Transmission Electron Microscopy Used to Diagnose Acute Toxoplasmosis in a Quarantined, Captive Born Cynomolgus Macaque

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A female Cynomolgus macaque that was born in captivity on Mauritius was brought to the USA and quarantined by the CDC. While in quarantine, the monkey was treated prophylactically with ivermectin and fenbendazole, and repeatedly tested by serology for tuberculosis, herpes B, SIV, SRV, STLV, measles and hepatitis A. As expected measles and hepA titers were positive; all other tests were negative. Standard quarantine treatment, including psychological and nutritional enrichment activities, was employed by the Veterinary scientists caring for the monkeys.

After several weeks in the care of BMS the monkey began to exhibit signs of disease. Clincal testing failed to yield a cause for the lethargy, weakness, anorexia, bruxism, and hunched posture exhibited by the monkey. The disease progressed and the animal was euthanized after one week of palliative treatment. The tissues taken for study at necropsy were limited to the abdominal viscera that had been obviously affected by the disease.

Histological examination of the pancreas detected variable sized diffuse foci of necrosis. Cellular and nuclear debris, and even fibrinoid material were abundant in these foci, but mononuclear inflammatory cell infiltrates were relatively scant. Purple-staining oval, or crescent-shaped protozoa up to 2 µm long were scattered in clusters intracellularly or dispersed extracellularly within, and adjacent to, necrotic foci. Samples of the formalin fixed pancreas were later post-fixed in osmium tetroxide and processed for transmission electron microscopy. The protozoa were positively identified by ultrastructural morphology as *Toxoplasma gondii*. Futher studies using immunohistochemistry were done to confirm the diagnosis and the extent of the infection in the tissues taken at necropsy.

A serological survey of the monkeys shipped with the infected female determined that only her cage mate had a positive titer (\geq 1:3200) for Toxoplasma. The titers for all the other monkeys were negative (< 1:25). This strongly suggests that the infection occured after the shipment was received by BMS and further suggests that the immune response of the female monkey was compromised somehow. The results of this study has led to changes in the preparation of whole produce given to the monkeys for psycological and nutritional enrichment. Toxoplasma infections in captive born macaques seems to be rare and immune competent individuals are considered resistant to fatal toxoplasmosis. In contrast to macaques, spontaneous fatal toxoplasmosis is well documented in neotropical (New World) primates.

References:

- [1] Carme B, Ajzenberg D, *et al.* Outbreaks of toxoplasmosis in a captive breeding colony of squirrel monkeys. Vet Parasitol. 2009 Jul 7;163(1-2):132-5.
- [2] Dietz HH, et al. Toxoplasmosis in a colony of New World monkeys. Vet Parasitol. 1997 Mar;68(4):299-304.

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- [3] Dubey JP, *et al.* Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. 1998 Apr;11(2):267-99.
- [4] Ekanayake DK, *et al.* Seroprevalence of Toxoplasma gondii in wild toque macaques (Macaca sinica) at Polonnaruwa, Sri Lanka. J Parasitol. 2004 Aug;90(4):870-1.

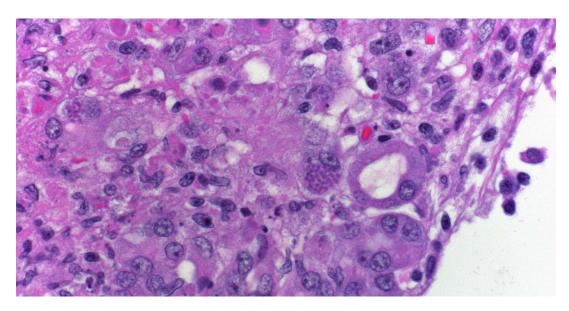


Figure 1. Histology of the pancreas of a female Cynologus macaque infected with Toxoplasmosis. A parsitophorous vacuole (arrow) is indicated. Bar = $25 \mu m$

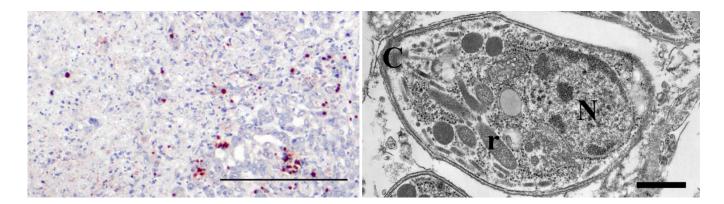


Figure 2. Left Panel: Immunohistochemistry of the pancreas with several areas of positive staning (arrow) for Toxoplasma gondii. Bar = $200 \mu m$. Right Panel: Characteristic ultrastructural morphology of Toxoplasma gondii includes the conoid (C), rhoptories (r) and a small eccentric nucleus (N). Bar = $500 \mu m$.