

BRAIN AND LUNG CRYPTOCOCCOMA AND CONCURRENT *Corynebacterium pseudotuberculosis* INFECTION IN A GOAT: A CASE REPORT

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ABSTRACT: A four-year-old male goat with a history of neurological disorder was euthanized. It presented uncommon nodules in the brain and lungs associated with multiple abscesses, predominantly in the spleen and liver. Histological examination of brain and lung sections revealed yeast forms confirmed to be *Cryptococcus gattii* after a combination of isolation and polymerase chain reaction (PCR) procedures. Moreover, *Corynebacterium pseudotuberculosis* infection was diagnosed by PCR of samples from the lung, spleen and liver. The present report highlights the rare concurrent infection of *C. gattii* and *C. pseudotuberculosis* in an adult goat from São Paulo state, Brazil, and indicates the necessity of surveillance in the treatment of goats with atypical pulmonary infections associated with neurological disorders.

KEY WORDS: *Cryptococcus gattii*, *Corynebacterium pseudotuberculosis*, meningoencephalitis, goat.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Cryptococcosis is an important cause of life-threatening meningoencephalitis both in human and other animals. *Cryptococcus gattii* is a basidiomycetes, a encapsulated yeast that is typically found in tropical and subtropical climate zones and predominantly infects immunocompetent hosts (1, 2). Traditionally, the natural habitat of *C. gattii* is mainly associated with several tree species (3). Moreover, cryptococcosis is characterized as an invasive fungal infection most commonly caused by one of two species normally defined as subacute or chronic, and often confused with viral or bacterial meningoencephalitis or other infections, including tuberculosis (3-5). Besides, there are substantial differences in the ecology that can influence directly on cryptococcosis epidemiology (3). Recently, *C. gattii* has been described to be associated with non-immunocompromised individual infection, resulting in a severe meningoencephalitis disorder in a domestic cat (5).

Corynebacterium pseudotuberculosis is the etiological agent of caseous lymphadenitis (CLA), a common disease in small ruminants throughout the world. This infection has been an important disease in the majority of sheep-rearing regions for over a century. Because of the chronic and often subclinical nature of CLA, it has proved to be difficult to control and its prevalence is high in many parts of the world, which, in turn, leads to significant economic losses for farmers (6, 7). Furthermore, to the best of our knowledge, the direct association between *C. gatti* and *C. pseudotuberculosis* infecting goats has never been described before.

CASE REPORT

A 4-year-old goat male, Boer breed, presenting a two-week history of gait and right head-tilt unresponsive to anti-inflammatory and antibiotic conventional treatment was sent to the Teaching Hospital of São Paulo State University, UNESP, in Araçatuba, SP, Brazil, and was killed for pathological examination.

Samples from the lung, brain and spleen were formalin-fixed and processed for histology by routine methods – sectioned (4 µm) and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS). Tissue suspensions, cerebrospinal fluid and pus from abscessed nodes were also submitted to microbiological analysis. Colonies resembling *C. pseudotuberculosis* were tested for biochemical properties. Some clinical samples were also evaluated by mycological exams, which revealed by direct microscopy and culture on Sabouraud dextrose agar at 30°C and 37°C the presence of *Cryptococcus* spp. After that, a multiplex-PCR was carried out to differentiate *C. neoformans* from *C. gatti* in lung

samples using the sets of primers, as previously described (8). An m-PCR was also carried out to identify *C. pseudotuberculosis* through sets of primers targeting on 16S rRNA, rpoB and pld genes, as previously described (9). Total DNA was extracted from all clinical samples; the primer sequences employed are shown on Table 1 (8, 9).

Table 1. Sequences of oligonucleotides employed to characterized the serotype of *Cryptococcus neoformans* and *C. gatii* and molecular identification of *Corynebacterium pseudotuberculosis*

Reference	Description	5' to 3' sequence
MVA 009 (ATCC 24066)	<i>Cryptococcus gatii</i> (genotype C)	CNa-70-A (59-ATTGCGTCCATGTTACGTGGC-39) CNa-29-S (59-CTCTTGACGTTGGCTCTTTC-39)
MVA 010 (ATCC 32045)	<i>Cryptococcus grubii</i> (genotype A)	CNa-70-S (59-ATTGCGTCCACCAAGGAGCTC-39) CNa-70-A (59-ATTGCGTCCATGTTACGTGGC-39)
MVA 011 (ATCC 28958)	<i>Cryptococcus neoformans</i> (genotype D)	CNa-29-S (59-CTCTTGACGTTGGCTCTTTC-39)
MVA 012 (ATCC 48184)	<i>Cryptococcus neoformans</i> (genotypes A and D)	29-A (59-CTACTGATGAAAAGCTCGCTG-39)
MPLD1	<i>Corynebacterium pseudotuberculosis</i>	16S rRNA gene 16S-F ACCGCACTTTAGTGTGTGTG 16S-R TCTCTACGCCGATCTTGTAT
MPLD2.1	<i>Corynebacterium. Pseudotuberculosis</i>	rpoB C2700F CGTATGAACATCGGCCAGGT C3130R TCCATTTCCGCCGAAGCGCTG pld PLD-F ATAAGCGTAAGCAGGGAGCA PLD-R1 ATCAGCGGTGATTGTCTTCC PLD-R2 ATCAGCGGTGATTGTCTTCCAGG

At necropsy, a 4.5 x 4.0-cm firm yellowish nodule filled with gelatinous substance associated with multiples 1 to 2-cm abscesses were found in the left lung lobe (Figure 1A). Ovoid abscesses and multiple coalescing translucent cysts in the spleen and liver were also observed. In addition, 2 to 3-mm translucent cysts were discovered dispersed in cerebral cortex, surrounded by necrosis (Figure 1B).

Cytology smears (PAS, staining) prepared from brain lesions and the large lung nodule revealed multiple yeasts with thick capsules arranged into clusters or not (Figures 1D and 1F). Histopathology sections prepared from lung specimens revealed severe pneumonia, associated mostly with yeasts localized at alveoli adjacent to bronchi, intracellular and

extracellular enmeshed in abundant amorphous material and nuclear debris (Figures 1C). Brain sections revealed extensive necrosis and yeast-like forms (Figure 1F). Furthermore, PAS positive yeast-like organisms, morphologically similar to *Cryptococcus* sp. were detected in the brain, spinal cord, encephalic white matter and lung nodule (Figure 1E). The molecular identification of *C. gatti* was confirmed, as it can be observed in Figure 2, in which is clearly demonstrated a fragment 448-bp specific for *C. gatti* in positive control (line1) and lung tissue sample (line 5). The fragment of 695-bp corresponds to ATCC samples of *C. neoformans*. Colonies isolated from lung and spleen abscesses which were suspected of being *Corynebacterium* genera were confirmed by m-PCR assay as being *C. pseudotuberculosis*, as demonstrated in Figure 3.

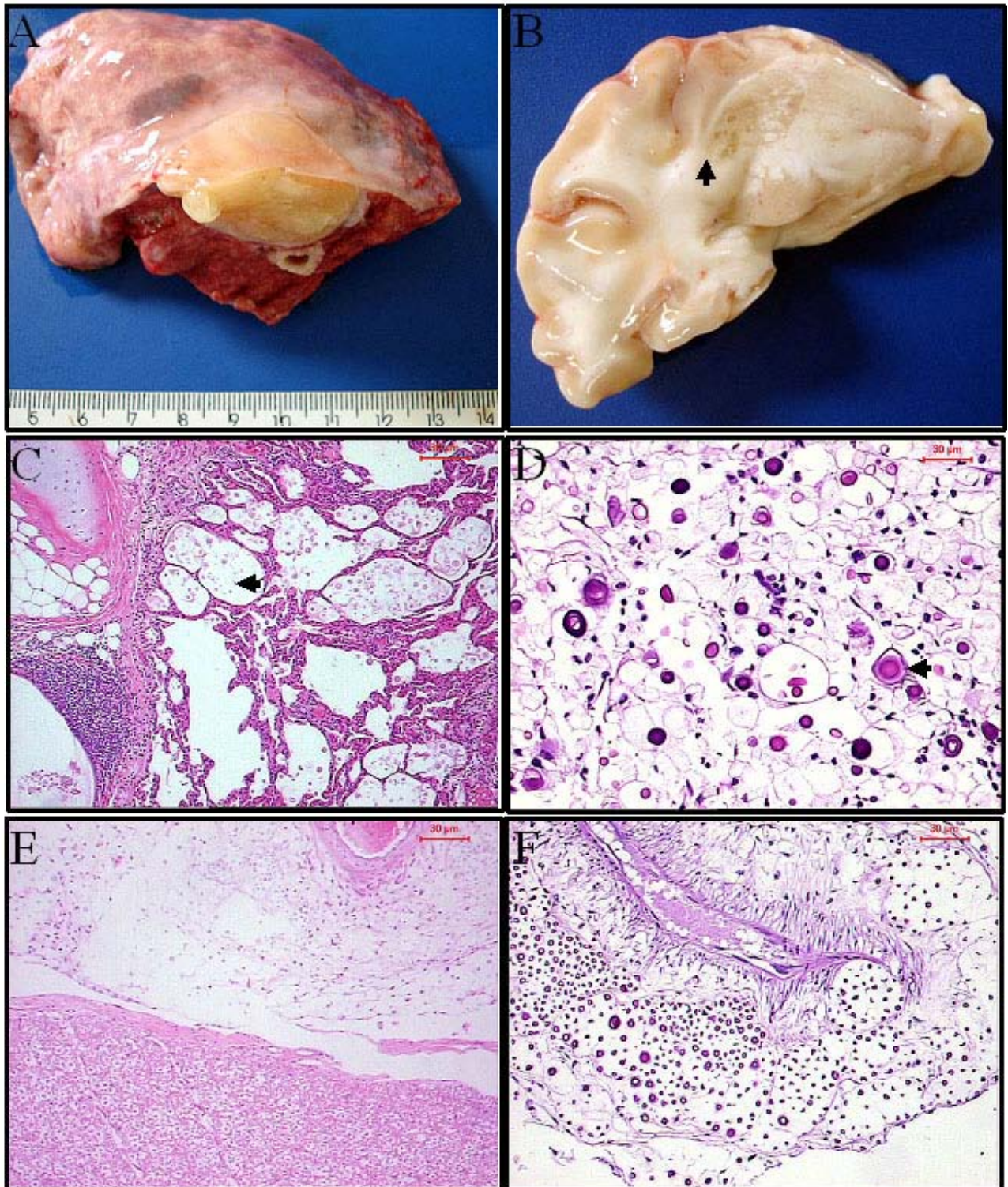


Figure1. (A) Nodule in the left lung lobe with yellow gelatinous material; (B) brain with multiple coalescing transparent cysts; (C) yeast-like organisms in alveoli (arrow) adjacent to a bronchus, HE 10x; (D) nodule in the lung with foamy macrophages associated with PAS positive yeast-like organisms (arrow) PAS, 40x; (E) yeast-like organisms in the brain, HE, 10x; (F) PAS positive yeast-like organisms in brain, 40x.

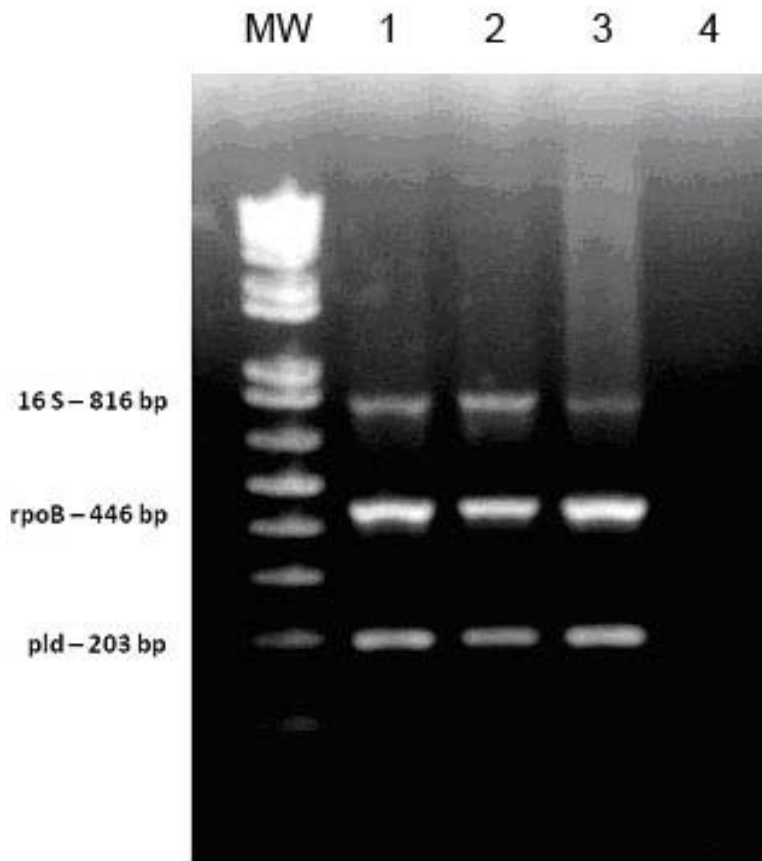


Figure 2. Multiplex polymerase-chain reaction (m-PCR). MW: molecular weight marker (1 kb plus ladder). Lines 1 and 2: samples from the lung and spleen positive for *C. pseudotuberculosis*; line 3: positive control (pure culture of *C. pseudotuberculosis*); line 4: negative control.

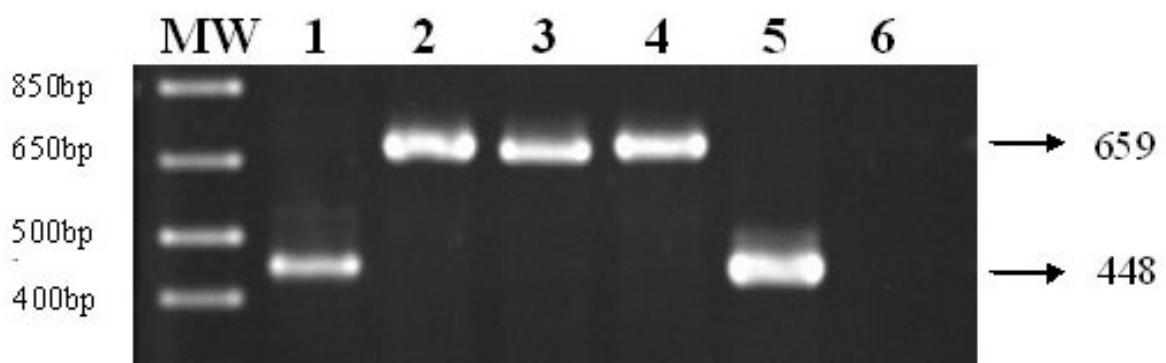


Figure 3. Molecular characterization of *Cryptococcus* spp. Lines 1 to 4: standard strains (ATCC 24066, ATCC 32045, ATCC 28958 and ATCC 48184, respectively); line 5: sample from examined lung; line 6: negative control.

DISCUSSION

Natural CLA infections in goats demonstrate several similarities with the disease in sheep, especially when the same biotype of *C. pseudotuberculosis* is responsible. However, consideration of the differences in clinical presentation between sheep and goats may be helpful in respect of pathogenesis and specific risk factors (6, 9). In both sheep and goats, the major sites of infection are the superficial lymph nodes. However, in the present study no superficial lymph node was clinically observed. Furthermore, visceral lesions have been described as rare and found only in a minority of animals (7). Additionally, in sheep, visceral lesions, and particularly lung lesions, occur more frequently and in greater numbers than in goats (6, 7, 10, 11). Herein, it seems that primary infection with *C. pseudotuberculosis*, in a different pathway, predisposed the animal to *C. gattii* infection. This is an important epidemiological aspect since *C. gattii* has been identified as a primary pathogen and led to other infections in both humans and other animals (2, 4, 5, 12-15). In addition, an association between *C. neoformans* and *Mycobacterium bovis* infection has already been described in goat, generating a granulomatous pneumonia (4).

There are few reports that describe *C. gattii* causing diseases in goats and it appears that chronic and often subclinical infections can predispose to *C. gattii* concurrent infections, even in non-immunocompromised individuals (16, 17). Different from CLA in goats, there are a small number of studies that report *C. gattii* infecting in this species. In Spain, the disease was described in outbreaks and caused severe pneumonia associated with cachexia in most affected animals (2). In Brazil, a survey on regional patterns of *C. neoformans* and *C. gattii* was carried out and found predominance of *C. gattii* in the northeast region (18). The present case seems to be the first one to report the occurrence of the disease in a Brazilian goat, caused by *C. gattii* serotype B. Since cryptococcosis is not well known in goats in the country, this infection may have predisposed to the concurrent infection by *C. pseudotuberculosis*, as it was herein presented. Both pathogens can be transmitted to non-immunocompromised hosts, both humans and other animals, and the surveillance of their natural occurrence should be stimulated aiming at preventing new infections.

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