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## Tuberculous Meningoencephalitis in a Wild Boar

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**ABSTRACT:** Bovine tuberculosis, caused by *Mycobacterium bovis*, is a zoonotic disease that affects cattle and wildlife worldwide. These animal hosts can serve as reservoirs of infection, thus increasing the risk of human exposure and infection. Tuberculous meningoencephalitis complicating disseminated tuberculosis is described in a 7-mo-old wild boar (*Sus scrofa*).

**Key words:** *Mycobacterium bovis*, spoligotype, *Sus scrofa*, tuberculous meningoencephalitis, wild boar.

A subadult male wild boar (*Sus scrofa*) was found moribund in a game estate bordering Monfragüe Natural Reserve (39°57'54"–36°37'58" N, 05°35'18"–06°19'51" W). The animal showed neurologic symptoms including dullness, confusion, turning movement, deviation of the head to the right, loss of coordination, and progressive ataxia.

At necropsy, the wild boar appeared in poor body condition. Mild enlargement of retropharyngeal and submandibular lymph nodes was observed in the head, and a widely disseminated pulmonary infection was characterized by multiple, 2 to 4-mm pale yellow, soft nodules throughout the lungs.

Smears prepared from suspect lesions and stained by the Ziehl-Neelsen acid-fast method had masses of slender acid-fast bacilli compatible with mycobacteria in lungs and several bacilli in central nervous system (CNS) when examined microscopically (1000×). Two grams of lymph node and two grams of brain tissue were collected from pathologic samples and the hexadecylpyridinium-chloride method (Corner and Trajstman, 1988) was used as a decontamination procedure. For each sample, two test tubes of Lowenstein-Jensen medium with pyruvate and two of Lowenstein-Jensen with glycerine (Pronadisa, Bodajoz, Spain) were inoculated.

Identification of the *Mycobacterium tuberculosis* complex was carried out on suspect colonies by molecular means (Cousins et al., 1991; Liebana et al., 1996). The spoligotyping technique was performed on the mycobacterium isolated as described by Kamerbeek et al. (1997). This method is based on the visualization of the spacer DNA sequences located between the 36-bp direct repeats (DRs) in the genomic DR region of *M. tuberculosis* complex strains. This DR region contains a variable number of DRs and also a variety of spacer DNA sequences between the DRs. This method determined that the isolate was a *M. bovis* strain belonging to the spoligotype SB1091. Previous studies by our team had found an identical spoligotype pattern in isolates from red deer (*Cervus elaphus*) and wild boar in the same area but without extrapulmonary dissemination (Parra et al., 2003).

Parallel samples were taken from CNS, lymph nodes, and lungs for histopathologic examination. Tissues were fixed in 4% buffered formalin, processed by standard paraffin-embedding methods, and 5- $\mu$ m-thick sections were cut and stained with hematoxylin-eosin (H-E) and Kinyoun's acid-fast technique.

Histopathologically, there was granulomatous meningoencephalitis. In the brain parenchyma (Fig. 1), numerous vessels had varying degrees of edema and perivascular cuffing, mostly composed of lymphocytes. Satellitosis and neuronophagia were also evident, along with spongiosis and reactive gliosis in the surrounding neural tissue. In the meninges (Fig. 2), there were poorly formed granulomas without central necrosis and dense infiltrations of lymphocytes and few plasma cells around focal small aggregates of

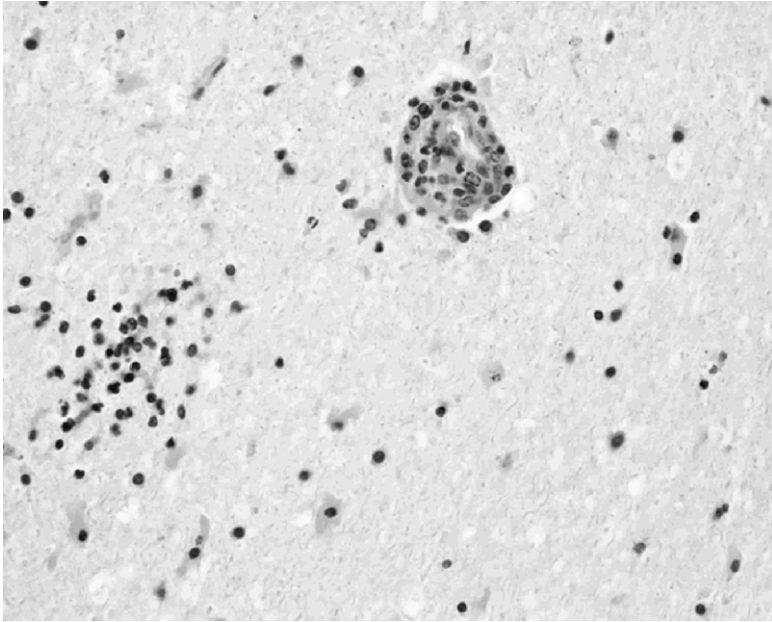


FIGURE 1. Brain. Tuberculous granulomas in different temporal stages, with one earlier and one more organized and later. H&E 200 $\times$ .

multinucleated giant cells. Marked vascular congestion and edema were also observed. Kinyoun's stain revealed several acid-fast bacilli surrounded by

the multinucleated giant cells in the lesions.

Lung and retropharyngeal and submandibular lymph node examination revealed

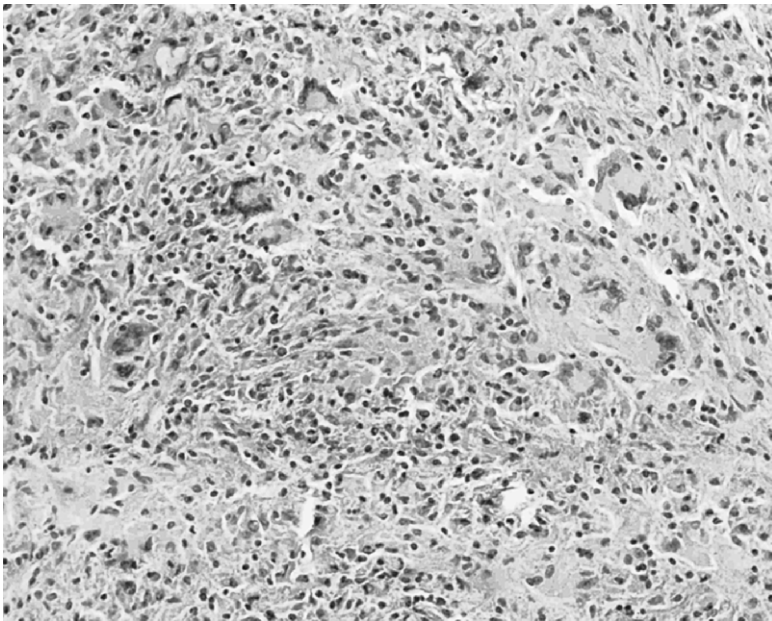


FIGURE 2. Meninges. Atypically organized granuloma with presence of Langhans cells. H&E 400 $\times$ .

the presence of multiple tubercles, characterized by a necrotic center, some with calcification, surrounded by multinucleated giant cells (Langhans cells) and macrophages, lymphocytes, and plasma cells.

The nature and distribution of lesions in tuberculosis vary according to the animal species affected, the species and strain of mycobacteria involved, the immunity of the host, the route of infection, and probably other ill-defined variables (Jubb et al., 1985).

The European wild boar is a major reservoir of *M. bovis* in some regions of Spain (Gortazar et al., 2003; Hermoso de Mendoza et al., 2006). In this species, mycobacteria apparently enter through the oropharyngeal tonsils or the lining of the intestine and then pass into the bloodstream, subsequently causing lesions in several organs, including the mandibular lymph nodes (MLN) (Segalés et al., 2005). The high prevalence of lesions reported in the retropharyngeal lymph nodes and lungs suggest that exposure to bovine TB in wild boars is most likely by the oronasal route (Gortazar et al., 2003), although there is also variation in individual boar susceptibility due to host genetic variation (Acevedo-Whitehouse et al., 2005).

Although miliary tuberculosis in immunocompetent adults is rare even in endemic areas, in this case the affected animal was <1 yr old and living in an enzootic bovine tuberculosis area where affected wild and domestic artiodactyls share common water and food supplies, which increases animal interaction and exposure to *M. bovis*. Expression of the disease in this case could be attributable to a massive bacterial proliferation resulting from a failure of the cellular immune response, a feature previously reported to occur in badgers (Clifton-Hadley et al., 1993). It has also been postulated that stress factors associated with continual movement associated with food shortage or the expulsion of subadult wild boar males from the family group might be

contributory factors (Fernandez-Llario et al., 2004).

*Mycobacterium bovis* infection can be modified by immunocompromise of the individual host, increasing the chances for atypical lesion distribution as well as more rapid disease progression. Atypical features can include diffuse lung involvement and extrapulmonary dissemination, suggesting that an immune mechanism(s) helps control dissemination of infected monocytes and/or maintain the integrity of the blood-brain barrier.

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