

## CONIDIOBOLOMYCOSIS IN SHEEP IN THE STATE OF PERNAMBUCO\*

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**ABSTRACT.** Mendonça F.S., Albuquerque R.F., Evêncio-Neto J., Dória R.G.S., Camargo L.M. & Freitas S.H. [Conidiobolomycose em ovinos no estado de pernambuco.] Conidiobolomycosis in sheep in the State of Pernambuco. *Revista Brasileira de Medicina Veterinária*, 34(3):241-246, 2012. Departamento de Medicina e Cirurgia Veterinária, Faculdade de Medicina Veterinária, Universidade de Cuiabá, Avenida Beira Rio, 3100, Cuiabá, MT 78065-480, Brasil. E-mail: shfreitas@terra.com.br

An outbreak of zigomycosis with rhinopharyngeal lesions involving fungal hyphae with characteristic of entomophthoromycosis due to *Conidiobolus* spp. is reported in the State of Pernambuco, Northeastern Brazil. The epidemiological, clinical, pathological and mycological findings are decrypted in a flock of Santa Inês sheep. The disease occurred at Nazaré da Mata from April to May 2009, all most cases occurred mainly during the rainy season affecting 5 sheep of 29, resulting in death of about 100% of the affected sheep. Clinical signs as noisy respiration and dyspnoea due to mechanical blockage of the nasal cavities, swelling of the nostrils and mucous or serosanguineous nasal discharge were observed. Two of 5 sheep showed exophthalmia, keratitis and unilateral corneal ulceration of the eye. The sheep either died of their infection or were euthanized after a clinical course of 7-20 days. At necropsy there was a firm whitish, lobulated, friable growth was evident in the ethmoidal region and nasal septum at the sagittal sections of the head from four affected sheep. Histopathologically findings were multifocal granulomas with lymphocytic infiltration, epithelioid multinucleated giant cells and fibrovascular tissue, surrounding an eosinophilic necrotic reaction (Splendore-Hoeppli phenomenon). The methenamine-silver stain uncovered fungal hyphae, rarely ramified with bulbous dilatation in their extremities. *Conidiobolus* sp. was isolated from nasal tissue lesions of two sheep. This is the first report of disease in the State.

**KEY WORDS.** Entomophthoromycosis, zygomycetes, sheep.

**RESUMO.** Os achados epidemiológicos, clínicos, patológicos e micológicos de um surto de zigomycose com lesões rinofaringeais envolvendo a presença de hifas fúngicas com características de entomofitomicose por *Conidiobolus* spp, são descritos no Estado de Pernambuco. A doença ocorreu no Município de Nazaré da Mata entre abril e maio de

2009, durante o período chuvoso afetando 5 ovinos de um total de 29, resultando na morte de 100% dos ovinos afetados. Os sinais clínicos consistiram em respiração ruidosa, dispnéia por obstrução das cavidades nasais, deformação facial e exsudação nasal mucosa ou serosanguinolenta. Os ovinos morreram ou foram eutanasiados após um curso clínico que

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variou de 7-20 dias. Vistos á secção sagital da cabeça, os achados de necropsia consistiram na presença de um crescimento granulomatoso, firme e esbranquiçado na região dos etmóides e septo nasal dos ovinos afetados. Os achados histopatológicos consistiram na presença de granulomas com infiltração linfocítica, de células gigantes multinucleadas e de tecido fibrovascular, circundados pelo fenômeno de Splendore-Hoeppli. Hifas fúngicas pouco septadas, com dilatação bulbosa das extremidades foram identificadas pela coloração da prata-metamina. *Conidiobolus* sp. foi isolado do tecido nasal de dois ovinos. Este é o primeiro relato da doença no Estado.

PALAVRAS-CHAVE. Entomofotoromicose, zigomicetos, ovinos.

## INTRODUCTION

Within the pathogenic zygomycetes two orders are of medical and veterinary importance and produce dramatically different infections: the Entomophthorales primarily causing chronic infection of the nasal submucosa and subcutaneous tissue of the nose and face (species of *Conidiobolus*) or causing chronic subcutaneous infection of the limbs (species of *Basidiobolus*) and the Mucorales (*Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Cunninghamella* and *Saksenaea*) commonly involved in angioinvasive infection called mucormycosis (Grooters et al. 2003, Prabhu & Patel 2004). Entomophthoromycosis due to *Conidiobolus* spp. is a granulomatous zygomycosis infection characterized by lesions that originate in the inferior turbinate, spread through ostia and foramina to involve the facial and subcutaneous tissues and paranasal sinuses (Galgóczy 2005, Gugnani 1992). The spores produced by these fungi are airborne and can be inhaled into the respiratory tract: this is the most common route for the infection of a susceptible host. Zygomycoses manifest primarily as rhinocerebral infections, but pulmonary and disseminated mycoses also occurred (Ribes et al., 2000).

*Conidiobolus* and *Basidiobolus* species generally cause subcutaneous and mucocutaneous infections in tropical and subtropical areas (Galgóczy 2005). Entomophthoromycosis due to *Conidiobolus coronatus*, *C. lamprauges* and *C. incongruus* have been identified as a cause of disease in horses (Humber et al. 1989, Boabaid et al. 2008), dogs (Grooters 2003), sheep (Boabaid et al. 2008, Carrigan et al. 1992, Riet-Correa et al. 2008, Silva et al. 2007a)

and humans (Frater et al. 1994). The illness has been reported in Australia as caused by *C. incongruus* (Ketterer et al. 1992) and in Brazil as caused by *C. coronatus* (Silva et al. 2007a,b).

The disease occurs in two different clinical forms: a rhinofacial infection affecting the nose vestibule, mucoepithelial junction, and upper lip (Silva et al. 2007a), and a nasopharyngeal infection affecting the ethmoidal region, turbinate bones, paranasal sinuses, hard and soft palates and pharynges (Riet-Correa et al. 2008, Silva et al. 2007b). In Brazil scientific data about the disease are still recent and were only recorded in the States of Piauí (Silva et al. 2007a,b), Mato Grosso (Boabaid et al. 2008) and Paraíba (Riet-Correa et al. 2008). For this reason this study aims to report the first cases of entomophthoromycosis due to *Conidiobolus* spp. and its epidemiological, clinical signs, mycological and pathological findings in a flock of sheep in State of Pernambuco, Northeastern Brazil.

## MATERIAL AND METHODS

Epidemiological, clinical and data about the nutrition and sanitary management of animals as well as morbidity and mortality were collected at a farm in the State of Pernambuco, Brazil, where 5 sheep became sick from April to May 2009.

Necropsies were performed on sheep that died of their infections and on those that were euthanized *in extremis*. Euthanasias were performed using 90 mg/kg of sodium pentobarbital by intravenous route (Pentobarbital Sódico 3%, Cristália Produtos Químicos e Farmacêuticos Ltda, Brazil) according the Brazilian guide for care and use of animals and ethical guidelines of animal welfare committee of the Universidade Federal Rural de Pernambuco (UFRPE). Samples from the nostril areas, peripheral lymph nodes, tissues from organs of the abdominal and thoracic cavities and central nervous tissues were fixed in 10% buffered formalin and were sent to the Laboratory of Veterinary Diagnosis of UFRPE where they were examined. For histological examination the tissues were embedded in paraffin, sectioned at 5µm, and stained with hematoxylin-eosin (HE) and Gomori's methenamine silver stains for fungi (Allen 1992). For microbiological examination, samples of lungs, submandibular lymph nodes and masses of nasopharynx and ethmoid bone of two ovine were washed with sterile saline solution more antibiotic (ampicillin 50 mg/L), placed on 2% Sabouraud Dextrose Agar (SDA) and Chloranphe-

nicol Sabouraud Dextrose Agar (CSDA). The cultures were incubated at 30°C for 7 days. The colonies were stained with lactophenol cotton blue (LPCB) for morphologic evaluation.

## RESULTS

The place where the outbreak occurred has tropical humid climate with annual rainfall ranging from 1,750 to 2,000 mm, with maximum intensity in May, June and July. The average temperature varies from 26 ° to 31 ° C. From October to January there is a dry period.

In April 2009, the veterinary service was requested on a farm located at Nazaré da Mata. According to the farmer 2 sheep showed depression, noisy respiration and dyspnea due to mechanical blockage of the nasal cavities, swelling of the nostrils and serosanguineous nasal discharge; both had a natural death from 7 to 20 days after symptoms appear. In May 2009 more 3 adult animals had the disease and all died naturally. In this property and neighbouring farms had never a history of similar disease. The herd was formed by 29 animals bred in a *Brachiaria decumbens* pasture bounded on by the Atlantic Coast Restinga Forests with several wet areas. The animals drank water from artesian reservatory and received mineral supplementation for sheep. The disease occurred in 5 (17.2%) of the adults sheep and all involved the nasopharyngeal area. Cranium-facial asymmetry because of exophthalmos of 1 eye was observed in 3 (10.3%) sheep. Farmer informed that in those cases the fatality rate was 100%. During the veterinary visitation 3 sheep, aged approximately between 9 months and 2 years had signs of the disease. All of those animals were examined and euthanized *in extremis* with an intravenous injection of 25 mg/kg sodium thiopental and intravenous injection of 30% potassium chloride.

In general clinical signs were mainly characterized by depression, anorexia and progressive weight loss. The sheep had serous or mucohemorrhagic nasal discharge, swelling of the nostril and severe dyspnea with noisy (Figure 1). Exophthalmos of 1 eye with increased volume of the eyeball, keratitis, blindness and corneal ulceration were observed too. Marked depression, with the animal maintaining the head down was observed in one sheep and severe tympanism in another sheep 12 hours before the death. In 2 sheep the clinical signs were similar to those above mentioned, but exophthalmia was not observed in these animals. The clinical manifesta-

tion period was from 1 to 4 weeks. All affected animals died after approximately a clinical course of 7 to 20 days.

The mainly lesions were observed after on sagittal section of the heads. A whitish or yellow friable mass with irregular/ lobulated and granular consistency were observed in the ethmoidal region and nasal septum (Figure 2). There were swelling of the nostrils, oedema of nasal mucosa surface, mucopurulent nasal discharge in frontal sinus, maxillary sinus and obstruction of choanae by granulomatous proliferation. The lesion extended from the basisphenoid and presphenoid bones to the pharynges, which full of necrotic yellowish material and exudates. In some sheep almost turbinate bones were changed for irregular granulomatous proliferation. In most cases the cribiform plate were also affected. Orbit was also affected, causing cranium-facial

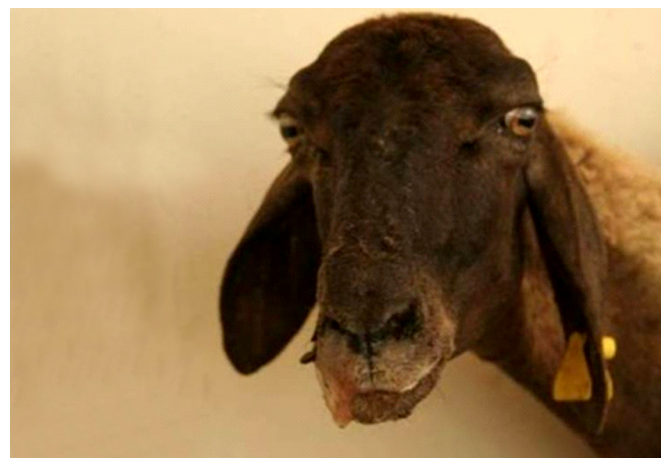


Figure 1. Sheep conidiobolomycosis. Nasal serosanguineous discharge and little cranium-facial asymmetry.

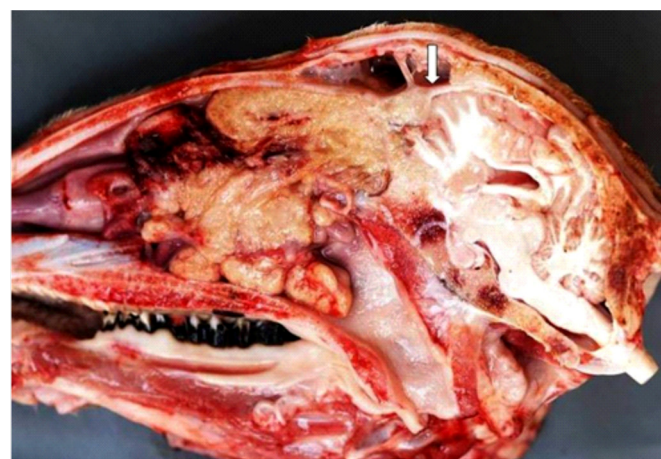


Figure 2. Sheep conidiobolomycosis. Sagittal section of the head. The ethmoid bone and most of the turbinate bones are destroyed and have been replaced by a yellow mass. The pharynx and larynx were invaded too. The lesion extended to the frontal meninges, which is thickened (arrow).



asymmetry and exophthalmos. Frequently the lesion invaded the anterior brain and meninges in the cranial cavity.

In the lungs, grayish multifocal granulomatous nodules with yellow central to red areas and a hard consistency, measuring 0.5-1cm in diameter were found in 3 sheep (Figure 3). These nodules were mainly evident on subpleural pulmonary surface tend to have a lobular distribution. In 1 sheep, the submandibular lymph nodes were enlarged, with multifocal yellowish areas. No macroscopic lesions were observed in anatomical areas other than the affected tissues.

In 4 sheep the main microscopic lesions were extensively multifocal areas of granulomatous proliferation, sometimes coalescent, with intensive connective tissue proliferation in tissues of nasal septum, nasal conchae, anterior brain and lung. In these areas there were inflammatory cells, mainly

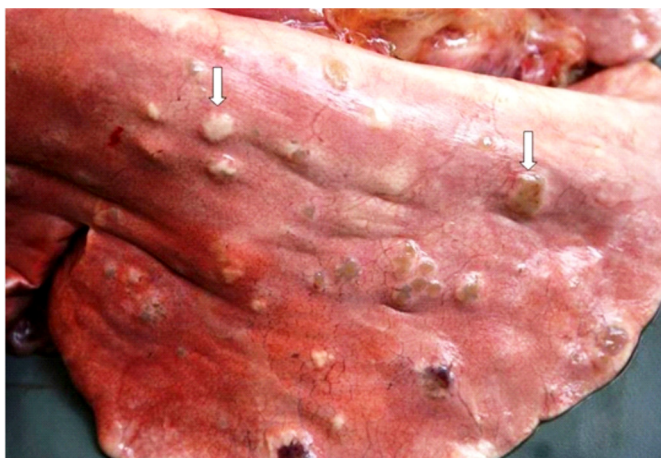


Figure 3. Sheep conidiobolomycosis. Lung showing numerous multifocal subpleural yellowish or whitish nodules, 0.5-1cm in diameter (arrows).

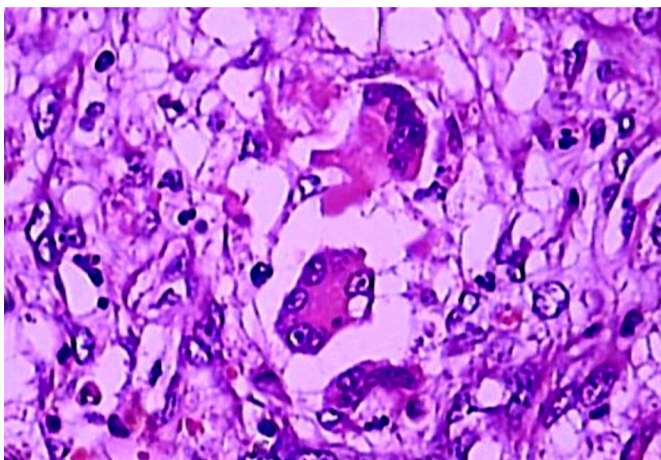


Figure 4. Sheep conidiobolomycosis. Histological aspect of lung with epithelioid cells and multinucleated giant cells (arrows). HE. Obj. 40X.

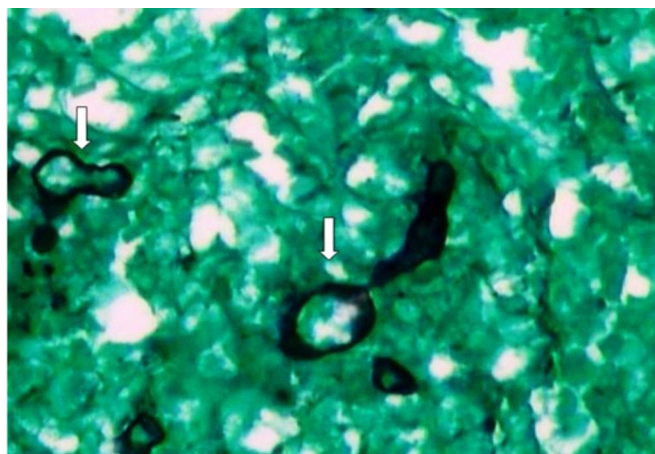


Figure 5. Sheep conidiobolomycosis. Nasopharyngeal tissues. A granuloma is observed containing rarely ramified fungi hyphae with bulbous dilatation in their extremities (arrows). Methenamine-silver stain. Obj. 40X.

eosinophils, neutrophils, mononuclear cells, and epithelioid and giant cells (Figure 4) bordered by caseous necrotic areas with negatively stained structures representing fungal hyphae surrounded by eosinophilic Splendore-Hoeppli material. Hyphalike structures were sometimes observed free or within giant cells, not associated with Splendore-Hoeppli material. Many vessels had segmental necrosis and thrombosis. Neovascularization, collagen deposit, congestion and hemorrhagia were frequently identified.

In the lungs the bronchiolar epithelium was hyperplastic with proliferation of fibrous tissue. Mixed inflammatory cells like lymphocytes, plasma cells, neutrophils, and Splendore-Hoeppli material were observed within the bronchioles or in peribronchial tissues. The meninges showed similar inflammatory reaction, thickened by a presence of lymphocytes, plasma cells, neutrophils and giant cells. In the anterior lobe of the brain occasionally microabscesses were observed with a cuffing of mononuclear cells. On Gomori's methenamine silver stains 6-25- $\mu$ m thick fungal hyphae, not often septated or ramified, thin-walled with black contoured, with bulbous dilatation in the extremities were identified (Figure 5).

In SDA, the growths of colonies were observed as flat whitish pale or cream color and smooth in the first days. These colonies have become powdery, corrugated and adhered to the agar after 5 days. In the microculture in stained of LPCB hyphae with septa were scarce and ends with bulbous dilatation in the extremities rounded prominent papillae. Based on the morphology fungal structures were classified in the genus *Conidiobolus*.

## DISCUSSION

The diagnosis of this outbreak of entomophthoromycosis in the State of Pernambuco was based in clinical, pathological and mainly in isolation of *Conidiobolus* spp. in many tissues of affected sheep.

Nasopharyngeal entomophthoromycosis due to *Conidiobolus* spp. in ruminants has only been reported in a few species including sheep in Brazil (Boabaid et al. 2008, Riet-Correa et al. 2008, Silva et al. 2007a,b), Australia (Carrigan et al. 1992, Ketterer et al. 1992), Trinidad and Tobago (Morris et al. 1991), equine (Humber et al. 1989, Mendoza et al. 1985) and llamas (French et al. 1994). In Australia the illness has been reported as caused by *C. incongruus* (Ketterer et al. 1992) and in State of Piauí, Brazil, as caused by *C. coronatus* (Silva et al. 2007a). The disease has recently been reported in sheep in State of Paraíba (Riet-Correa et al. 2008), however to date, in Pernambuco there were not records of this disease.

Almost cases of conidiobolomycosis in sheep at Pernambuco occurred from April to May, when the humidity is high and the temperature increased to 26 °C, with intense rainfall. These data are the most commonly reported as important in the epidemiology of the disease (Boabaid et al. 2008, Silva et al. 2007a,b). Further more, the presence of considerable amounts of decaying vegetation in a wet season is a probably risk factors for the occurrence of the disease in sheep (Prabhu et al. 2004). Differently of the outbreaks reported in Brazil that both forms of conidiobolomycosis were observed on the same farm (Boabaid et al. 2008, Riet-Correa et al. 2008, Silva et al. 2007a,b), all those sheep in Pernambuco had rhinopharyngeal lesions, extending through the ethmoid bone to the frontal lobe of the brain. The mortality rate in sheep was of 100%, similar to others reports. The rhinofacial form of disease until to date, did not occur.

Typical clinical signs of conidiobolomycosis such as serous or mucohemorrhagic nasal discharge, severe dyspnea with noisy, cranium-facial asymmetry and exophthalmos of 1 eye were identified in 3 sheep of this study. These same clinical signs were reported by different researchers (Boabaid et al. 2008, Carrigan et al., 1992, Frater et al. 1994, Ketterer et al. 1992, Morris et al. 2001, Riet-Correa et al. 2008, Silva et al., 2007b). Such as others reports an interesting feature of the disease was the rapid clinical progression of the lesions (7-20 days in most animals) (Gugnani et al. 1992, Riet-Correa

et al. 2008). Apparently, the disease in State Pernambuco does not have an enzootic character like in semi-arid northeastern Brazil. The disease occurs like an isolated entity, since there was no history of similar disease in previous years in the farms where outbreaks of conidiobolomycosis occurred.

The mainly gross lesions described here, such as necrogranulomatous rhinitis with mycotic invasion to ethmoidal region, nasal septum and choanae characterizes the nasopharyngeal form of entomophthoromycosis due to *C. incongruus* in Australia (Ketterer et al. 1992) and due to *C. coronatus* in Brazil (Silva et al. 2007a). In Australia, the infection by *C. incongruus* in sheep affected also the lung, lymph nodes, and brain, but not other organs (Carrigan et al. 1992, Ketterer et al. 1992). These same organs were affected in 4 sheep of this study (other organs were also not affected).

In human the diagnosis of zygomycoses is very challenging: most such infections are identified only as zygomycosis or mucormycosis, without at least genus determination (Eucker et al. 2001, Ribes et al. 2000). In this paper the definitive diagnosis of disease in sheep was made based on microbiological and histological study of the tissue lesions where were typical entomophthoramycetous fungi, with negatively stained structures representing fungal hyphae surrounded by eosinophilic Splendore-Hoeppli material around 6-25µm in diameter thick, rarely septated, and thin walled ribbon-type fungal elements.

The observation of new cases of conidiobolomycosis in Pernambuco suggests that the problem, despite being recent, can affect a larger number of sheep in the region although its occurrence is restricted.

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