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Original Article
Livestock Diseases

Conidiobolomycosis in goats[1]

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ABSTRACT.- Macêdo J.T.S.A., Biscarde C.E., Pescador C.A., Nakazato L., Fonseca N.D-S., Ubiali D.G., Riet-Correa F. & Pedroso P.M.O. 2021. **Conidiobolomycosis in goats.** *Pesquisa Veterinária Brasileira 41:e06978, 2021*. Laboratório de Patologia Veterinária, Fundação Universidade de Brasília, Campus Universitário Darcy Ribeiro, Via L4 Norte s\n, Brasília, DF 70910-970. E-mail: pedrosovet@yahoo.com.br

The present article presents cases of conidiobolomycosis in adult goats with clinical signs characterized by serous nasal discharge, dyspnea, apathy, and weight loss. Two goats were necropsied. Necropsy displayed increased volume on the sagittal section of the head and an ulcerated surface containing a yellow friable mass with irregular and granular consistency in the nasal septum and in the ventral nasal turbinate. One goat also presented lesions on the ear's skin and the right pelvic limb. Microscopically, lesions were characterized by multifocal granulomas with a central necrotic area containing non-stained fungal hyphae images surrounded by a granulomatous infiltrate. Samples of the lesions examined by immunohistochemistry and polymerase chain reaction were positive for *Conidiobolus lamprauges*. This is the first report of conidiobolomycosis in goats, and the disease should be considered in the differential diagnoses for rhinitis and dermatitis in goats.

INDEX TERMS: Conidiobolomycosis, goats, granulomatous rhinitis, dermatitis, immunohistochemistry, PCR.

RESUMO.- [**Conidiobolomicose em caprinos.**] O presente artigo apresenta casos de conidiobolomicose em cabras adultas com sinais clínicos caracterizados por secreção nasal serosa, dispneia, apatia e perda de peso. Dois caprinos foram necropsiados. Na necropsia, em corte sagital da cabeça, foi observado aumento de volume e superfície ulcerada contendo massa amarela e friável com consistência irregular e granular no septo nasal e conchas nasais ventrais. Uma cabra apresentou também lesões na pele da orelha e no membro pélvico direito. Microscopicamente, as lesões foram caracterizadas por granulomas multifocais com área central de necrose, contendo imagens de hifas fúngicas não coradas, circundadas por infiltrado inflamatório granulomatoso. Amostras das lesões submetidas à imuno-histoquímica e reação em cadeia da polimerase foram positivas para *Conidiobolus lamprauges*. Este é o primeiro registro de conidiobolomicose em caprinos e deve ser considerado no diagnóstico diferencial de rinite e dermatite em caprinos.

TERMOS DE INDEXAÇÃO: Conidiobolomicose, caprinos, rinite granulomatosa, dermatite, imuno-histoquímica, PCR.

INTRODUCTION

Conidiobolus spp. a fungus of the class Zygomycetes, order Entomophthorales, causes zygomycosis, a chronic invasive fungal rhinosinusitis, affecting both animals and humans, mainly in tropical countries (Vilela et al. 2010, Pestana et al. 2019). *Conidiobolus* spp. are saprophytes fungi found mainly in soil, decaying vegetation, and insects of tropical and subtropical regions (Kimura et al. 2011, Carmo et al. 2015). *Conidiobolus coronatus*, *C. lamprauges* and *C. incongruus* have already been identified as a disease cause in animals (Carrigan et al. 1992, Silva et al. 2007a, 2007b, Morris et al. 2001). Sheep display

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two clinical forms of the disease caused by *Conidiobolus*. The rhinofacial form is characterized by a volume increase in the nasal vestibule, cutaneous mucus union of the nose, the skin of the anterior forehead region, and the higher lip. Another form is called nasopharyngeal, as it mainly affects the ethmoidal region, pharynx, and paranasal sinuses (Silva et al. 2007a). Nasopharyngeal ringworm displays grainy surface nodules, yellow or white, with necrosis of the ethmoidal region. Microscopically there is multifocal chronic granulomatous inflammation, with a necrotic center containing large hyphae surrounded by Splendore-Hoeppli material (Morris et al. 2001), fibrovascular tissue, lymphocytic infiltration, neutrophils, eosinophils, epithelioid macrophages, and multinucleated giant cells (Morris et al. 2001, Riet-Correa 2007). The current study describes the epidemiological, clinical, pathological, immunohistochemistry, and molecular findings in goats affected by rhinofacial and dermatitis by *C. lamprauges*.

MATERIALS AND METHODS

Two goats "Parda Alpina" breed (Cases 1 and 2), 3-year-old, kept under semi-extensive breeding, were necropsied after showing respiratory clinical signs and no response to treatment. Case 1 was euthanized *in extremis* according to the "Brazilian guide for the care and use of animals," and Case 2 died of infection. During the necropsy, tissue samples from the rhinofacial region and other organs (brain, heart, lungs, kidneys, spleen, liver, skin, and lymph nodes) were collected and fixed in buffered 10% formalin, processed routinely for histology, and stained with hematoxylin-eosin (HE). Rhinofacial tissues were stained with Grocott's Methenamine Silver stain (GMS). Epidemiological information and clinical signals were provided by the farmers.

Rhinofacial tissues of Case 1 were submitted for immunohistochemistry (IHC) to identify *Conidiobolus lamprauges* and *Pythium insidiosum* using polyclonal antibodies against the two agents. These antibodies were prepared (Ubiali et al. 2013) by subcutaneous inoculation in rabbits of exoantigens from purified cultures of *C. lamprauges* (previously verified by PCR) (De Paula et al. 2010) or zoospores of *P. insidiosum* (previously verified by PCR) (Azevedo et al. 2012). Antibodies on rabbit sera were confirmed by immunodiffusion and enzyme-linked immunosorbent assay (ELISA), respectively (Santurio et al. 2006, Ubiali et al. 2013). The IHC tests for *C. lamprauges* were performed as previous protocols (Ubiali et al. 2013).

The formalin-fixed paraffin-embedded tissue samples nasal lesions from Case 2 were examined by polymerase chain reaction (PCR) for *C. lamprauges* (De Paula et al. 2010) and *P. insidiosum* (Azevedo et al. 2012).

RESULTS

The first case occurred in April 2012, in the municipality of Conceição do Coité (11°33'50" S, 39°16'58" W), in the semiarid region of the state of Bahia, northeastern Brazil. The goat had displayed serous nasal discharge, dyspnea, stridor, and weight loss during the last three months. The clinical course was approximately three months. The goat was unsuccessfully treated with tetracycline, and the goat was euthanized *in extremis*. The farmer informed that another goat from the same herd had died previously, displaying serous nasal discharge, apathy, dyspnea, stridor, and weight loss.

The second case occurred in January 2014, in the municipality of Cruz das Almas (12°40'12" S, 53 39°06'07" W), in the Recôncavo region of the state of Bahia. The goat had displayed apathy, dyspnea, congested ocular and oral mucosa, and mucopurulent nasal discharge. This goat died spontaneously. The skin, the pinna internal and external regions on both sides, and the metatarsophalangeal region of the right hind limb displayed several multifocal to coalescing nodules with diameters of approximately 0.5cm. The clinical course was approximately 105 days.

The Case 1 necropsy pointed out an increased volume on the mid-sagittal section of the head and an ulcerated surface containing a yellow friable mass with irregular and granular consistency in the rostral portion of the nasal septum. Necropsy of Case 2 revealed a bilateral yellow friable mass extending from the nasal vestibule to the frontal portion of the nasal cavity (Fig.1). The nodules of the ear (Fig.2) showed a subcutaneous tissue expanded by multifocal to coalescent and irregular, yellow areas interspersed by hemorrhages and necrosis (Fig.3), and the right hind limb (Fig.4) showed a yellow cut surface interspersed by hemorrhages and necrosis. No gross changes were observed in other organs.

Microscopically, the nasal cavity lesions in both goats were multifocal granulomas with a central necrotic area containing non-stained fungal hyphae images surrounded by eosinophilic Splendore-Hoeppli material and moderate inflammatory infiltrate, composed mainly of macrophages, neutrophils, epithelioid cells, and multinucleated giant cells (Fig.5). Similar histopathologic findings were observed in the ear and hind limb. In Case 2, the lesion revealed multifocal vasculitis with hyphae inside vessels (Fig.6). In the GMS of the rhinofacial tissue, we observed thin walled, rarely septated, irregular branching hyphae, ramified, with a varying degree of parallelism, sometimes with bulbous dilatation in the extremities. The hyphal diameter was about 8µm, bulbous was about 25µm (Fig.7). The lesions examined by IHC were positive for *Conidiobolus lamprauges* (Fig.8) and negative for *Pythium insidiosum*. In a sample from Case 2, the identity of the hyphal elements was confirmed by PCR using oligonucleotides encoding the partial sequence of the 18S rDNA gene for *C. lamprauges*, with 540 base pairs amplicons. This sample was negative to *P. insidiosum* by PCR.

DISCUSSION

Diagnosis of conidiobolomycosis in goats was based on epidemiology, clinical signs, pathological findings, positive immunostaining, and PCR for *Conidiobolus lamprauges*. This is the first report of conidiobolomycosis in goats. The use of IHC (Ubiali et al. 2013) and PCR (Santurio et al. 2008, De Paula et al. 2010, Azevedo et al. 2012, Ubiali et al. 2013) to discriminate between *C. lamprauges* and *Pythium insidiosum* provides a means for differential diagnosis.

Conidiobolomycosis is an endemic disease of sheep in the semiarid region of northeastern Brazil (Silva et al. 2007a, 2007b, Riet-Correa et al. 2008, Mendonça et al. 2012). In this region, the production of goats is an important economic activity. However, the importance of conidiobolomycosis in goats is unknown.

Conidiobolus spp. are found in soil, in vegetation decomposed and as insect parasites, occurring preferentially in tropical and subtropical climates (Carrigan et al. 1992, Silva et al. 2007a). Three factors are essential for fungus development: humidity, temperature, and dead plant matter. For this

reason, the occurrence of this disease coincides with the rainy season (Silva et al. 2007a, 2007b). Both cases presented in this study occurred during the rainy season. The possible forms of conidiobolomycosis infection in sheep that should be considered in this report are inhalation of spores present in the environment, which implant in the nasal mucosa; traumatic spores implantation due to the insects' bite (Carrigan et al. 1992, Gugnani 1992, Ketterer et al. 1992); or yet, through spikey plants containing conidia (Ketterer et al. 1992).

In animals and humans, *Conidiobolus* spp. displays a positive tropism for the nasal cavity (Silva et al. 2007a, Furlan et al. 2010, Pestana et al. 2019). Clinical signs of goat conidiobolomycosis, characterized by serous nasal discharge, dyspnoea, and weight loss, were similar to those described in sheep. However, cranium facial asymmetry and exophthalmos, which are common in sheep conidiobolomycosis (Silva et al. 2007a, 2007b, Riet-Correa et al. 2008, Pedroso et al. 2009, Furlan et al. 2010, Ubiali et al. 2013, Schild et al. 2016) were not observed in these goats making the clinical diagnosis more difficult in this species. In these goats, granulomatous lesions of nasal septum were in the nasal vestibule (rhinofacial); different from that observed in sheep in which the lesions are preferably located in the rhinopharynx (Silva et al. 2007a, 2007b, Boabaid et al. 2008, De Paula et al. 2010, Kimura et al. 2011, Schild et al. 2016, Pestana et al. 2019).

In Midwest Brazil, in sheep, rhinopharyngeal lesions, located in the ethmoidal region were observed in 86.7% (13/15) of the cases (Ubiali et al. 2013), in contrast with pythiosis, which has a predilection for the rhinofacial region (Santurio et al. 2006, Ubiali et al. 2013). Cutaneous lesions observed in the Goat 2 are similar to those reported in goats with nasal and cutaneous aspergillosis (Carmo et al. 2014) and should be embraced in the differential diagnosis of conidiobolomycosis in goats. Extra-nasal lesions, including the brain, eyes, lymph nodes, kidneys, lungs, heart, abomasum, and gallbladder typically found in sheep and other species (Silva et al. 2007a, Boabaid et al. 2008, De Paula et al. 2010) were not found in these goats. However, Case 2 displayed dermatitis.

Fig.1-4. Conidiobolomycosis in goats. Goat 2. (**1**) Left cranial septum of the nasal cavity with a yellow friable mass extending from the nasal vestibule to the rostral region. (**2**) Nodules in the skin of the ear. (**3**) Cut surface of a nodule in the ear skin. The subcutaneous tissue is expanded by multifocal to coalescent and irregular, yellow areas interspersed by hemorrhages and necrosis. (**4**) Right hind limb. Cut surface: subcutaneous tissue with yellowish areas interspersed by hemorrhage and necrosis (arrow).

In conidiobolomycosis, lethality is 100%, and treatment has been inefficient, suggesting euthanasia in affected animals (Riet-Correa et al. 2011). In this study, one goat died, and the other was euthanized after a chronic clinical course of approximately three months. In sheep, the disease is subacute and invariably fatal (Silva et al. 2007b, Furlan et al. 2010, Mendonça et al. 2012, Schild et al. 2016). These differences in clinical evolution between the two species can be related to variations in virulence of the agent or higher susceptibility of sheep. In addition, the late diagnosis and possibly the rapid clinical course can contribute to lethality in sheep (Silva et al. 2007b).

In humans, the treatment of coniodiobolomycosis with potassium iodide may relapse, and the use of itraconazole, ketoconazole, or amphotericin B for a prolonged period, from six months to a year, could be effective (Riet-Correa et al. 2011). In sheep, the treatment with potassium iodide is usually unsuccessful, maybe because the disease shows clinical signs when the lesions are already widespread in the ethmoidal region, retro-orbital fossa, and pharynx when they are difficult to recover (Pestana et al. 2019). Information about the animal immune response in *C. lamprauges* infection is scarce. However, treatment studies about Conidiobolus infections in sheep and goats are required. Silva et al. (2015) demonstrated antigenic proteins of *C. lamprauges* and specific IgG in sheep sera by the immunoblot technique. The knowledge about antigenic profile against infected sheep may assist in developing early diagnostic methods and the use of protein as candidate vaccines for the control and prevention of infection.

Microscopically, was observed granulomas with a central necrotic area with images of fungal hyphae surrounded by inflammatory cells and eosinophilic Splendore-Hoeppli material. Several papers described similar histologic patterns in cases of conidiobolomycosis in sheep (Boabaid et al. 2008, De Paula et al. 2010, Kimura et al. 2011, Mendonça et al. 2012, Ubiali et al. 2013, Schild et al. 2016, Pestana et al. 2019). The histological exam provides differentiation between conidiobolomycosis

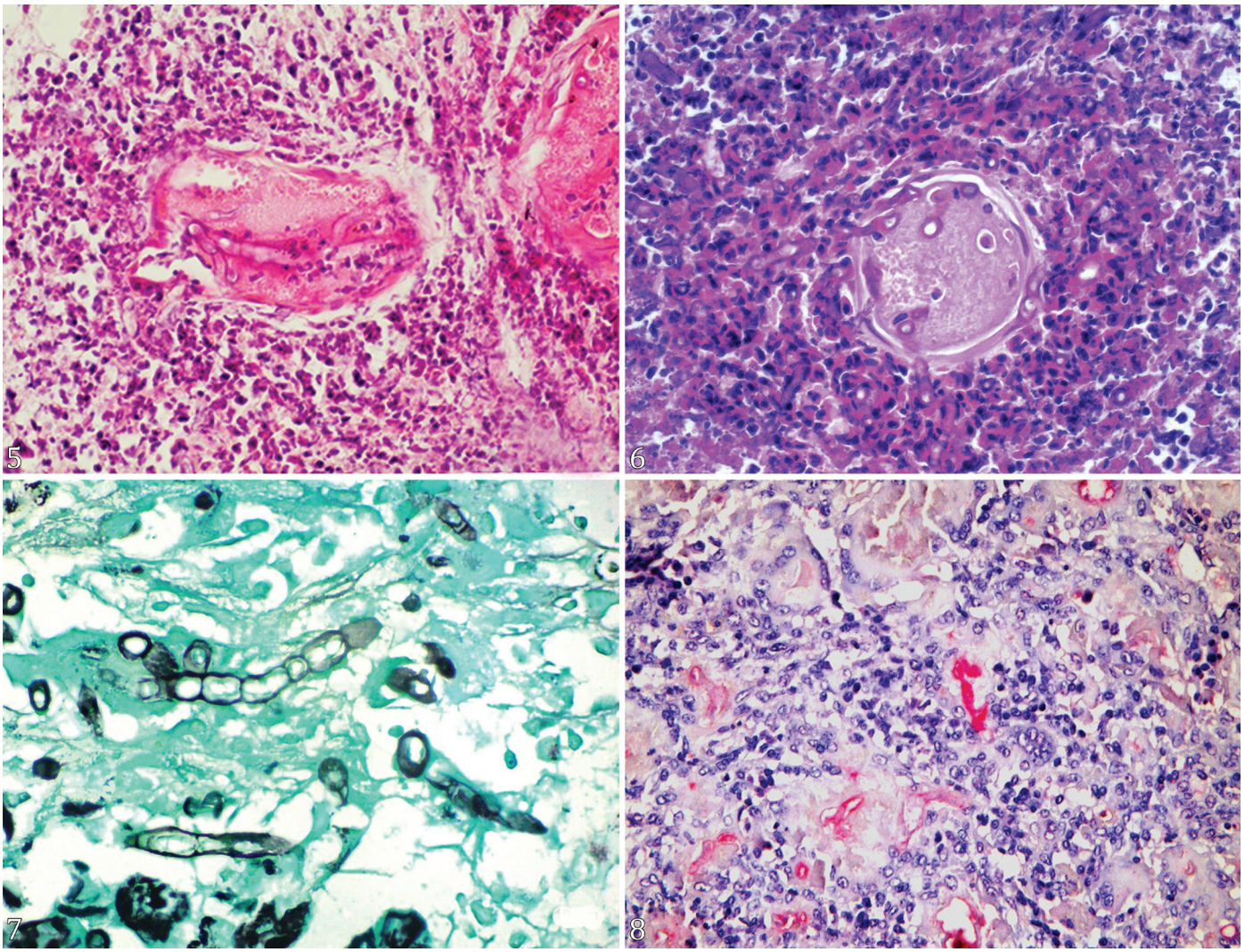

Fig.5-8. Conidiobolomycosis in goats. (5) Goat 2. Nasal cavity. Granuloma with a central necrotic area containing negative images of fungal hyphae surrounded by Splendore-Hoeppli material and pyknotic debris . HE, obj.40x. (6) Goat 2. Vasculitis and hyphae are observed inside vessels. HE, obj.40x. (7) Goat 1. Nasal mucosa showing hyphae irregular in shape, rarely septate or ramified, with bulbous dilatation in the extremities. GMS, obj.63x. (8) Goat 1. IHC showing multiple hyphae of *Conidiobolus lamprauges* stained red, within multinucleated giant cells. HE, obj.40x.

and pythiosis: both cause granulomatous lesions with intralesional hyphae surrounded by Splendore-Hoeppli material. However, in conidiobolomycosis, the Splendore-Hoeppli reaction material is amorphous and comprises the antigen-antibody complex. In pythiosis, Splendore-Hoeppli material is granular and means eosinophils degranulation (Ubiali et al. 2013). Also, in phytiosis, the infiltration of eosinophils is more prominent than in conidiobolomycosis (Santurio et al. 2006, Ubiali et al. 2013). In human cases of infection by *C. lamprauges* uniform and septate hyphae with dichotomous branching, resembling those of *Aspergillus* are observed inside blood vessels. Thus, histologic features of conidiobolomycosis must be interpreted with caution (Furlan et al. 2010). The use of IHC (Ubiali et al. 2013) and PCR (Santurio et al. 2008, De Paula et al. 2010, Azevedo et al. 2012, Ubiali et al. 2013) to discriminate provides a differential diagnosis between *C. lamprauges* and *P. insidiosum*. These tests may provide a rapid diagnosis compared to the common fungal culture, which usually requires a laboratory structure and takes an extended amount of time (Vilela et al. 2010). The PCR technique is a safe option diagnosis but may reveal false-negative results as tissue fixation with formalin denatures DNA (Gatta et al. 2012). The immunohistochemical technique, considered a quick and accurate diagnostic method for conidiobolomycosis, reveals hyphae in the cytoplasm of giant cells or the center of granulomas (Ubiali et al. 2013).

In Brazil, other agents causing similar lesions to conidiobolomycosis in goats are aspergillosis by *Aspergillus niger* (Carmo et al. 2014) and protothecosis by *Prototheca wickerhamii* (Macêdo et al. 2008). Aspergillosis histological lesions consist of piogranulomas with hyphae, a central necrotic area surrounded by neutrophils and outermost layers of epithelioid macrophages, lymphocytes, and rare multinucleated giant cells (Carmo et al. 2014). In protothecosis, histologic lesion consists of necrotizing, pyogranulomatous dermatitis, and rhinitis, with infiltration by lymphoid cells, macrophages, giant cells, and neutrophils. In addition, myriads of ovoid-to-spherical, non-budding, walled structures (sporangia) are observed (Macêdo et al. 2008).

Other diseases in the Northeast region of Brazil should be considered as cases of nasal cryptococcosis in sheep were diagnosed by *Cryptococcus neoformans* and *Cryptococcus gattii* (Silva et al. 2010, Macêdo et al. 2020). In cryptococcosis the histological lesions consist of areas of necrosis with myriads of rounded or oval structures, yeast-like, with thin basophilic wall. Surrounding the yeasts is a clear halo not stained neither by hematoxylin nor eosin, giving a characteristic "soap bubble" appearance (Silva et al. 2010). Another disease that should be included in the differential diagnosis is basidiobolomycosis caused by *Basidiobolus ranarum*, which promotes subcutaneous eosinophilic infections in the face, limbs, and other anatomical areas (Greene et al. 2002).

CONCLUSION

Conidiobolus lamprauges is a potential cause of granulomatous rhinitis and dermatitis in goats.

Conflict of interest statement.- The authors have no competing interests

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