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## AN OUTBREAK OF TOXOPLASMOSIS IN WALLAROOS (*Macropus robustus*) IN A CALIFORNIA ZOO

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**Abstract:** Four wallaroos (*Macropus robustus*) from a central California zoo colony of 22 died within a three week period. Histopathologic findings in all four cases were suggestive of toxoplasmosis. Brain, lung and heart were the most frequently involved organs. Within 4 months of the last death sera from nine colony members were tested for antibodies against *Toxoplasma gondii* using the indirect hemagglutination test. All nine had serological titers ranging from 1:64 to 1:131,072.

### INTRODUCTION

Few reports are available on diseases affecting macropod marsupials (kangaroos, wallabies and wallaroos) in captivity. In a report on the diseases of Australian laboratory marsupials, nocardiosis was the most common fatal disease,<sup>1</sup> but several sporadic cases of toxoplasmosis also have been noted, usually involving kangaroos.<sup>3,4,6,8,11</sup> In one serologic study, evidence of toxoplasmosis was not found in kangaroos in their native habitat.<sup>2</sup> In a recent survey of exotic animals in zoos and in private ownership, serologic evidence of infection with *Toxoplasma gondii* was found in 33% of the animals.<sup>9</sup> Twelve wallaroos were included in this study and six were seropositive with titers ranging from 1:128 to 1:2048.<sup>9</sup> Recently, four wallaroos from a central California zoo were submitted for necropsy within a three week period. This report presents the pathology of toxoplasmosis in these wallaroos.

### HISTORY

A colony of approximately 22 wallaroos (*Macropus robustus*) has been maintained at a central California zoo for 35 years. Colony size is maintained by selling 4 to 6 animals per year. The

only recent introduction was a male wallaroo obtained from a Texas zoo in 1971. During the fall of 1974 they were moved to a new enclosure that is partially shaded and the ground is bare of vegetation. Water is supplied in a low trough and pelleted alfalfa is fed *ad libitum*. Animals showing clinical signs receive veterinary care while those found dead are submitted to the Department of Pathology, School of Veterinary Medicine, University of California (UCVM) for thorough necropsy. Sporadic deaths have occurred and necropsy reports from this zoo were found by reviewing the UCVM pathology files for the past ten years. The mortalities were random and were attributable to a variety of causes.

Recently, four wallaroos died within a three week period. On 8 March 1975 an adult female wallaroo was found dead without prior clinical signs and was submitted for necropsy. Her offspring (approximately 5 months old) was removed from the group for hand-rearing. The following week, two 6 month old wallaroos were found dead, also without prior clinical signs, and submitted for necropsy. On 29 March the joey died. It had been doing poorly, had intermittent diarrhea and sustained a weight loss. After gross examination, selected tissues were fixed in 10% buffered formalin,

embedded in paraffin, sectioned and routinely stained with hematoxylin and eosin. Selected tissues also were stained with periodic acid-Schiff (PAS), gram-Weigert, Giemsa and Goodpasture.

Within 4 months following the last death, blood was randomly collected from nine wallaroos, allowed to clot, and the serum tested at dilutions ranging from 1:64 to the end point for antibodies against *Toxoplasma gondii* using the indirect hemagglutination (IHA) test.<sup>5,7</sup>

In early July 1975, a feral female short hair cat (*Felis domesticus*) was seen in the feed trough in the wallaroo enclosure. The animal was caught and kept for observation to determine if she was shedding oocysts and/or had a positive titer for toxoplasmosis.

## RESULTS

### Pathology

The gross findings in all four wallaroos were minimal except for the respiratory system. Lungs tended to be mottled red-pink, heavy and wet. In two cases portions of the pulmonary tissue sank when placed in formalin.

Histopathologic examination revealed diffuse toxoplasmosis in the three young animals and minimal lesions in the adult female. Organisms occurred either individually in the tissue (Fig. 1), within the cytoplasm of macrophages, or within a structure with a defined wall (Fig. 2).

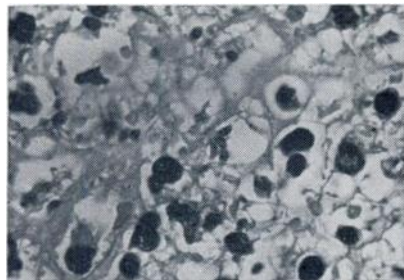


FIGURE 1. *Toxoplasma* organisms in a lymph node of a 6 month old wallaroo (hematoxylin and eosin, original magnification 1000X).

*Toxoplasma* organisms were found in the lung in all four cases (Fig. 3). Extensive necrosis and fibrin exudation often were found in association with the organisms. An infiltrate of mononuclear cells and fibrin in alveoli also were found (Fig. 4). In the three young animals the brain contained multiple glial nodules (Fig. 5) and occasional *Toxoplasma* cysts were found in the glial nodules (Fig. 6). The brain of the adult was not examined. A review of the slides from necropsy cases of wallaroos from the past 10 years failed to reveal any additional cases of toxoplasmosis, nor was *Toxoplasma* found in animals dying since the outbreak.

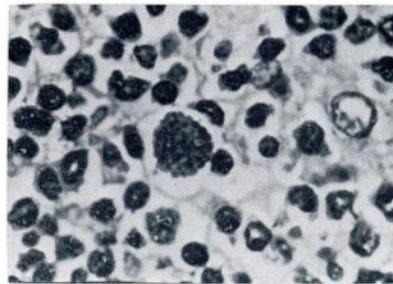


FIGURE 2. Lymph node from a 6 month old wallaroo containing a *Toxoplasma* cyst (hematoxylin and eosin, original magnification 1000X).

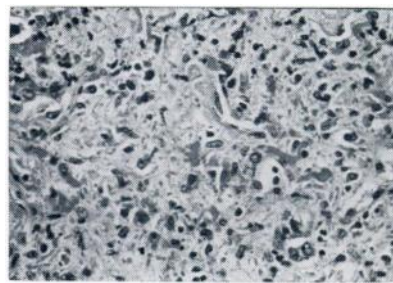


FIGURE 3. Lung from wallaroo showing marked accumulation of cells and protein rich fluid in alveoli. Note *Toxoplasma* cyst (arrow) (hematoxylin and eosin, original magnification 250X).

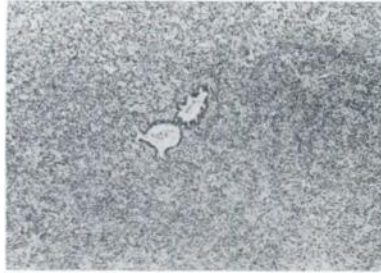


FIGURE 4. Lung from wallaroo showing diffuse pulmonary involvement (hematoxylin and eosin, original magnification 25X).



FIGURE 6. Brain of a 6 month old wallaroo with a glial nodule. Note *Toxoplasma* cyst (arrow) (hematoxylin and eosin, original magnification 250X).



FIGURE 5. Brain from a 6 month old wallaroo showing glial nodules (hematoxylin and eosin, original magnification 100X).

#### Serology

All nine wallaroos were positive for antibodies directed against *gondii* using the IHA test as shown in Table 1. Titers ranged from 1:64 to 1:131,074. One animal tested twice showed a rising titer.

The feral cat caught in the wallaroo enclosure was examined for serologic evidence of toxoplasmosis by the IHA method. Two serum samples, taken over a three week period, were both negative.

TABLE 1. Indirect hemagglutination antibody titers to *Toxoplasma gondii* in Wallaroos (*Macropus robustus*).

Animal Identification	Sex	Titer*
96	F	131,072
97	F	2,048
98	F	16,384
100	F	4,096
100**	F	32,768
103	F	32,768
110	F	32,768
117	M	512
86	F	512
Offspring of 86		64

\* Reciprocal serum titer

\*\* Same animal sampled two months later

## DISCUSSION

Generally, reports of toxoplasmosis in macropods involve isolated cases and we are aware of only one report in a wallaroo.<sup>8</sup> In this outbreak, all four cases occurred within three weeks. We have no evidence of toxoplasmosis in animals dying in the previous years and no new cases have occurred in this colony in the past year. This would suggest either a common source of infection or some factor precipitating the disease. Six of 12 wallaroos from the colony had serologic evidence of infection in 1973<sup>9</sup> indicating that this was not the first exposure to *Toxoplasma* and toxoplasmosis had been reported in Pallas cats (*Felis manul*) from this same zoo.<sup>10</sup>

Feral domestic cats have been observed occasionally in the wallaroo enclosure and are certainly a possible source of exposure, although the cat examined was negative. A recent survey

of domestic cats in this area revealed that approximately 14% are seropositive for toxoplasmosis (Riemann, H. P. and Behymer, D. E., personal communication).

Three of the four wallaroos were six months of age or less, indicating that the infection may have taken place *in utero* or neonatally. Additional evidence is the finding of *Toxoplasma* in the mother of one of the dead infants. A case of toxoplasmosis involving mother and infant also has been reported in a kangaroo.<sup>3</sup>

In a recent report, 33% of captive exotic mammals were seropositive for *Toxoplasma*.<sup>9</sup> It would seem that the true extent of this disease in our zoo populations is not fully appreciated. Necropsies of animals dying in zoos and serological surveys will help define the problem, but epizootiological studies are needed to pinpoint sources of spread and thus suggest methods of prevention.

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