DIAGNOSTIC EXERCISE

From The Davis-Thompson Foundation*

Case #: **334**; Month: **April**; Year: **2024**

Answer Sheet

Title: Rhinopharyngeal zygomycosis in sheep with a systemic presentation

Contributors: Tessie B.M. Möck[1], Karla M.R. Guedes[2], Igor Alexandre H.F.S. Peres[2], Raquel S. Juliano[2], Larissa L. de Souza[1], Thaisa X. e Silva[1], Claudio S.L. Barros[1].

[1]Laboratório de Anatomia Patológica, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.
[2]Embrapa Pantanal, Corumbá, MS, Brazil.
Corresponding author: tessiebeck@hotmail.com

Clinical History: On July 18, 2018, a female Pantaneira sheep presented blindness and mild exophthalmos of the left eye. Two days later, it also had severe dyspnea and remained in sternal decubitus, with lateral torticollis. The sheep died on July 21. On July 24, two additional sheep of the same flock were observed with a mild nasal mucohemorrhagic discharge. When rechecked on July 28, the discharge had become more severe, and the ewes were dyspneic and gasping. There was skull and facial asymmetry due to unilateral exophthalmos. The protrusion of the eyeball favored keratitis and corneal ulceration on Sheep 2. Due to poor prognosis, the ewes were euthanized 34 days after the onset of clinical signs.

To help the reader to properly compare the disease presentation in the three sheep, the clinical and pathological data are summarized in the Table below.

Animal ID / Clinico-pathological picture	Sheep 1	Sheep 2	Sheep 3
Exophtalmos	x	x	x
Nasal discharge	x	x	x
Dyspnea	x	x	x
Nasal cavity lesions	x	x	x
Brain lesions		x	x
Lung lesions	x	x	x
Kidney lesions			x
Outcome	Spontaneous death	Euthanasia *in extremis*	Euthanasia *in extremis*
Observations	Freezing and autolysis artifacts		

Gross Findings: The three sheep had similar *postmortem* gross lesions differing mainly in severity. The head lesions affected essentially the left side in Sheep 1 and 3 and the right side in Sheep 2.

The carcasses had poor nutritional status and scant fat reserves. Sheep 1 and 3 had mild and moderate exophthalmos, respectively. In Sheep 2, a severe increase in volume in the orbital region added to mild tumefaction over the right nasal bone resulted in marked cranial asymmetry (Fig. 1A). The eyeball was not visible since it was covered by reddish, swollen, ulcerated conjunctiva (Fig. 1B). It was pressed and displaced by a granulomatous white mass that occupied part of the orbit. The eyeball was shrunken and structurally disorganized, and the cornea and sclera were thickened by fibrosis (*atrophia bulbi with shrinkage*) (Fig. 1C).

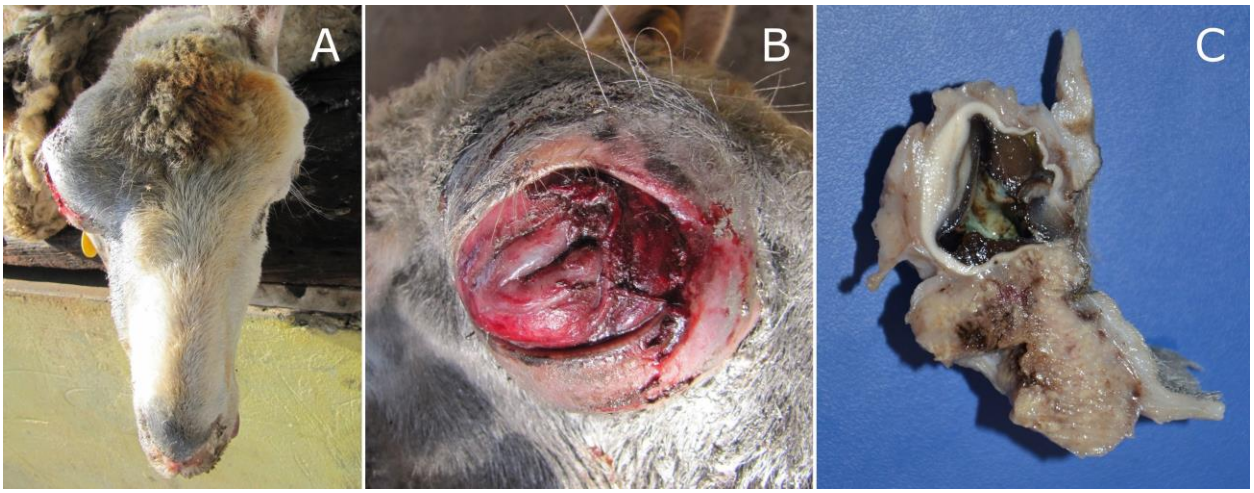


Figure 1. Sheep 2. **A.** Cranium-facial asymmetry, with exophthalmos of the right eye and tumefaction over the nasal bone. **B.** Right eye. The orbital region is severely enlarged. The conjunctiva is markedly swollen, red, and ulcerated. The eyeball can not be seen. **C.** Formalin-fixed specimen. The eyeball is shrunken and disorganized, and the cornea and sclera are thickened by fibrosis.

The nasal bone tumefaction was caused by a 2x0.5 cm mass that expanded the subcutaneous and resembled that from the orbit. Mucohemorrhagic discharge was apparent in Sheep 3 (Fig. 2A), where rare larvae of the bot fly *Oestrus ovis* could be seen in the exudate and scant in the other. On a mid-sagittal section of the head, the nasal conchae (left side on Sheep 1 and 3 and right side on Sheep 2) were replaced by a large gray, firm-to-soft, multifocal, friable mass of irregular contours (Fig. 2B).

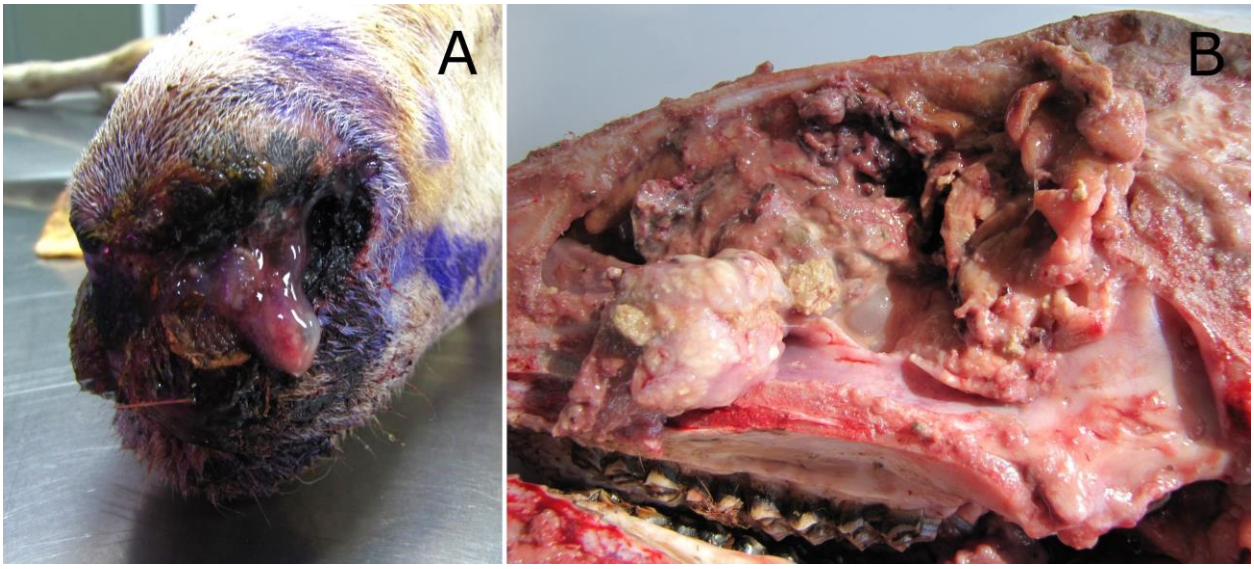


Figure 2. A. Sheep 3. Mucohemorrhagic discharge is apparent in the left nostril. **B.** Sheep 2. Mid-sagittal section of the head, right side. The nasal conchae are destroyed and replaced by a large soft mass of irregular contours. There are multiple foci of necrosis and purulent exudate within the mass.

The mass extended to the pharyngeal region and destroyed the turbinates, invading the cranial vault and reaching the frontal lobe of the brain (Fig. 3A). In Sheep 2 and 3, after the brain hemispheres were removed, an increase in the volume of the olfactory bulb was apparent, which was swollen and dark, and had a caseous lesion at its junction with the frontal lobe (Fig. 3B).

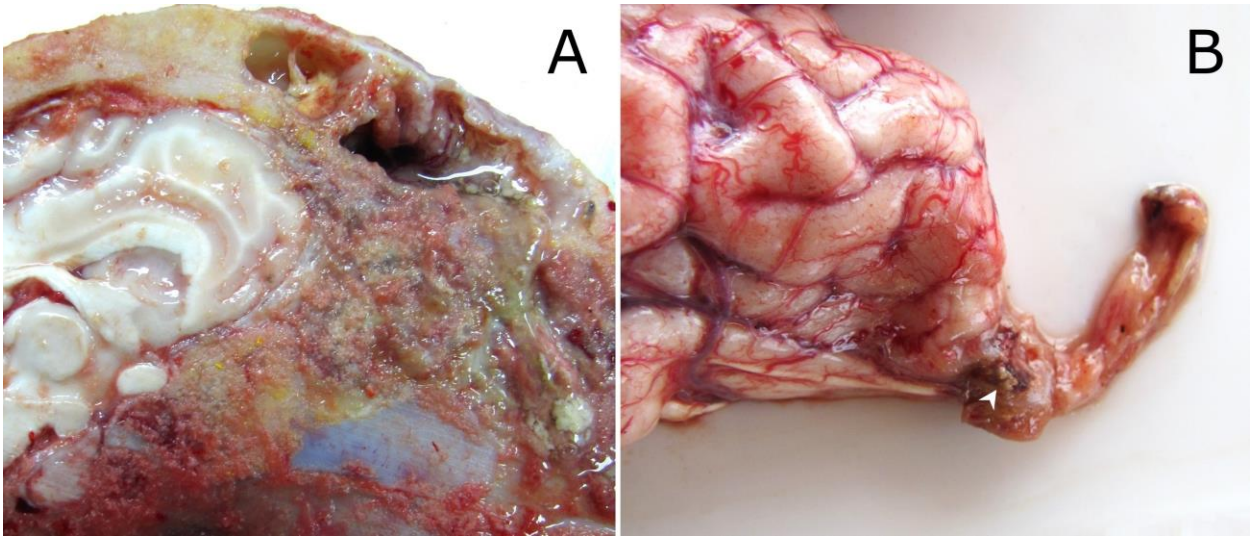
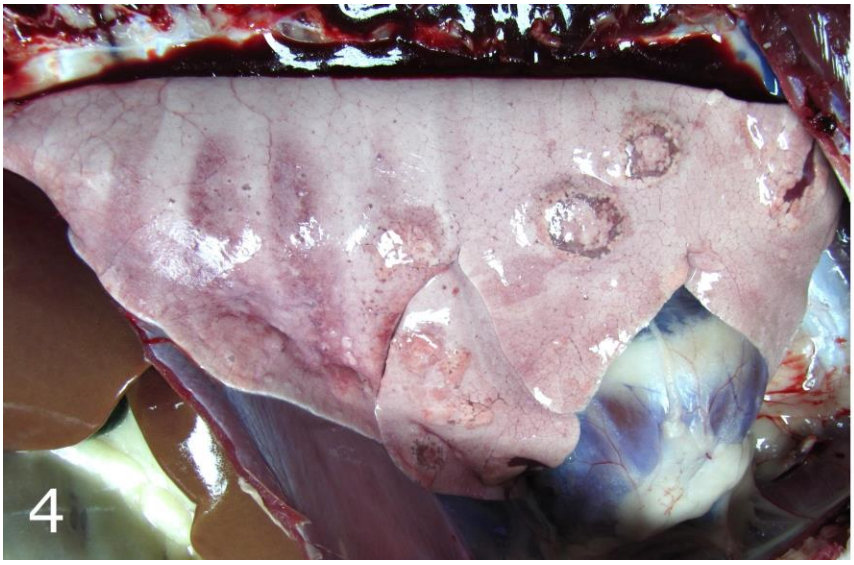


Figure 3. A. Sheep 3. Mid-sagittal section of the head. The left side is completely destructed with loss of nasal conchae, which are replaced by a large mass of irregular contours. The mass invaded the cranial cavity and reached the frontal brain. Note the thickening of meninges covering the frontal lobes. **B.** Sheep 2. The right olfactory bulb is swollen and dark and has a nodular caseous lesion at its junction with the frontal lobe (arrowhead).

In the frontal portion of the cerebral hemisphere, the meninges were thickened, and there was a focal loss of gray matter. The remaining nasal conchae (contralateral side) were intact but moderately congested in all sheep.

In both lungs, several soft, yellowish subpleural nodules of variable sizes (0.5-1 cm in Sheep 2 and 0.5-3 cm in Sheep 1 and 3) were surrounded by a red rim (Fig. 4). In Sheep 3 only, both kidneys had circular pale areas of 0.5-1 cm in the natural surface that extended to the cut surface, occasionally reaching the corticomedullary interface (Fig. 5).



Figures 4 and 5. Sheep 3. **Figure 4.** Right lung. Multifocal yellowish subpleural nodules of variable sizes (0.5-3 cm) surrounded by a red rim. **Figure 5.** Kidney. Circular pale areas of 0.5-1 cm in the natural surface.

Other lesions found at necropsy were considered incidental and included calcified nodules of *Oesophagostomum* sp. larvae in the omentum and one small hydatic cyst in the liver.

Histologic description:
Similar findings were observed in the nasal conchae, lungs, eye (eyelid, conjunctiva, sclera, periocular muscles, and adipose tissues; Sheep 2), kidneys (Sheep 3), and brain frontal lobe (Sheep 2 and 3). The tissues were replaced (nasal conchae) or expanded by nodular aggregates or sheets of epithelioid macrophages and Langhans giant cells admixed with eosinophils and interspersed with foci of liquefactive necrosis. As seen in the kidneys, the pyogranulomatous exudate would sometimes be organized as discreet nodules with a core of eosinophils surrounded by macrophages and little to no necrosis associated. In the brain frontal lobe, the leptomeninges were expanded by coalescent collections of epithelioid macrophages, lesser numbers of eosinophils, lymphocytes, plasma cells, and a few Langhans giant cells. The granulomatous component was admixed with plump fibroblasts, thin

bundles of fibrous tissue, and multifocal aggregates of granular, deep basophilic, and dense material (mineral) (Fig. 6).

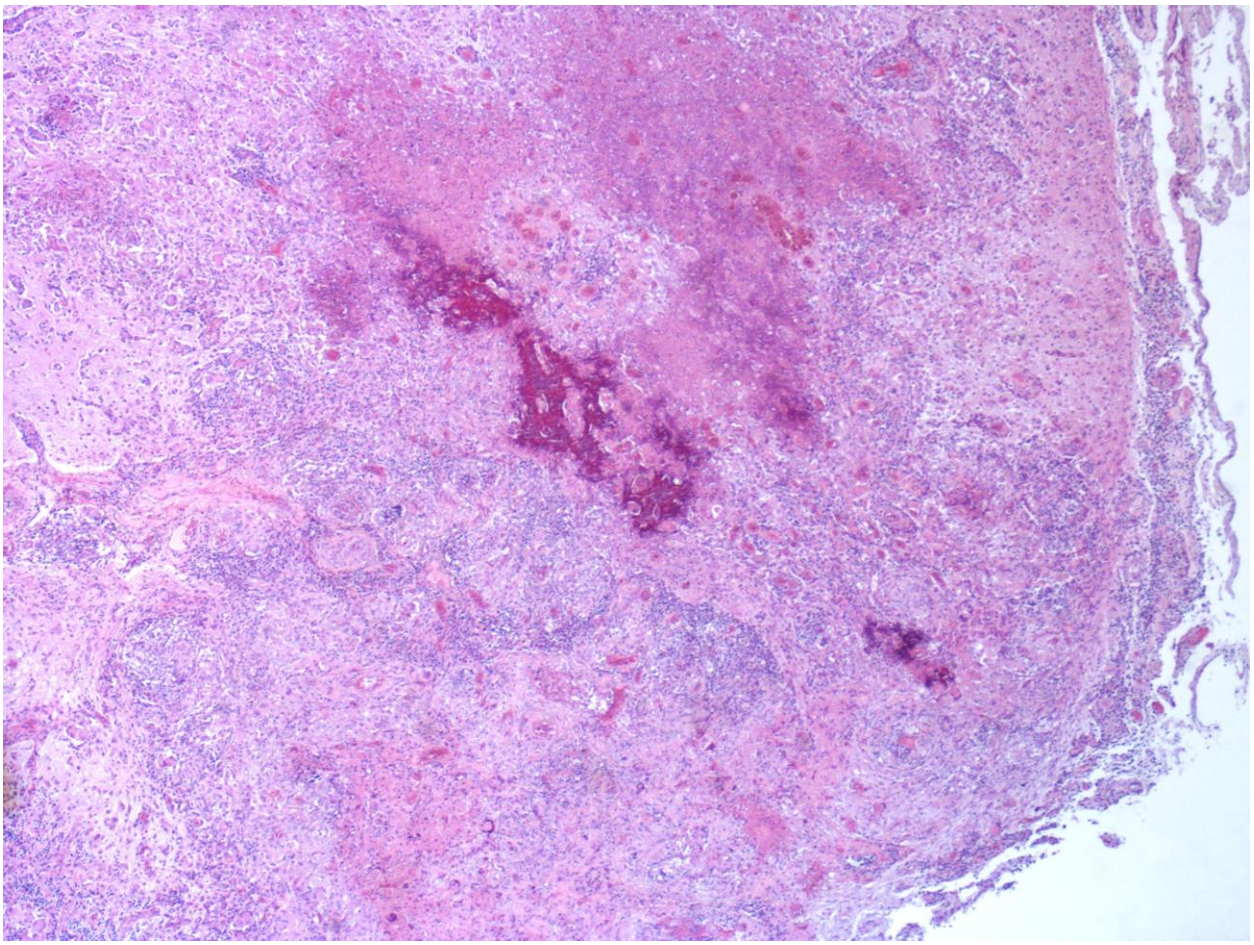


Figure 6. Sheep 2. Brain right frontal lobe. Collections of epithelioid macrophages, Langhans giant cells, and eosinophils densely infiltrate the leptomeninges and parenchyma. Amidst the pyogranulomatous infiltrate, there are multiple necrosis foci and mineral deposits. HE, 40x.

There was a focally extensive area of necrosis of liquefaction, swelling of astrocytes, and cavitation of nervous tissue, where the parenchyma had been replaced by proliferating microglial cells, many of which already differentiated into foamy macrophages. Perivascular cuffs containing up to three layers of lymphocytes were also observed (Fig. 7).

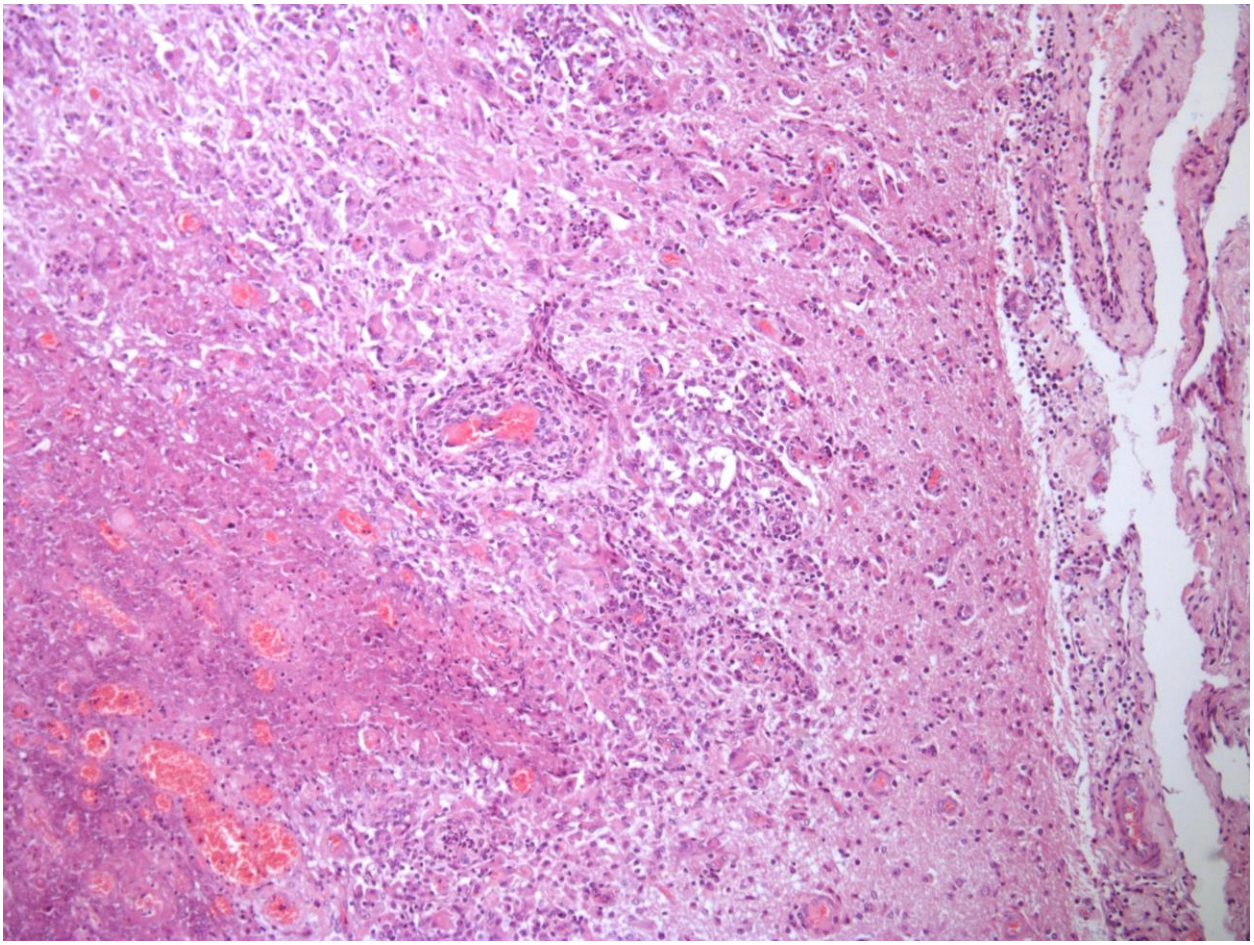


Figure 7. Sheep 2. Brain right frontal lobe. Rarefaction of the parenchyma and proliferation of microglial cells, with numerous foamy macrophages, are evident, as are perivascular cuffs. There is a focally extensive area of necrosis of liquefaction on the picture's left side. HE, 100x.

Among the cell debris or in the cytoplasm of the multinucleated cells, there were tubularly elongated (when longitudinally sectioned), circular or oval (when transversally sectioned) structures barely stained and hard to visualize in hematoxylin and eosin (HE) stained slide ("negative" images of fungal hyphae). The hyphae were characterized by a body that was hollow or filled with eosinophilic or basophilic granular material and surrounded by varying, but usually scarce, amounts of strongly eosinophilic, smooth, or granular material (Splendore-Hoeppli reaction). The wall of medium-sized arteries in the affected areas was segmentally or circumferentially expanded by acellular, bright eosinophilic, and homogeneous material (fibrinoid necrosis), and occasionally, hyphae infiltrate the vessel walls or associated with thrombosis in the vessel lumen (mycotic thrombi).

The organisms stained well by the Grocott-Gomori's methenamine silver (GMS) method as slightly and irregularly branched, thin-walled hyphae with nonparallel sides, rare septa, and an irregular diameter of 6-10 μm, associated with foci of necrosis (Fig. 8A) or within giant cells (Fig. 8B). Occasionally, a rounded structure

of larger diameter (up to approximately 30 µm) was found at the end of the hyphae.

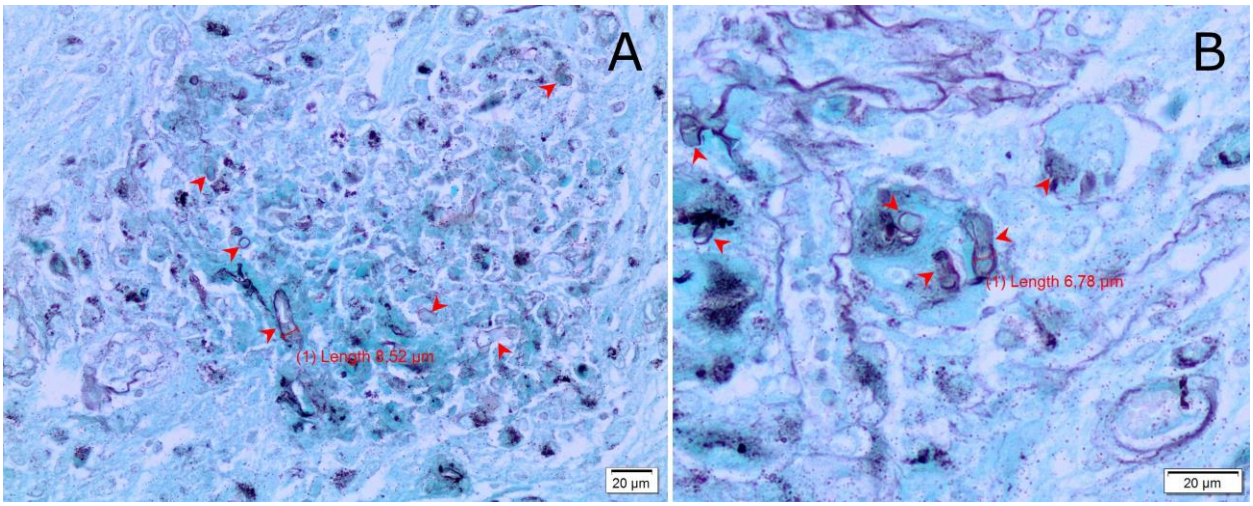


Figure 8. Sheep 2. Brain right frontal lobe. Thin-walled hyphae with nonparallel walls (arrowheads) associated with a focus of necrosis (**A**) and within a Langhans giant cell (**B**). GMS, 400x.

Answers:

● *Morphologic diagnoses*

1. Nasal cavity and pharynx, pyogranulomatous rhinopharyngitis, focally extensive
2. Brain, pyogranulomatous meningoencephalitis, focally extensive
3. Eye, pyogranulomatous panophthalmitis, unilateral
4. Lung, multifocal pyogranulomatous pneumonia
5. Kidney, multifocal pyogranulomatous nephritis

● *Most likely cause*

Conidiobolus sp.

● *Differential diagnoses*

1. Rhinopharyngeal conidiobolomycosis
2. Rhinofacial pythiosis
3. Aspergillosis
4. Protothecosis
5. Neoplasia (myxoma and enzootic intranasal tumors)

Comments: The lesions in the ewes are consistent with nasal zygomycosis of sheep, a common disease in the semiarid Region of Northeastern and Midwestern Brazil (5,7).

The fungi of Phylum Zygomycota (zygomycetes) that are important as a cause of disease in animals (6) and humans (2) belong to three orders: Mucorales, Mortierellales, and Entomophtorales. Zygomycetes include widely distributed saprophytes capable of producing opportunistic diseases, and the term zygomycosis describes the illnesses caused by these organisms (2,6). Historically, phycomycosis

referred to infections induced by zygomycetes and by the fungus-like organism *Pythium insidiosum*, now classified as an Oomycete from the kingdom Chromista (6). *P. insidiosum* is a well-known cause of eosinophilic subcutaneous granulomas associated with the Splendore-Hoeppli phenomenon and slender, sparsely pulmonary granulomas in horses and other animal species, including humans (7), and necrotic rhinitis in sheep (10).

Fungi within the order Entomophtorales (entomophthoromycosis) causing animal diseases include (a) *Basidiobolus* spp., reported as a cause of cutaneous granulomas in horses and dogs (7) and (b) *Conidiobolus* spp. reported as responsible for granulomatous inflammation in the subcutaneous tissue of horses; subcutaneous, gastrointestinal and pulmonary granulomas in dogs (6) and nasal granulomas in sheep and horses (4).

In Brazil, granulomatous rhinitis produced by *Conidiobolus* spp. is a common affection of sheep with the highest prevalence in the Northeast (1,5,7,8) and Midwest (10), but also occurs in the South (3). The disease can cause significant losses in sheep due to its high lethality rate, up to 100% (1,8).

Conidiobolomycosis is not a contagious disease (2). Infection may occur through spore implantation in the nasal cavity by minor traumas such as insect bites, as was described in *C. coronatus* infection. The presumed means of infection of the nasal cavity is the inhalation of fungal conidia from the pasture or possibly inoculation by the sharp-pointed plant parts. From there, the microorganism invades adjacent tissues by direct dissemination (4,10). The extension of the inflammatory reaction to the orbit causes exophthalmia and other injuries to the eye (4).

The inflammatory process destroys the ethmoid bone, causing the invasion of the brain's olfactory bulb and frontal lobe. Brain compromise occurs in the rhinopharyngeal form of *Conidiobolus* spp. infection, with a prevalence of about 20% (10) to 40% (8). In this report, two (67%) of the affected sheep had mycotic meningoencephalitis.

In all sheep reported here, the spread also occurred to the lungs, which occurs in this disease with a prevalence of about 15% (3) to 45% (8-10) of the cases and is probably due to aspiration (10). The solid effort for breathing may cause granulomatous mass fragments to detach and lodge in the lungs, developing new granulomatous foci (10). However, the less frequent dissemination to distant organs such as the kidney, as seen here in one sheep (33%), has probably occurred by lymphatic or hematogenous spread (8). There is evidence for hematogenous dissemination, including mycotic lesions in the vessel walls and invasion of the lumen of vessels associated with thrombosis. Spread of the lesions to distant sites has at least been reported to lungs (1,5,8-10), kidneys (5,9), colon (5), liver (10), heart, and gall bladder (9). Also, regional lymph nodes may be affected (1,5,9,10).

The fungal or fungal-like produced granulomatous rhinitis in sheep occurs in two presentations: (a) rhinofacial, which affects the nose and the entrance of the nasal fossae and the upper lip, and (b) rhinopharyngeal, which affects the turbinates, paranasal sinuses, hard and soft palates, and pharynx (3,7,9). Over 85% of rhinofacial mycotic granulomatous rhinitis cases are due to *P. insidiosum*, and over 90% of the cases of rhinopharyngeal presentation to *Conidiobolus* spp. (10). In Brazil, *C. lamprauges* (3, 10) and *C. coronatus* (1,8) are the species isolated from cases of rhinopharyngeal conidiobolomycosis. In humans, subcutaneous infections confined to the nasal mucosa and adjacent tissues have *C. coronatus* as the single causative agent (2).

The clinical manifestations usually develop within one to five weeks (8) but may vary from two to 45 days (1). Respiratory clinical signs consist of serous nasal discharge that evolves to mucous or mucohemorrhagic (4), or severe epistaxis (1); there is difficulty breathing with stridor, anorexia, and swelling of the anterior or posterior nasal cavity (4). Larvae of the bot fly (*Oestrus ovis*) can lead to mild eosinophilic, catarrhal to mucopurulent rhinitis in sheep, with eventual sneezing and difficult, noisy breathing in severe cases. They may have contributed to the condition seen in Sheep 3, but probably very little, given the minimal number of specimens found. Other clinical signs include, but are not limited to, cranium-facial asymmetry due to unilateral exophthalmos, marked apathy, sometimes with the head held in a lowered position, head pressing (8), reluctance to move (3), torticollis, fever (1), and weight loss (8).

The tissues of the case reported here were formalin-fixed when they arrived at the laboratory, precluding fungal cultures. Immunohistochemistry and PCR (10) would be the best options to confirm the diagnosis. So, we had to engage in a diagnosis exercise that allowed us to reach etiology with a reasonable margin of certainty. A comprehensive review of the lesions affecting the nasal cavity of ruminants in Brazil (5) listed the following five differentials in small ruminants: rhinopharyngeal conidiobolomycosis, craniofacial pythiosis, aspergillosis, protothecosis and neoplasia (myxoma and enzootic intranasal tumors). After the histopathological examination, only the first two diseases presented a challenge in the differential diagnosis. However, significant clinical and pathological differences between rhinopharyngeal conidiobolomycosis and rhinofacial pythiosis will allow one to reach a final diagnosis. Due to retrobulbar granuloma, the clinical sign of exophthalmos is a valuable indication of rhinopharyngeal zygomycosis (1,4,7,8,10).

Although both causal agents may be associated with rhinopharyngeal or craniofacial presentation of granulomatous rhinitis in sheep, the location of the lesions is reliable circumstantial evidence to incriminate a specific causal agent. Diseases produced by infection of *Conidiobolus* spp. are almost always rhinopharyngeal and appear as a firm yellow or white mass; pythiosis is almost always located in the rhinofacial region, producing a necrotic and friable cellular exudate. Histologically critical morphological differences between the aspects of the two diseases include the morphology of the two microorganisms. The hyphae in pythiosis have thick walls that are almost parallel and sparsely septate. The hyphae of *Conidiobolus* spp. are thin-walled, non-parallel, and sparsely septated, with ballooning dilations in

their extremities. The zygomycetes' morphological and tinctorial characteristics usually enable the microscopist to identify these fungi (2). Detailed descriptions of these can be found elsewhere (2).

Enzootic intranasal tumors of ruminants are occasionally diagnosed in cattle and sheep in Brazil (5), and rhinopharyngitis caused by *Conidiobolus* spp. has been mistakenly diagnosed as enzootic intranasal tumor. Although these two conditions have similar aspects, they can be set apart on the grounds of histological examination.

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