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DETECTION OF AGGLUTINATING ANTIBODIES TO *TOXOPLASMA GONDII* IN SERA FROM FREE-RANGING EASTERN BARRED BANDICOOTS (*PERAMELES GUNNII*).

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ABSTRACT: Sera from 150 eastern barred bandicoots (*Perameles gunnii*) were collected from two study sites in southern Tasmania between 1992 and 1995. Samples were tested for antibodies to the protozoan parasite, *Toxoplasma gondii*, using formalin-treated tachyzoites as the antigen in direct (DAT) and modified agglutination tests (MAT). Cut-off titers were set based on confirmed cases of toxoplasmosis in this species. A total of 133 animals (89%) were classified as negative, seven (4.6%) had suspicious reactions, and 10 (6.7%) were diagnosed as positive. Five of the 10 positive animals were not retrapped after initial seroconversion; another three animals recorded high MAT titers on two consecutive bleedings, three months apart. Of the remaining two seropositive bandicoots, one was found dead in a trap and generalized toxoplasmosis was diagnosed at necropsy, while the other animal had central nervous system disabilities consistent with toxoplasmosis but was accidently released and never recaptured. Based on these findings we propose that eastern barred bandicoots are likely to be highly susceptible to primary *T. gondii* infection.

Key words: Serologic survey, *Toxoplasma gondii*, agglutination test, antibody, eastern barred bandicoot, *Perameles gunnii*.

INTRODUCTION

Toxoplasmosis is an significant disease of Australian marsupials commonly causing mortality in captive and free-ranging populations (Obendorf and Munday, 1983; Canfield et al., 1990). The apicomplexan protozoa, *Toxoplasma gondii* is found throughout the world with felids being the only known definitive host (Dubey, 1994). Australia's terrestial fauna have evolved in the absence of felids and many species of marsupials are highly susceptible to *Toxoplasma* sp. infection (Canfield et al., 1990).

The impact of this disease is 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). Toxoplasmosis has been reported in free-ranging eastern barred bandicoots, (*Perameles gunnii*) in southern Tasmania (Obendorf and Munday, 1990) and from its last remnant population on the Australian mainland (Lenghaus et al., 1990).

Over a 2-yr period, two populations of eastern barred bandicoots were the subject of a intensive ecological study to investigate the conservation priorities for the species, particularly any threatening processes which affect population survival. Our objectives were to assess the prevalence of *T. gondii* infection in these populations using a serological test and review the likely impact of the parasite on bandicoots.

MATERIALS AND METHODS

Between July 1992 and March 1995, 150 bandicoots were caught in wire cage traps (Mascot Wire Works, Enfield, New South Wales, Australia) at two localities in the Huon valley, southern Tasmania (Huonville site: 43°03′S, 147°02′W; Grove site: 42°59′S, 147°05′W). Both sites were agriculturally modified grassland habitats with areas of forest and native understory as shelter. Every 3 mo a grid of 55 traps was established at each site. Traps were baited and set for five consecutive days (275 trap-nights/site). Each trapped bandicoot was identified by a numbered ear tattoo. The bandicoots were bled by puncturing the lateral ear vein with a sterile lancet. At each bleeding approximately 0.25 ml of blood was collected into a Microtainer serum separator tube (Becton Dickinson & Co., Rutherford, New Jersey, USA) with a gel interface to ensure maximum serum collection.

Direct (DAT) and modified (MAT) agglutination tests were conducted on all sera using

reagents supplied by BioMerieux (Charbonnieres les Bains, France). In the DAT, a 25 μl serum sample was initially tested at 1:8 and, if positive at that dilution, the serum was serially diluted to an end-point. Formalin-treated *T. gondii* tachyzoites were used as the antigen. In the MAT, a 25 μl serum sample was initially mixed with 25 μl of 0.2 M 2-mercaptoethanol (2-ME) (British Drug Houses, England) in phosphate buffered saline (PBS) before being similarly tested by serial dilution. The kit included positive and negative control sera and the tachyzoite antigen was checked for spontaneous agglutination in wells in which PBS was substituted for serum. Microtiter plates were incubated at 20 C (room temperature) and read at 5 and 18 hr. An agglutination reaction was considered positive when the *T. gondii* organisms formed a diffuse layer overlying at least half the well's base and negative when a small distinct aggregate of sedimented organisms formed in the bottom of the well.

The results of the agglutination test were expressed as the reciprocal of the highest serial dilution giving a positive reaction for a given test sera. Based on the test results for several confirmed toxoplasmosis cases in *P. gunnii*, the results were classified as negative when the DAT titer was <64 and no reaction in the MAT, suspicious when the DAT titer was ≥64 and no reaction in the MAT, and positive where both the DAT and the MAT titers were both ≥64.

During the study, several dead eastern barred bandicoots were also examined by necropsy. They were collected from various locations in Tasmania; one bandicoot was a trap death from the Huonville site (H53). At the necropsy examination samples of brain, heart, skeletal muscle and lung were preserved in 10% formol-saline for histopathological evaluation. After dehydration through ethyl alcohol, tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. Mounted slides were examined by light microscopy. Samples of heart blood were also evaluated for *T. gondii* antibodies using DAT and MAT serology.

RESULTS

Based on necropsy and histopathological examinations of eight dead bandicoots, toxoplasmosis was confirmed in seven animals. In each case the serological reaction in the agglutination tests gave a DAT > 64 combined with a MAT > 64 (range: DAT 256 to 64,000; MAT 64 to 64,000). This data set was used as the basis for classification of positive reactions in the bandicoots from the two study sites.

We trapped 150 bandicoots (Huonville site: n = 54; Grove site: n = 96), with many animals being bled on several occasions. Sera from 133 (89%) of 150 bandicoots had no detectible antibodies in either agglutination test. A further 10 (6.7%) sera had positive reactions in both the DAT and MAT, and seven (4.6%) were classified as suspicious reactions.

Five of the 10 antibody-positive animals were not retrapped after their initial sero conversion. The other five animals had antibodies on two consecutive occasions 3 mo apart. One of these antibody-positive bandicoots appeared docile and weak when subsequently retrapped. When this animal was released it displayed erratic movements with a tendency to fall to one side; the animal was never recaptured. Another bandicoot which had a DAT:MAT titer of 256: 256 at the previous trapping was found dead in the trap. At necropsy the bandicoot had generalized gingivitis, tooth loss and periodontitis. On gross examination of the internal body cavities there was diffuse pulmonary consolidation with exudation into the airways. Microscopically, cysts typical of *T. gondii*, sometimes in association with infiltrates of mononuclear and lymphocyticinflammatory cells, were seen in the brain, heart, lung, and skeletal muscles.

None of the seven bandicoots with serological reactions designated as suspicious were recaught at the next three monthly trapping. By comparison, most (68%) bandicoots with negative serology were retrapped on subsequent trapping periods.

DISCUSSION

The agglutination tests have been used to diagnose *Toxoplasma* sp. infection in humans (Desmonts and Remington, 1980), domesticated animals (Dubey and Beattie, 1988), and marsupials (Dubey et al., 1988). A limitation of the agglutination test has been that the DAT lacks specificity. False-positive DAT reactions are re-

portedly due to non-specific class M immunoglobulin (IgM) binding to the formalin-treated tachyzoites (Desmonts and Remington, 1980). By contrast, the MAT detects only class G immunoglobulin (IgG) because 2-ME destroys all IgM in sera (specific and non-specific).

Experimental studies monitoring the development of agglutinating antibodies to *T. gondii* have been conducted in two macropodid marsupials: the eastern grey kangaroo, (*Macropus giganteus*) (Johnson et al., 1989) and tammar wallaby (*Macropus eugenii*) (Lynch et al., 1993). In these species, an IgM-related seroconversion, defined as a rising DAT titer with no MAT, commenced 7 to 10 days after infection. Thirty days after infection, surviving macropods had increasing MAT titers indicative of an IgG-related seroconversion. Macropods previously exposed to *T. gondii* and those maintaining inapparent infections subsequently have DAT and MAT titers> 64 (Johnson et al., 1989).

Both agglutination tests have been used successfully to aid in the diagnosis of acutely fatal toxoplasmosis in the common wombat (*Vombatus ursinus*), the Tasmanian pademelon (*Thylogale billardierii*), and the Bennett's wallaby (*Macropus rufogriseus*). Some animals die with moderate to high DAT titers (generally between 256 and 1024) and low or negative MAT titers (<256). Sera from other cases of toxoplasmosis have identical DAT and MAT reactions at high to very high titres (up to 16,000) suggesting that some animals survive the early IgM-related seroconversion (D. L. Obendorf, unpubl.).

Sera of some eastern barred bandicoots reacted only at low to moderate titrations in the DAT. These were initially classed as suspicious reactors as it was uncertain if these reactions were due to specific or non-specific agglutination of IgM antibodies. On subsequent samplings, however, these individuals recorded negative DAT reactions; at no time did these bandicoots develop MAT titers. For this reason these reactions were considered non-specific and these bandicoots were reclassified as antibody-negative.

Seven bandicoots had moderate to high DAT reactions which would be consistent with early stages of seroconversion; none had clinical symptoms. None were retrapped at the next three monthly trapping period. Five of the 10 bandicoots with moderate to high MAT titers were also not retrapped. Another seropositive animal which died in the trap had extensive microscopic lesions associated with *T. gondii* infection.

Toxoplasma gondii infection can cause central nervous system and systemic disease leading to death of eastern barred bandicoots (Obendorf and Munday, 1990). The loss of agility and their noctural foraging behavior have been thought to increase their susceptibility to predation by raptors, large dasyurid marsupials, or feral cats (Obendorf and Munday, 1990; Lenghaus et al., 1990). Feral cats were regularly caught or sighted at both study sites.

An absence of bandicoots with DAT and MAT titers >64 for periods in excess of three months is evidence that this species of bandicoot does not act as a long-standing, inapparent carrier for *T. gondii*. These findings are closely analogous to a serological survey conducted on the brown hare (*Lepus europaeus*) in Sweden (Gustafsson and Uggla, 1994). The brown hare is highly susceptible to *T. gondii* which causes an acutely fatal disease (Borg 1961; Gustafsson et al., 1988), and the failure to detect any *Toxoplasma* sp. antibodies by agglutination test or ELISA in 176 hares was taken as evidence that the population consisted only of unexposed susceptible individuals (Gustafsson and Uggla, 1994).

The presence of moderate to high MAT reaction titers in confirmed toxoplasmosis cases is evidence that at least some bandicoots survive the initial period of acute infection and seroconversion.

In future research, we plan to experimentally infect eastern barred bandicoots with *T. gondii* oocysts and follow the course of the disease and seroconversion.

It is likely that bandicoots become infected through ingestion of soil-associated invertebrates (Obendorf and Munday, 1990). Factors such as the body weight of the host and the number of infective organisms ingested may determine the longevity of individual bandicoots.

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