

## BRIEF COMMUNICATIONS and CASE REPORTS

### *Cryptococcus neoformans* and *Mycobacterium bovis* Causing Granulomatous Pneumonia in a Goat

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**Abstract.** An adult Blanca-Celtibérica doe originating from a goat herd with a high prevalence of tuberculosis was presented with respiratory signs. At necropsy, this goat had a diffuse and severe mycotic pneumonia associated with the presence of *Cryptococcus neoformans* concomitant with pulmonary focal caseous nodules from which *Mycobacterium bovis* was isolated. Microscopically, the mycotic lesion was a granulomatous pneumonia with many large foamy macrophages containing intracellular yeast bodies. The extensive mycotic changes, their granulomatous nature, and the lack of positive response to different immunologic tests for mycobacterial infection suggested an impaired immune status in this animal.

**Key words:** Cryptococcosis; *Cryptococcus neoformans*; goats; *Mycobacterium bovis*; pneumonia; tuberculosis.

*Cryptococcus neoformans* is a yeastlike fungus with worldwide distribution; it causes diseases in both animals and humans. The organism occurs as a saprophyte in nature, usually in soil contaminated with pigeon and chicken manure, and is considered an opportunist pathogen.<sup>2</sup> In cattle, reports of *C. neoformans* are relatively common, and infection is restricted to mammary tissue and adjacent lymph nodes.<sup>9</sup> Sporadic cases of mastitis have been also described in other domestic ruminants, including goats.<sup>11</sup> Apart from cattle, clinical cryptococcosis is mainly recognized in cats and has been described as a localized or disseminated disease, with a predilection for the respiratory system (particularly the nasal region) and the central nervous system.<sup>2</sup> In Spain, cryptococcosis has been previously reported in cats,<sup>4</sup> pigeons,<sup>8</sup> and goats in association with caprine pleuropneumonia.<sup>1</sup>

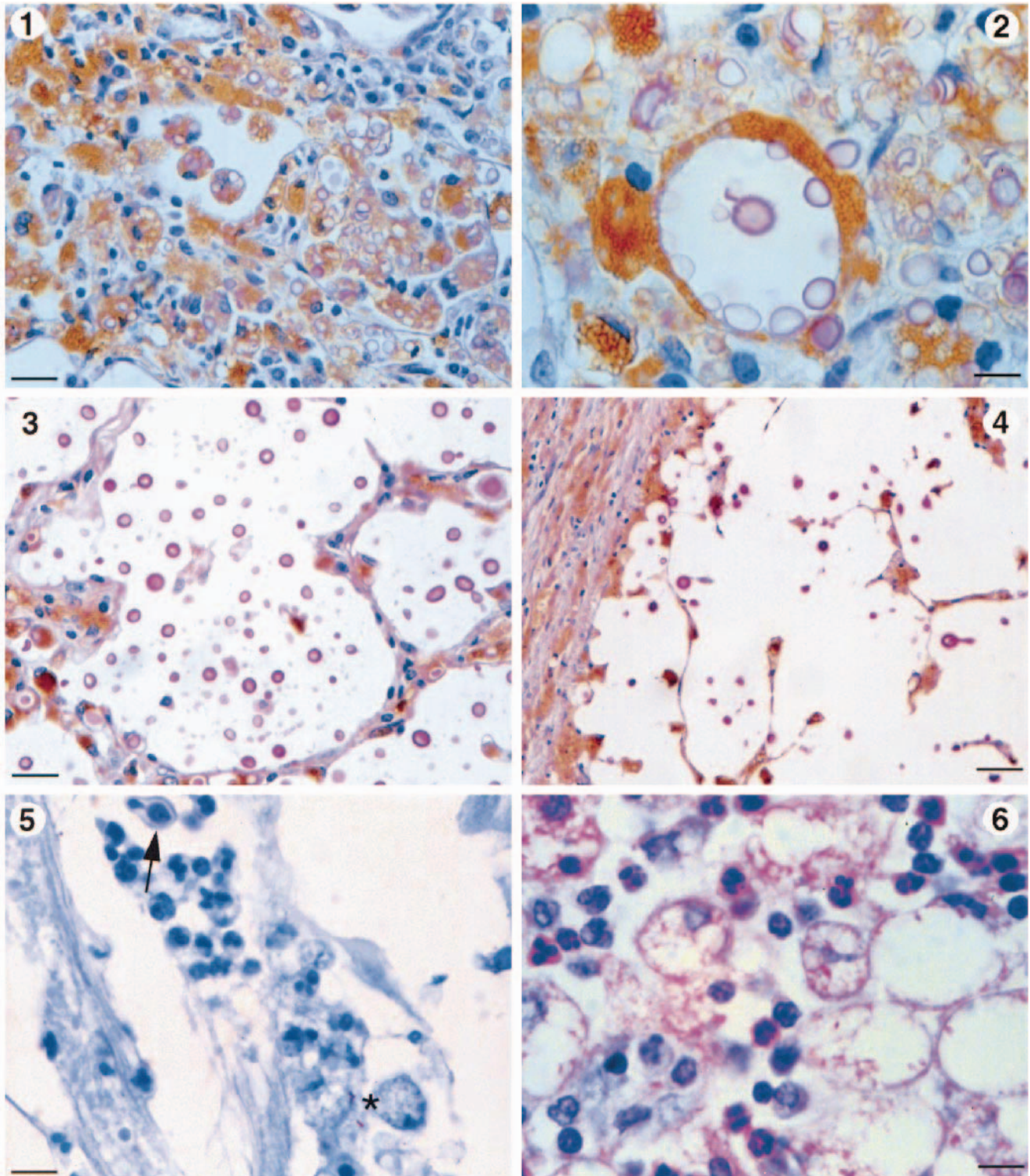
*Mycobacterium bovis* infection is usually recognized as the cause of bovine tuberculosis (TB), but the range of possible hosts is wide and includes humans and a variety of domestic and wild animals.<sup>2</sup> In Spain, bovine TB eradication campaigns have resulted in a low prevalence of TB in cattle.<sup>5</sup> However, *M. bovis* infection is widespread in some goat herds, and there are no sound statutory schemes for its control in this species. As in cattle, TB lesions in the goat usually are found in the lungs and regional lymph nodes, but generalized forms can also occur.<sup>5</sup>

During the course of a study of TB in a goat herd with high prevalence of this disease, a 7-year-old Blanca-Celtibérica doe presented with clinical signs of weight loss, coughing, and respiratory distress. Responses to immunologic tests for mycobacteria<sup>7</sup> were negative (tuberculin skin test, serologic bovine purified protein derivative enzyme-linked immunosorbent assay) or inconclusive (TB gamma-interferon assay). At postmortem examination, gross lesions were restricted to the lungs and mediastinal and bronchial lymph nodes. The lungs were gray, enlarged, and diffusely

consolidated. Several gelatinous gray, slightly raised, well-demarcated nodules 0.5–2 cm in diameter were scattered in the lungs, with a smaller number of white, firm caseous foci approximately 0.2–1 cm in diameter found in association with bronchi. Bronchial and mediastinal lymph nodes were enlarged and contained milliary caseous white foci.

Microscopically, most of the pulmonary parenchyma was occupied by a diffuse, severe granulomatous pneumonia, with many large foamy, frequently multinucleated macrophages in the alveolar septa and lumina. These macrophages were strongly positive for the ovine macrophage marker VPM32<sup>6</sup> by immunoperoxidase staining. They usually contained a variable number (1–10) of yeast bodies, but extracellular yeast bodies were seldom seen (Figs. 1, 2). In contrast, in some groups of alveoli the yeast bodies appeared free, with a scant inflammatory reaction associated with them that consisted of clusters of neutrophils and solitary macrophages in the alveolar spaces (Fig. 3). The macroscopic gray nodules appeared microscopically as large cyst-like cavitations with encapsulation, within which numerous organisms were found extracellularly with remnants of alveolar septa. The inflammatory response was minimal in these areas (Fig. 4). The free yeast bodies were ovoid to spherical, 4–10  $\mu$ m in diameter with thick periodic acid-Schiff (PAS)-positive capsules and occasionally presented a narrow-base budding (Figs. 3, 4). Most yeast bodies within macrophages looked the same as the extracellular organisms, including a thick capsule, but some yeast bodies had anomalous shapes and partial PAS reaction (Figs. 1, 2). The yeast was confirmed by culture to be *C. neoformans*.

Microscopically, the white foci appeared as tubercles with a caseous necrotic center surrounded by a thin layer of epithelioid and multinucleated giant cells, with infiltration of lymphocyte and plasma cells. Small granulomata formed by epithelioid cells and giant cells were seen in the periphery of the tubercles, some of them located in the submucosa of



**Fig. 1.** Lung; goat. Granulomatous pneumonia due to *Cryptococcus neoformans* infection. Large foamy macrophages in the alveolar septa and lumina with numerous intracellular yeast bodies. Immunostaining with monoclonal antibody VPM32 against ovine macrophages, avidin-biotin-peroxidase complex method, diaminobenzidine chromogen. PAS, Harris's hematoxylin counterstain. Bar = 62.5  $\mu$ m.

**Fig. 2.** Lung; goat. Granulomatous pneumonia due to *Cryptococcus neoformans* infection. Macrophages and giant cells with intracellular yeast organisms showing different shapes and intensity of PAS reaction. Immunostaining with monoclonal antibody VPM32 against ovine macrophages, avidin-biotin-peroxidase complex method, diaminobenzidine chromogen. PAS, Harris's hematoxylin counterstain. Bar = 25  $\mu$ m.



the associated bronchi. Acid-fast organisms were present in moderate numbers in the caseous necrotic areas or within giant cells and more abundantly in the exudate of the associated bronchi (Fig. 5). Yeast bodies and foamy macrophages were found in the bronchial exudate (Fig. 5), but they were not found in association with the tuberculous lesions. *M. bovis* was cultured from these foci.

In the bronchial and mediastinal lymph nodes, granulomatous lymphadenitis was observed with the medulla and paracortex, which were largely occupied by macrophages containing a variable quantity of fungal material. Partially digested remnants of yeast with no PAS reaction were common (Fig. 6). No organisms were found outside macrophages. The cortex had some scattered tuberculous foci, either small granulomata or large tubercles with a thick fibrotic reaction. Microscopically, TB lesions were also seen in the lymphoid tissue of the ileocecal valve, but mycotic lesions were found only in lungs and bronchomediastinal lymph nodes.

*C. neoformans* pulmonic lesions are sometimes described as resembling those of TB.<sup>9</sup> However, the lesions caused by the fungus and those caused by the mycobacteria in this goat were clearly differentiable; the mycobacterial lesions were localized and well demarcated, with a distinctive caseous component. An acinar or acinonodose pattern is common in caprine TB,<sup>5</sup> and if present here the distinction between the two types of lesions would have been less clear, particularly if superimposition in the same areas had occurred.

*C. neoformans* infection has been occasionally described in combination with other diseases, such as caprine arthritis encephalitis,<sup>3</sup> caprine pleuropneumonia,<sup>1</sup> or neoplasia.<sup>4</sup> However reports of either *C. neoformans* or *M. bovis* infection in goats are not very common, and this is the first one of a concomitant infection with these organisms. The true incidence of disease associated with these agents in goats is, however, unknown. Yeast bodies resembling *C. neoformans* have been observed in the lungs of two other goats with tuberculous lesions (unpublished data). The yeast bodies were extracellular and limited in number, and the host cellular response was minimal. The latter features are typical of cryptococcosis and were significantly different from the pattern exhibited in the present case.

The granulomatous reaction with intercellular organisms as seen in this goat has been called atypical cryptococcosis,<sup>10</sup> to differentiate it from the more common histologic pattern of cystlike cavitations and interstitial pneumonia with free

yeast bodies. The atypical changes may be related to the competency of the host's immune system.<sup>10</sup> The alveolar macrophages were the most prominent inflammatory cells involved in the mycotic lesions in this goat. Although very numerous and able to phagocytize yeast, these macrophages did not appear to be effective in destroying the yeast bodies or in controlling their spread. This failure in turn could have facilitated the development of the extensive pneumonia observed. This goat also failed to respond adequately to TB tests based on cellular and serologic immune phenomena, which suggests that she had an impaired immune response. The concomitant presence of *C. neoformans* and *M. bovis* in this goat may have resulted in some kind of synergism between the two pathogenic agents that contributed to the severe atypical fungal pneumonia and to the lack of cellular and humoral immune response to TB tests.

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### References

- 1 Aller B, Santiago E, Escudero A, Rey M: Criptococcosis pulmonar en cabras. *Rev Patronato Biol Anim* **15**:387–297, 1971
- 2 Dungworth DL: The respiratory system. In: *Pathology of Domestic Animals*, ed. Jubb KVF, Kennedy PC, and Palmer N, 4th ed., pp. 539–699. Academic Press, San Diego, CA, 1993
- 3 Ellis TM, Robinson WF, Wilcox GE: The pathology and aetiology of lung lesions in goats infected with caprine arthritis-encephalitis virus. *Aust Vet J* **65**:69–73, 1988
- 4 Ferrer L, Ramos JA, Bonavia R, Cabañes J, Pumarola M: Cryptococcosis in two cats seropositive for feline immunodeficiency virus. *Vet Rec* **131**:393–394, 1992
- 5 García Marín JF: Tuberculosis caprina. *Ovis* **46**:1–91, 1996
- 6 González C: Immunobiology of sheep macrophages. MPh Thesis, University of Edinburgh, Edinburgh, Scotland, 1989

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**Fig. 3.** Lung; goat. Abundant extracellular yeast bodies in alveoli. Scant inflammatory reaction with some macrophages in the alveolar walls. Immunostaining with monoclonal antibody VPM32 against ovine macrophages, avidin–biotin–peroxidase complex method, diaminobenzidine chromogen. PAS, Harris's hematoxylin counterstain. Bar = 62.5 µm.

**Fig. 4.** Lung; goat. Cystlike cavitation with extracellular yeast bodies and minimal inflammatory cellular response. Immunostaining with monoclonal antibody VPM32 against ovine macrophages, avidin–biotin–peroxidase complex method, diaminobenzidine chromogen. PAS, Harris's hematoxylin counterstain. Bar = 125 µm.

**Fig. 5.** Bronchial lumen; goat. Inflammatory exudate containing fibrin, neutrophils and macrophages. Note yeast body (arrow) and three acid-fast organisms (\*). Ziehl-Neelsen method. Bar = 25 µm.

**Fig. 6.** Mediastinal lymph node; goat. Granulomatous lymphadenitis. Macrophages are loaded with yeast remnants. PAS. Bar = 25 µm.

- 7 Gutiérrez M, Tellechea J, García Marín JF: Evaluation of cellular and serological diagnostic tests for the detection of *Mycobacterium bovis*-infected goats. *Vet Microbiol* **62**:281–290, 1998
  - 8 Hermoso de Mendoza M, Miranda García A, León Vizcaíno L, Perea Remujo JA, Carranza Guzmán J, Gázquez Ortiz A, del Pino J: Criptococcosis espontánea en palomo. *Arch Zootec* **33**:27–41, 1984
  - 9 Kaplan W: Epidemiology of the principal systemic mycoses of man and lower animals and the ecology of their etiologic agents. *J Am Vet Med Assoc* **163**:1043–1047, 1973
  - 10 Lichtensteiger CA, Hilf LE: Atypical cryptococcal lymphadenitis in a dog. *Vet Pathol* **31**:493–496, 1994
  - 11 Pal M, Randhawa HS: Caprine mastitis due to *Cryptococcus neoformans*. *Sabouraudia* **14**:261–263, 1976
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