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Short communication

Naegleria fowleri-associated encephalitis in a cow from Costa Rica

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Abstract

Species of *Naegleria*, *Acanthamoeba*, and *Balamuthia* are soil amoebae that can cause encephalitis in animals and humans. Of these, *Naegleria fowleri* is the cause of often fatal primary meningoencephalitis in humans. *N. fowleri*-associated encephalitis was diagnosed in a cow that was suspected to have rabies. Only formalin-fixed brain was available for diagnosis. There was severe meningoencephalitis involving all parts of the brain and numerous amoebic trophozoites were present in lesions. The amoebae reacted with *N. fowleri*-specific polyclonal antibodies in an indirect immunofluorescent antibody test. This is the first report of amoebic encephalitis in any host from Costa Rica.
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Keywords: *Naegleria fowleri*; Cattle; Encephalitis; Costa Rica

1. Introduction

Species of the genera *Acanthamoeba*, *Balamuthia*, and *Naegleria* are soil-dwelling free-living amoebae that can cause encephalomyelitis in animals and humans (Martinez and Visvesvara, 1997; Visvesvara, 1999). Of these soil amoebae, *Naegleria fowleri* is highly pathogenic and is the cause of primary amoebic meningoencephalitis (PAM) in humans. PAM has been reported in young healthy children or young adults exposed to thermally polluted waters. Most cases of PAM are fatal. *N. fowleri* is thought to gain entry to the central nervous system (CNS) through invasion of olfactory bulbs via nasal–pharyngeal route (Martinez et al., 1973; Martinez and Visvesvara, 1997).

Recently, *N. foweleri*-associated PAM was diagnosed in a herd of Holstein cattle from California (Daft et al., 2005; Visvesvara et al., 2005), and in a captive South American tapir (*Tapirus terrestris*) in a zoo in Arizona, USA (Lozano-Alarcón et al., 1997). We report *N. fowleri*-associated encephalomyelitis in a cow from Costa Rica.

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2. Materials and methods

2.1. Naturally infected cow

A Jersey cow developed anorexia, ataxia, and paralysis, and died 3 days later. Whole brain and cervical spinal cord were removed at necropsy because of the suspicion of rabies. One-half of the brain was fixed in buffered 10% formalin and the rest was submitted to the Department of Rabies, Ministerio de Agricultura y Ganaderia for diagnosis of rabies.

2.2. Histpathological examination

For histopathogical studies paraffin-embedded sections were cut from the cervical spinal cord, medulla oblongata (obex), midbrain, hypothalamus, hippocampus, occipital and frontal cortex and bulb

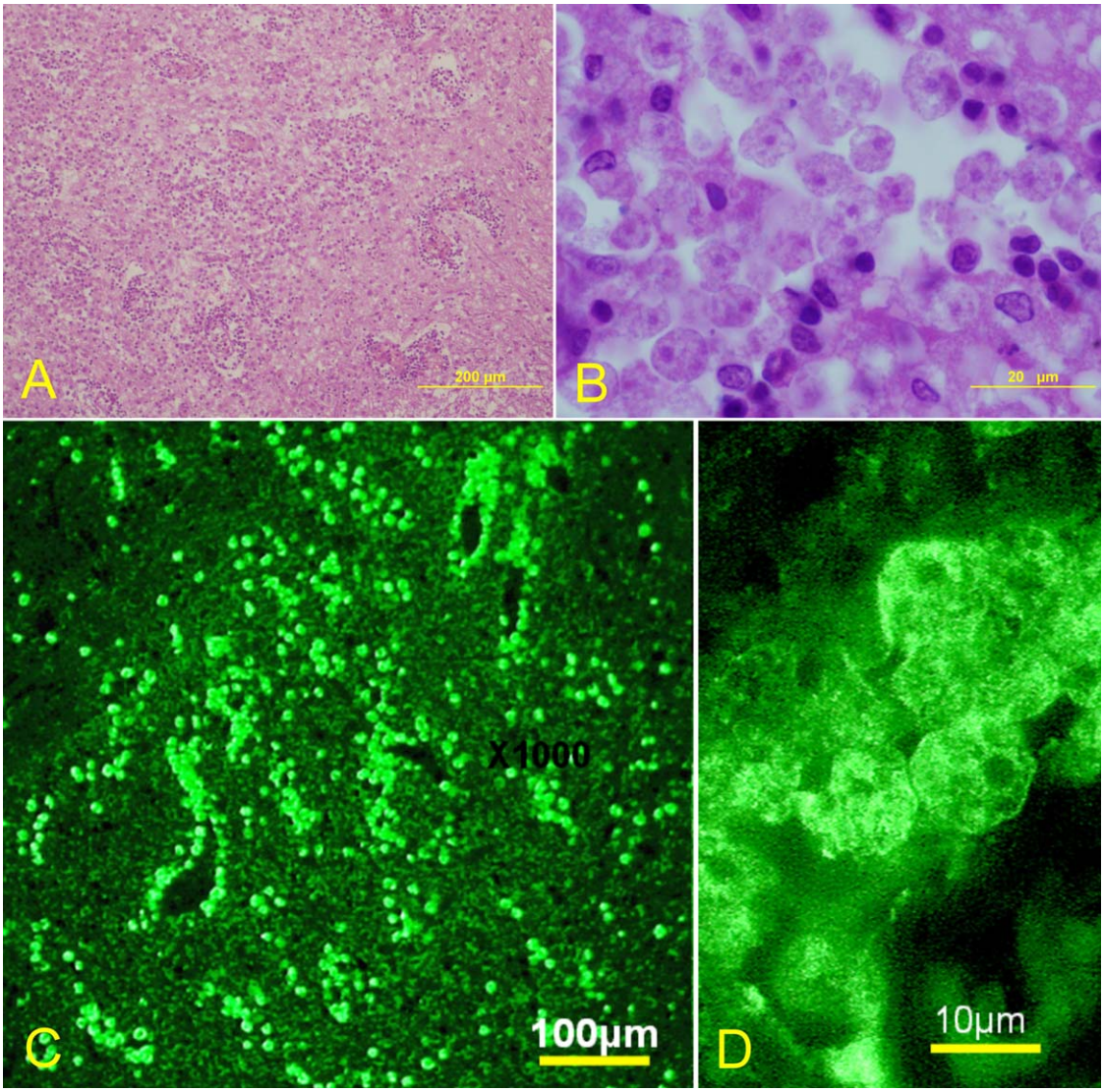

Fig. 1. Encephalitis and amoebae in the brain of the cow from Costa Rica. (A) Severe encephalitis in the cerebellum of the cow. H and E. (B) Higher magnification of numerous trophozoites. (C and D) Numerous amoebae stained with antibodies to *N. fowleri* in an indirect fluorescent antibody test.

olfactory, stained with hematoxylin and eosin, and examined microscopically.

2.3. Immunofluorescence for amoeba species

Immunofluorescence tests were conducted by reacting sections of brain with polyclonal anti-*Acanthamoeba castellanii*, anti-*N. fowleri* and anti-*Balamuthia mandrillaris* antibodies generated at CDC. Methods of antibody preparation and their specificity and sensitivity have been described previously (Visvesvara et al., 1990).

3. Results and discussion

Microscopic examination revealed a nonsuppurative, granulomatous meningoencephalomyeltis with multifocal encephalomalacia (Fig. 1). Medulla, midbrain, hypothalamus, and hippocampus had most severe lesions. The meninges were thickened by infiltrates of lymphocytes, macrophages, and a small number of neutrophils. In the medulla and midbrain, there were extensive areas of necrosis and the accompanying inflammatory response consisted of gliosis and perivascular cuffing (lymphocytes, plasma cells, macrophages, and few eosinophils), scattered foci of polymorphonuclear leukocytes, and sporadic giants cells. Mononuclear cell infiltrates were present in choroids plexis and ventricles.

Numerous amoebic trophozoites were seen in lesions throughout the brain including olfactory bulbs, and they were especially aggregated around perivascular spaces (Fig. 1B). In H- and E-stained sections these protozoa resembled enlarged macrophages. With high illumination they could be distinguished from macrophages by their eccentric nucleus and a large nucleolus based on optimally stained sections (Fig. 1B). A vacuole around the amoebae was also helpful in locating amoebae. Amoebic trophozoites were round to ovoid, 5–12 μm in diameter; cysts were not seen. Rabies testing was negative.

The amoebae in the section treated with anti-*N. fowleri* antibodies reacted brightly (Fig. 1C and D), but showed no reactivity with the other antisera.

Review of records of the 108 bovine brains submitted to the Department of Pathología, Escuela Medicina Veterinaria, Universidad Nacional Autonóma en, Heredia, Costa Rica in 2003 for the diagnosis of bovine spongiform encephalopathy (BSE) or rabies, revealed that none had BSE. However, 23 cows were positive for rabies and one of the rabies suspect (present case) was positive for amoebae. No other information was available for the amoebic cow to speculate on the sources of infection. Outbreaks of PAM in cattle from California were associated with high ambient temperatures sometimes exceeding 42°C and warm waters (30 °C or higher) which favor multiplication of *N. fowleri* (Daft et al., 2005). In the California outbreaks, of more than 21 young heifers with neurological signs, brains were examined from nine and amoebic trophozoites were demonstrated in brains of all of nine brains, indicating that PAM may not be a rare disease of cattle.

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