Congenital Encephalitozoonosis in a Squirrel Monkey
(*Saimiri sciureus*)

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Abstract. Multifocal granulomatous encephalitis associated with invasion of the brain by *Encephalitozoon cuniculi* was present in a squirrel monkey less than 24 h old. This appeared to be a congenital infection. The infant's dam was clinically normal.

*Encephalitozoon (Nosema) cuniculi*, a protozoan in the order *Microsporidium*, is a common parasite of laboratory rabbits, rats, and mice. The organisms characteristically induce granulomatous and nonsuppurative inflammation both in the central nervous system and nonneural tissues. They may also be present without accompanying host response [3]. Usually, regardless of microscopic lesions, the infection is latent or subclinical [4].

Encephalitozoonosis has been reported in other species such as dogs, cats, guinea pigs, and hamsters, and has recently been reviewed by SHADDUCK and PAKES [9]. The protozoan is not known to be common in primates. There have been only four reports to date: (1) nonfatal encephalitis in a 9-year-old boy [5]; (2) fatal, congenitally acquired encephalomyelitis and chorioretinitis in a 4-week-old female human infant (morphology of organisms on light microscopy and certain staining properties were similar to those of *Encephalitozoon*) [11]; (3) an asymptomatic infection of intestinal mucosal cells in a *Callicebus moloch* [8]; and (4) granulomatous encephalitis, nephritis, and hepatitis, thought by the authors to be congenital [1], in a 2-month-old squirrel monkey.

This report is of the second case of encephalitozoonosis in a squirrel monkey, a congenital infection in a perinate.
Case History

In June 1970 a male newborn squirrel monkey (Leticia) less than 24 h old was found dead at the New England Regional Primate Research Center. This was his dam’s first observed pregnancy since her arrival in the colony in 1968. She was a clinically healthy monkey and alive at the time of this report. Her hematologic values and results of urinalysis were normal. The sire of the infant was unknown as the monkeys were housed in an outdoor gang cage.

Materials and Methods

A postmortem examination was performed, and tissues collected were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. The following additional stains were used on sections of brain: Gram, Giemsa, Gomori methenamine silver, periodic acid-Schiff, Ziehl-Neelsen, and (MacCallum) Goodpasture.

Formalin-fixed pieces of brain were cut into 1-mm cubes and placed in chilled 2% paraformaldehyde -2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 (one half strength Karnovsky’s fixative) for 5 h. These tissue cubes were subsequently rinsed in 0.1 M cacodylate buffer, immersed in 1% osmium tetroxide in the same buffer for 1 h, rapidly dehydrated, and embedded in Epon 812. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with an RCA EMU-3 H electron microscope.

Results

The infant was premature by birth weight (70 g) [7] and had a crown-rump measurement of 13.5 cm. The lungs were dark red and uninflated. The adrenals were dark red, and a fetal zone was present. No other gross lesion was seen.

Granulomatous encephalitis was present in the brain at all levels, most extensively in the mesencephalon and telencephalon. Various-sized foci of microglia, macrophages, and lymphocytes, often surrounding a core of liquefactive necrosis (fig. 1), were in gray and white matter. Capillaries in the brain parenchyma were dilated.

Organisms approximately 1.5×2.5 μm were found singly and in clumps within and adjacent to the microgranulomas (fig. 2). Organisms were also present in spherical, cyst-like collections unassociated with lesions (fig. 3). They stained poorly with HE, were Gram-positive, acid-fast, and purple with Goodpasture stain. The wall of the spherical collections did not stain with Gomori methenamine silver or periodic acid-Schiff.
Fig. 1. Large and small foci of granulomatous encephalitis in the thalamus. HE.

Fig. 2. Focus of granulomatous encephalitis composed of microglia, macrophages, and lymphocytes. Organisms are present singly (small arrow) and in spherical clumps (large arrow). Goodpasture stain.
Fig. 3. Spherical collections of organisms unassociated with an inflammatory response. Thin section, toluidine blue.

Fig. 4. Spore form of *Encephalitozoon cuniculi* containing cross sections of the characteristic polar filament (arrows) and circumferential laminations of the polarplast surrounding a portion of the polar filament.
Electron microscopic examination showed that the fine structure of these protozoan organisms was identical to that previously described for *Encephalitozoon cuniculi* [10]. Sporont, sporoblast, and spore forms of the organism were present in the dilated spaces in the cerebral cortex. The spore forms of the organism contained a well-defined polar filament, a structure characteristic of microsporidia (fig. 4).

No lesions were present in the tongue, stomach, small intestine, colon, pancreas, trachea, lung, liver, heart, thyroid, and eye. Glomerulogenesis, indicative of tissue immaturity [7], was present in the kidney. Early germinal centers were present in lymph nodes and spleen. Congestion and focal hemorrhages occurred in the adrenal cortex and thymus. The placenta had been eaten by the dam and was not available for examination.

**Discussion**

Lesions of granulomatous encephalitis in this monkey were similar to those seen in encephalitozoonosis in rabbits. They were also identical to those in the case reported by BROWN *et al.* [1]. Our infant, however, did not have the protozoa disseminated in other visceral organs. Organisms present in the brain closely resembled *E. cuniculi*, both morphologically and by staining properties. They could be differentiated from *Toxoplasma gondii* because they were Gram- and Goodpasture-positive, acid fast, and did not have a stainable cyst wall. The ultrastructural features of the organism were identical to those described for *E. cuniculi*.

The routes of spontaneous transmission of *Encephalitozoon* in its common hosts (rats, rabbits, mice) have not been established. Experimental horizontal transmission has been accomplished by both oral and parenteral routes [4, 6, 9]. Evidence for vertical transmission was described by HUNT *et al.* [2] in gnotobiotic rabbits. Finding a severe diffuse encephalitis in this monkey that was less than 1 day old and in a hand-reared 2-month-old monkey [1] is strong evidence of congenital infection in this species as well.

Encephalitozoonosis of the central nervous system has not been previously reported in adult nonhuman primates. This may indicate either that the infection is not common or that the organism is well adapted to the adult host and therefore is inconspicuous. Thus, one should examine brain and viscera of *Saimiri sciureus* with the knowledge that *Encephalitozoon* may occur in subclinical form. The healthy status of our infant’s mother is suggestive of the latter. The infant of BROWN *et al.* [1] was orphaned at birth, but the authors did not report pathological findings in its dam.
The severity of brain lesions in our newborn monkey and the lack of significant lesions in any other organs suggest that the protozoan infection caused the infant's death.

Acknowledgement

This work was supported by National Institutes of Health Grants 5F03RR50457-02 and RR 00168-10.

References


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