Rev. Inst. Med. trop. S. Paulo
45(3):163-166, May-June, 2003

CASE REPORT

MYCOTIC BOVINE NASAL GRANULOMA

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SUMMARY

A case of mycotic bovine nasal granuloma in a 10 year-old Jersey cow, produced by *Drechslera halodes* is presented.

Histopathological sections showed abundant hyaline and pigmented extra and intracellular fungal structures together with a polymorphic cellular granuloma formed by neutrophils, lymphocytes, plasmocytes, histiocytes and giant cells of the Langhans type.

It is the first case of mycotic bovine nasal granuloma recognized in Uruguay although this disease seems to be frequent according to the opinion of veterinarian specialists.

Another similar clinical case also in a Jersey cow from the same dairy house with an intense cellular infiltrate rich in eosinophils without granulomatous image, together with extracellular hyaline and fuliginous fungal forms, is also referred for comparative purposes. *Geotrichum* sp. was isolated.

The need of an early diagnosis and treatment of the disease is stressed.

KEYWORDS: Mycotic granuloma; Nasal; Bovines, *Drechslera;* Trichosporon.

INTRODUCTION

Mycotic bovine nasal granuloma (mbng) is a granulomatous disease characterized in the chronic, late stage, by large single or multiple polypoid nodules in the nasal cavity. It must be differentiated from bovine nasal granuloma of probable hypersensitivity origin.

Since the lesions may reach several cm in size, the animals show evident signs of nasal obstruction with a difficult and noisily strident breath. Also they reveal suffering and anxiety, adopting a curious position of stretched neck. Breathing is more abdominal than thoracic. The general condition may be deteriorated at this stage and prognosis is always severe. Death can then be produced as a consequence of the deficient nourishment and respiratory distress.

The aim of the paper is to refer the first proven Uruguayan case of the disease pointing up its mycological and histopathological aspects. Another similar case but without histopathological characteristics of granuloma is included.

MATERIALS AND METHODS

Case 1: A Jersey cow, 10 year-old from a rural dairy house in Canelones county, Uruguay, was sent to the Veterinary School of Montevideo city, for presenting a general bad condition with important stertoreous breathing and large intranasal nodules well visible to the naked eye. Death became a few days later. At autopsy, specimens from nasal polyps, liver and lung were fixed in 10% formalin and sent to the pathology department; there, fragments were paraffin–embedded, sectioned at 5 microns and further on stained with hematoxilin and eosin (H&E), periodic-acid Schiff (PAS) and Gomori methenamine silver technique (GMS). Pieces of polyps were also sent to the microbiology laboratory, inoculated on Sabouraud glucose agar tubes and on Sabouraud plus chloramphenicol and cicloheximide tubes and incubated at laboratory temperature. Furthermore, a direct examination between slide and coverslip and apposition smears fixed in methyl alcohol and stained by Giemsa for 20 minutes were made.

Case 2: Jersey cow, 9 year-old, from the same dairy farm, pregnant of 7 months with an intense strident breathing and several cm in diameter

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polyps in both nasal cavities. The lesions had a yellowish smooth surface covered by a mucous-purulent exudate. A biopsy taken from one of the polyps was cut in several portions; some of them fixed in 10% formalin and processed as in the first case while others were used for mycological study also as in the case 1. The cow died two months later after delivering a female healthy calf.

RESULTS

Case 1:

Mycological fresh examination and stained smears of nasal polyps: In both studies abundant fuliginous and hyaline, mostly round and thick wall fungal structures, some of them producing short and irregular filaments segmented in oval or rounded forms, were noticed. More details are presented below.

Histopathology: The histological sections of nasal lesions stained by H&E showed an intense polymorphic inflammatory cellular infiltrate with granulocytes, lymphocytes, plasmocytes, histiocytes and giant cells of the Langhans type together with some areas of necrobiosis. Abundant extracellular and intracellular fungal structures were easily observed in every microscopic field. They were grossly rounded, 15-16 microns in diameter (range 6-28), with thick cellular walls and cytoplasm sometimes vacuolated. In some extracellular forms a well defined brown pigmentation of the cell wall was seen. The intracellular structures were nonpigmented and their walls showed an intense pinky color. They appeared isolated or until 6 in number with a different degree of morphological alteration (Fig. 2).

All the fungal cells became intensely stained by the PAS and GMS techniques. While some extracellular forms showed buds (Fig. 1), other produced short unbranched hyphae, 4-5 microns in diameter, with ovoid or round enlargements of 9-11 microns. A small group of branched hyphae was also observed.

Mycology (cultures): In the Sabouraud slants, a grayish–black mycelial fungus with a predominant abundant black immersed mycelium grew in a few days together with scarce colonies of *Trichosporon* sp. Microscopically the black fungus showed dark pigmented, regularly and abundantly septated, 4 microns in diameter, hyphae. No conidia were observed. Subcultures on corn meal agar evidenced chlamidospores on short lateral branches and conidia in small groups or isolated with the following characteristics: straight, long-ellipsoidal (Havana-like) with rounded ends and a little higher diameter at the center tapering uniformly to the ends, straw-colored, pseudoseptated with end cells paler than the intermediate ones. Such characteristics permitted us to consider the organism as a dematiaceous fungus of the genus *Drechslera*[2].

The morphological details of the conidia, mostly with 6-8 pseudosepta, with end cells hyaline or pale, cut off by thick, dark septa and intermediate cells golden brown, 65-70 microns long, 14-16 microns thick in the broadest part and a protuberant hilum, led us to the diagnosis of *D. halodes* (Drechsler) Subran & Jain, 1966 (Figs. 3-4). The strain is maintained at the Microbiology Department of the Veterinarian School of Montevideo with the number 155 (DMFV 155).

Case 2:

Histopathology: In the H&E sections a dense cellular infiltrate rich in eosinophils without any histopathologic image of granuloma, was observed. Inside necrobiotic areas and coexisting with bacterial clusters extracellular hyaline and fuliginous fungal structures were seen. They included: short unbranched hyaline hyphae 10-15 microns long by 2-4 microns thick, scarce longer filaments, yeast forms some of them budding, arthrospores-like cells and ovoid or rounded thick cells with fuliginous thick walls (Figs. 5-6).

Mycology (cultures): In both, Sabouraud and Sabouraud plus antibiotics slants developed in 3-4 days, abundant colonies of a cream color fungus with a flat, dry and floury surface. The microscopic examination showed hyaline hyphae with abundant rectangular and round ends arthrospores what made the diagnosis of *Geotrichum sp*. No dematiaceous fungi were isolated.

DISCUSSION

Both cases had similar symptomatology: a noisy, stertorous breathing and large smooth yellowish surface polyps on their interior nasal cavities without apparent lesions in other organs. Also both animals had a fatal outcome but it was impossible to attribute the nasal disease as the final cause of death.

The presence of large lesions at the nasal cavities differentiates the mbng from the so called bovine nasal granuloma, atopic rhinitis, chronic granular rhinitis or bovine allergic granular rhinitis characterized by multiple small nodules of 1-2 mm in diameter, large numbers of eosinophils and lymphocytes, scarse fibroblasts and epithelioid cells and absence of fungal organisms, macrophages and giant cells[6,11]. Atopic rhinitis would be passible of experimental reproduction by means of a immediate type hypersensitivity in the nasal mucosa[8]. The microbial allergens might be identified by simultaneously monitoring the pasture particle content and assessing nasal eosinophilia in cattle with the disease[1].

Contrarily, the mbng is clinically comparable with the nasal rhinosporidiosis commonly observed in horses but also described in mules and cows[5]. Laboratory studies (mycology and histopathology) are obviously necessary to establish the correct diagnosis.

Our two cases clearly differed by the histopathological picture. While in the first one a typical granulomatous reaction was observed with giant cells, histiocytes, lymphocytes, plasmocytes and neutrophiles, the second showed a dense leukocytic infiltrate with many eosinophils.

The mycotic origin in both cases appears evident for the presence of fungal structures.

In the first one extraordinarily abundant intra and extracellular hyaline and fuliginous fungal cells were observed. The isolation in cultures of a species of *Drechslera* confirms previous observations. In 1977 MCKENZIE & CONNOLE[6] referred seven cases of mbng diagnosed in the period 1966-1975 in Queensland, Australia, pointing out their clinical and histopathological similarity with Australian and North-American cases previously published. In 2 of the 7 cases *D. rostrata* (*Exserohilum rostratum*)[4], was isolated. Also in 1977, PATTON described nasal granulomas in a cow associated to cutaneous granulomatous nodules on ears, tail, vulva and thigh. The histopathology showed rounded intra and extracellular 5 microns in diameter fungal structures, together with

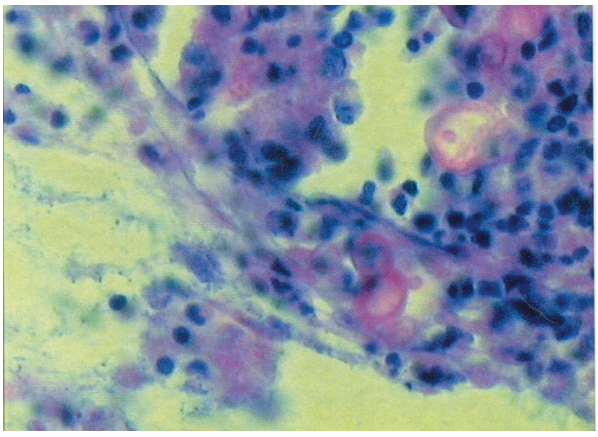

Fig. 1 - Mycotic bovine nasal granuloma. Histopathological section of the first case. Several extracellular rounded and thick walled PAS positive fungal cells are observed inside a deep polymorphous granulomatous reaction. Some of them are budding cells. PAS stain. (x400).

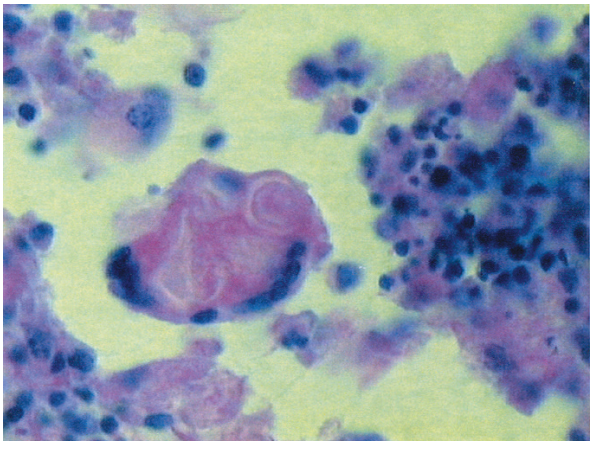

Fig. 2 - Same case of Fig.1. Langhans cell with thick walled fungal structures inside. Some cells show morphological alterations. PAS stain. (x400).

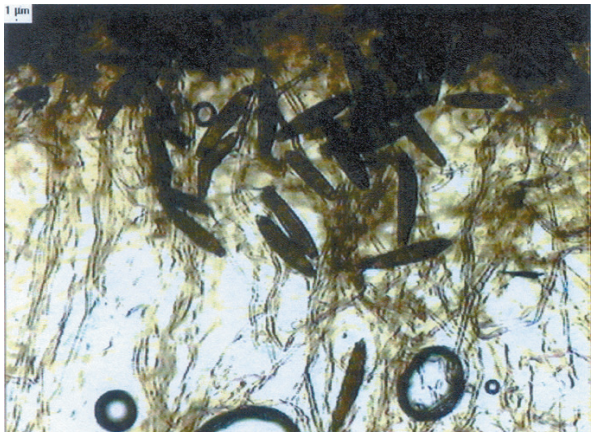

Fig. 3 - Culture on corn meal agar of the fungus *Drechslera halodes* isolated from case number one. Numerous long-elipsoidal (Havana-like), with rounded ends and a little higher diameter at the center tapering uniformly to the ends, are observed. (x100).

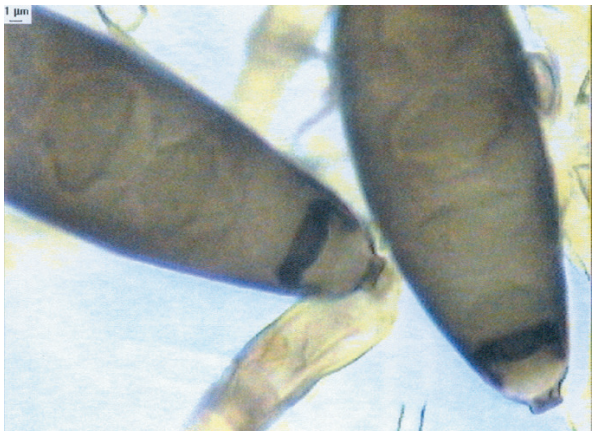

Fig. 4 - Details of the *D. halodes* conidia. The pale end cells, cut off by thick and dark septa as well as their protuberant hilum are clearly observed. (1,000).

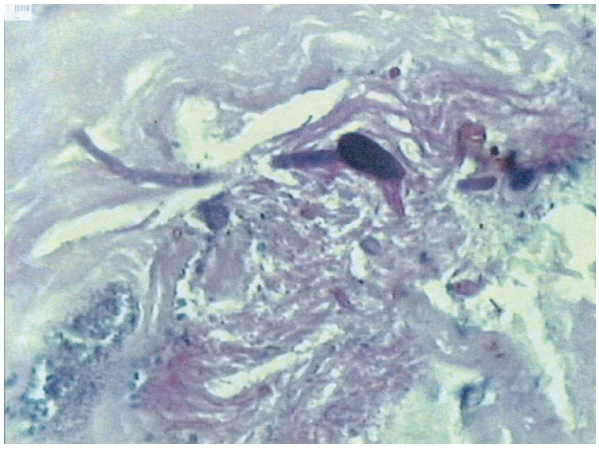

Fig. 5 - Histopathological section of case number two. Inside of a necrobiotic area several PAS positive fungal structures are seen. One of them appears as a chlamydospore-like cell producing filaments by both ends. Other smaller yeast-like cells are also observed. No granulomatous reaction was present. Instead of a dense cellular infiltrate rich in eosinophils were observed. PAS stain. (x400).

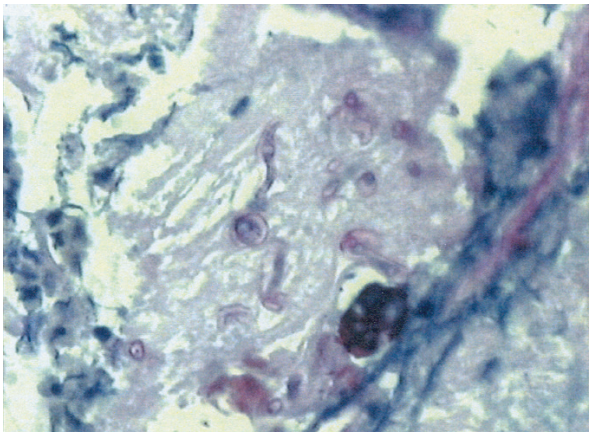

Fig. 6 - Same of Fig. 5. Here, many round filamenting and non filamenting PAS positive cells are observed, also inside a necrobiotic area. PAS stain. (x 400).

other ovoid 12 by 19 microns cells some of them producing buds or septated and branched hyphae. In cultures *Helminthosporium speciferum* (sic) was isolated[7]. It is good to establish that *Helminthosporium* has been frequently confused with *Drechslera*. According to ELLIS[2] the species *H. spiciferum* and *Curvularia spiciferum* must be considered as the state *Drechslera* of *Cochliobolus spicifer.*

Furthermore, PENRITH *et al*.[9] published the isolation of dematiaceous fungi of the genus *Bipolaris* and *Drechslera* from two cases of mbng. Non pigmented cells identified as chlamidospores and also hyaline hyphae were observed.

The isolation in our case of *D. halodes* and the other already mentioned papers seems to confirm the pathogenic potentiality of the genus. The *Trichosporon* sp. strain also developed on the Sabouraud slants would probably be considered as a contaminant or as a secondary invader. However we must recall the importance of *Trichosporon* species mainly *T. beigelii*, as agents of outbreaks of mastitis in dairy herds[3].

In our second case we could not isolate dematiaceous fungi although the observation of pigmented fungal tissular structures together with hyaline rounded and hyphal forms. Instead of, *Geotrichum* sp. grew abundantly in the inoculated slants. This genus has been implicated as cause of adenitis in pigs, intestinal disease in an ocelot, disseminated disease in dogs, cutaneous lesions in horses and mycotic abortions[10].

Until the present time no cases of mbng have been described in Uruguay but according to several consultations to veterinarian doctors, the disease might be unrecognized. Consequently, laboratory diagnosis would be advisable in front of suspicious clinical presentations.

It is good to remember that species of *Nocardia* may also be agents of mbng. While the nocardial nasal granuloma described by SHIBAHARA *et al*.[11] in a Holstein heifer corresponded to a severe eosinophilic infiltration with multinucleated giant cells, the nasal nocardiosis published by TAKAHASHI *et al*.[12] in a female Japanese Black calf appears to be well comparable in its histopathology to our first case.

Both, fungal agents and nocardial strains have their normal habitat in the external environment (soil, vegetable debris, etc). From these sites they would infect the nasal mucosa of cattle mainly through microtraumatisms. Later on, the immunological host reaction might lead to the granulomatous lesions already described. Early diagnosis by means of appropriate laboratory techniques followed by the corresponding specific treatment would avoid the development of the severe disease.

ACKNOWLEDGEMENTS

The authors present their appreciation to Prof. Raúl Casas Olascoaga, V.D. for his worthy suggestions and to Mariel Méndez for her kind help in the bibliographic revision.

RESUMO

Granuloma micótico nasal bovino

É apresentado caso de granuloma micótico nasal bovino, em vaca Jersey, com 10 anos de idade, produzido por *Drechslera halodes.*

Cortes histopatológicos mostraram abundantes estruturas fúngicas hialinas e pigmentadas extra e intracelulares junto com granuloma polimorfo celular formado por neutrófilos, linfócitos, plasmócitos, histiócitos e células gigantes de Langhans.

É o primeiro caso de granuloma micótico nasal bovino diagnosticado no Uruguai embora esta doença pareça ser freqüente de acordo com a opinião de veterinários especializados.

Outro caso clínico semelhante, também em vaca Jersey da mesma fazenda de criação de gado leiteiro, com intenso infiltrado celular rico em eosinófilos, sem imagem granulomatosa, junto com formas fúngicas fuliginosas hialinas extra celulares é também relatado para fins de comparação. *Geotrichum* sp. foi isolado.

A necessidade de diagnóstico precoce e tratamento da doença é enfatizada.

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Received: 16 January 2003
Accepted: 13 May 2003