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SHORT COMMUNICATIONS

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Pathology of Experimental Toxoplasmosis in Eastern Barred Bandicoots in Tasmania

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ABSTRACT: Wild-caught eastern barred bandicoots (*Perameles gunnii*) initially seronegative to *Toxoplasma gondii,* were inoculated orally with approximately 100 *T. gondii* oocysts. The bandicoots were maintained in indoor pens under laboratory conditions and observed daily. Serial blood samples were tested for agglutinating antibodies to *T. gondii.* Inoculated bandicoots died 15 and 17 days post infection. A rise in Direct Agglutination Test (DAT) titres was detected at the time of death (1:256, 1:64 respectively). Clinical observations, serological changes, gross findings at necropsy, and histopathological changes were consistent with acute toxoplasmosis. The findings indicate that eastern barred bandicoots are likely to die from primary *T. gondii* infection, often even before detectable antibodies are produced, reinforcing the significance of toxoplasmosis as a potential contributor to the reduction in numbers of wild populations of eastern barred bandicoots.

Key words: Eastern barred bandicoot, marsupial, pathology, *Perameles gunnii, Toxoplasma gondii,* serology.

Toxoplasmosis is recognized as an important opportunistic pathogen in immunologically vulnerable hosts. There are many reports of this infection in captive and free-ranging Australian marsupials (Canfield et al., 1990; Obendorf and Munday, 1983, 1990). The impact of toxoplasmosis has been of particular importance for rare and endangered marsupials, especially in captive breeding programs, and in the management of small free-ranging populations in remnant habitats (Lenghaus et al., 1990). One such marsupial is the eastern barred bandicoot (*Perameles gunnii*), defined as a threatened marsupial on the Australian continent. Only on the island of Tasmania does the species remain abundant. Loss of habitat and the impact of introduced predators, such as the European fox (*Vulpes vulpes*) and the domestic cat, are predominantly responsible for reduced populations of this marsupial (Brown, 1989; Seebeck et al., 1989). Toxoplasmosis is known to cause deaths of free-ranging bandicoots in Victoria and Tasmania (Obendorf and Munday, 1983; Lenghaus et al., 1990; Obendorf et al., 1990). A recent serological study of the prevalence of *T. gondii* antibody in free-ranging *P. gunnii* populations concluded that >95% of animals were seronegative and therefore unexposed, the few individuals that developed detectible antibody were not retrapped (Obendorf et al., 1996). The conclusion drawn was that infected bandicoots do not survive primary infection with *T. gondii.* This field study prompted an experimental investigation to study the course of the disease and formation of antibodies following oral inoculation with a known number of viable *T. gondii* oocysts.

In 1995, four adult eastern barred bandicoots (1 female, 3 males) were captured from a wild population at Kingston (Tasmania, Australia: 43°S, 147°03′E) in accordance with established guidelines and protocols of Parks and Wildlife Service permit to take protected wildlife (Hobart, Tasmania, Australia; permit numbers 95249 and 95256). The animals were captured using wire cage traps (Mascot Wire Works, Enfield, New South Wales, Australia) and housed in the Central Animal House at the University (Hobart, Tasmania; Animal Ethics Committee approval number 94010). Each animal was initially placed in a single-animal holding cage with a nest

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box. The diet consisted of commercial kitten biscuits (softened in sterile water), rolled oats, sultanas, fresh apple pieces, peeled raw carrot, meal worms, occasional pieces of hard boiled egg and water *ad libidum.* Care was taken to exclude any extraneous infections at all stages of food handling and preparation.

Two bandicoots were inoculated orally with *T. gondii* oocysts of the P89/Veg strain (kindly provided by Dr. J. P. Dubey, USDA, Beltsville, Maryland, USA). This strain was selected for its virulence in mice, one oocyst is known to be lethal to mice by any route of administration (Dubey et al., 1995). The concentration of oocysts was determined by direct microscopic counts using a Neubauer haemocytometer counting slide; 100 cysts were given *per os* to each animal. Five days post-infection animals were transferred to individual pens (2.5 × 1 m); their diet remained the same with water *ad libidum*; each day the bandicoots were observed for any changes in behaviour.

Blood samples were collected from all animals by lancing the lateral ear vein into a Microtainer serum separator tube with gel interface. (Becton Dickinson & Co., Rutherford, New Jersey, USA). All bandicoots were seronegative before the experiment commenced. On each sampling session at 0, 6 and 13 days post infection (DPI) bandicoots were weighed, observed and their body temperature taken by digital thermometer inserted into the rectum. The Direct Agglutination Test (DAT) and Modified Agglutination Test (MAT) were used to test all sera using the commercial kit Antigene Toxo-AD and microtiter plates reagents (bioMerieux SA, Marcy-l'Etoile, France). Formalin-treated *T. gondii* tachyzoites were used as the antigen. The tests were performed at Mt Pleasant Laboratories (Department of Primary Industry and Fisheries, Launceston, Tasmania, Australia) using methods as described by Obendorf et al., (1996). In the DAT, a 25 μl serum sample was initially tested at 1:8 and titrated in two-fold serial dilutions to 1:4096. In the MAT, a 25 μl serum sample was initially mixed with 25 μl of 0.2 M 2-mercaptoethanol (2-ME) (BDH, Poole, England) in phosphate buffered saline (PBS) before being similarly tested in serial dilutions.

A selection of organs and tissues from animals that died was fixed in 10% buffered formol-saline; histological specimens (prepared in the Division of Pathology, University of Tasmania) were embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (H&E) for microscopic examination. Duplicate sections were prepared on Chrome Alum slides and stained using avidin-biotin peroxidase complex (ABC) using the commercially available Vectastain AB Rabbit IgG kit (Vector Laboratories, Bulingame, California, USA). Sigma Fast DAB (Sigma Chemical Co., St. Louis, Missouri, USA) was used to simplify final preparation of slides. Rabbit-anti *T. gondii* serum was used as the secondary antibody. Positive tissue controls included brain with *T. gondii* from an eastern barred bandicoot (*P. gunnii*), Bennett's wallaby (*Macropus rufogriseus*) and common wombat (*Vombatus ursinus*). Negative tissue controls included tissue sections from eastern barred bandicoots with no microscopic pathology.

The two bandicoots (1 male and 1 female) orally dosed with oocysts died 15 and 17 DPI. The presence of toxoplasmosis was confirmed by gross and microscopic examination. During the post-infection interval there was no weight loss or change in body temperature. From 10 DPI the inoculated animals were more likely to be outside their nest boxes during daylight hours and increased their water intake.

Both inoculated bandicoots had a DAT titre at the time of death (1:256 and 1:64) but negative reactions in the MAT. At 6 and 13 DPI, one bandicoot had a DAT titre of 1:256 the other was negative. Both control animals remained seronegative throughout the experiment and no behavioural changes were observed.

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Notable necropsy findings were congestion, edema and patchy consolidation of the lungs, excess slightly blood tinged abdominal fluid, petechial haemorrhages to gastric and small intestinal serosa, edematous mesentery and enlargement of the mesenteric lymph nodes. Both animals had distinctly enlarged spleen and liver, the latter with a distinct lobular pattern to the parenchyma.

The lungs showed extensive interstitial pneumonia associated with cellular infiltrates into alveolar tissues, acute inflammatory changes, and areas of focal fibrinous necrosis. The heart contained focal areas of non-suppurative interstitial inflammation and myonecrosis with infiltrates of lymphocytes and histiocytes. Numerous tachyzoites and tissue cysts were found in association with these lesions and stained positively in the avidin-biotin peroxidase reaction. The liver had multifocal areas of hepatocellular necrosis associated with the presence of crescentic tachyzoites. The spleen and lymph nodes showed moderate to severe necrosis of the lymphoid follicles with aggregates of *T. gondii* tachyzoites, sometimes in the form of close clusters, in areas of necrosis. Acute inflammation and cell necrosis associated with tachyzoites or cysts were found in skeletal muscles, pancreas, as well as the muscle and submucosal layers of the small intestine.

The direct and modified agglutination tests can be used on any serum sample irrespective of the species from which it was collected. The agglutination test, as outlined by Desmonts and Remington (1980), has been used to monitor the development of specific *T. gondii* antibodies following experimental exposure to *T. gondii* organisms in the Forester kangaroo (*Macropus giganteus*) (Johnson et al., 1989) and the Tammar wallaby (*Macropus eugenii*) (Lynch et al., 1993). Both these experimental studies demonstrated that toxoplasmosis was accompanied by an initial IgM antibody response. This was characterised by a steadily rising DAT titre in the absence of any detectible MAT titre commencing approximately 7 to 10 DPI. At about 30 DPI, macropodids which survived, developed rising MAT titres indicative of an IgG antibody seroconversion. In this study bandicoots given 100 *T. gondii* oocysts *per os* died at 12 and 17 DPI; at the time of death both had a definite DAT titre (1:256 and 1:64 respectively) and no detectible MAT titre.

Based on these studies it has been concluded that necropsies on free-ranging marsupials with moderate to high DAT titres (generally between 1:256 and 1:1024) and low or negative MAT titres (<1:256) have died from acute primary exposure to *T. gondii.* This suggests that they die within weeks of infection. Sera from other necropsy cases in which toxoplasmosis was also considered the principle cause of death had identical DAT and MAT reactions with high to very high titres (up to 1:64000). It is likely these animals survived the early IgM-related seroconversion and then developed a strong IgG response (Obendorf et al., 1996).

Agglutination tests have also been conducted on free-ranging *P. gunnii* known to have died of toxoplasmosis (Obendorf et al., 1996). Of the eight confirmed cases, seven had IgG-related seroconversions (MAT range 1:256 to 1:64000); only one individual showed a mixed IgM and IgG seroconversion (DAT 1:256; MAT 1:64). In this trap/release study, the agglutinating antibodies to *T. gondii* in free-living populations of *P. gunnii* were monitored for 3 yr. The study showed the bandicoots that developed antibodies, were less likely to be retrapped compared with seronegative bandicoots. The conclusion drawn was that eastern barred bandicoots were likely to die from primary *T. gondii* infection.

In the present study, the inoculum of 100 *T. gondii* oocysts caused the deaths of both eastern barred bandicoots. Gross lesions noted in these orally inoculated bandicoots were similar to those of experimentally infected Tammar wallabies (*M. eugenii*) (Reddacliff et al., 1993). The his-

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topathology was characterised by extensive cellular necrosis, acute inflammation predominated by neutrophil infiltrations and the presence of large numbers of tachyzoites either as dispersed single organisms or in close aggregates or cysts. The generalised cell necrosis of lymphoreticular tissues may have been responsible for the weak humoral antibody response in the infected bandicoots. The severity of microscopic changes seen in many organs and tissues in association with proliferation of the organisms would suggest that the dose and strain of *T. gondii* administered was extremely pathogenic for *P. gunnii.* An earlier attempt to infect bandicoots with a lower dose of 10 oocysts of this strain was unsuccessful. Due to the restrictions imposed on the study, we were unable to repeat the experiment using additional animals inoculated with smaller numbers of oocysts.

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