr. Ivon Macedo Tabosa, da Universidade Federal de Campina Grande, Patos, Paraíba presta um importante serviço de diagnóstico nas diferentes espécies de animais, principalmente na região semiárida da Paraíba. Com relação às doenças diagnosticadas na cavidade nasal de ruminantes e equídeos, verifica-se que todas cursam com sinais clínicos semelhantes e inespecíficos, como dispneia, ruído respiratório, secreção nasal, massas obstruindo a passagem de ar, assimetria e deformidade de face, o que dificulta o diagnóstico clínico e macroscópico.

Para tanto, a realização do exame histopatológico constitui uma importante ferramenta de diagnóstico, seja para confirmar ou descartar um diagnóstico clínico, além de sugerir uma nova condição. Na maioria das doenças observadas neste estudo o diagnóstico só foi possível através da avaliação *post mortem*, mas que também devem fazer parte no momento do estabelecimento dos diagnósticos diferenciais.

A imuno-histoquímica foi fundamental para a confirmação de agentes previamente identificados no exame histopatológico, uma técnica de fácil aplicação e amplamente utilizada na patologia veterinária. Além disso, as técnicas microbiológicas e moleculares devem ser empregadas quando há envolvimento de agentes infecciosos, principalmente aqueles que cursam com doenças raras e que pouco se sabe sobre sua patogenia.

O estabelecimento e a divulgação da variedade de enfermidades, sejam elas infecciosas ou neoplásicas aqui observadas, que podem acometer animais de produção têm por objetivo auxiliar médicos veterinários clínicos e patologistas à campo no estabelecimento de diagnóstico das enfermidades que afetam a cavidade nasal destes animais, bem como no estabelecimento de medidas de controle e prevenção, melhorando desta forma a qualidade e expectativa de vida desses animais e diminuindo os prejuízos ao produtor rural.