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AGGLUTINATING ANTIBODIES TO *TOXOPLASMA GONDII* IN SERA FROM CAPTIVE EASTERN BARRED BANDICOOTS IN AUSTRALIA

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ABSTRACT: Toxoplasmosis is considered a severe health risk for many marsupial species. The mainland Australian population of bandicoot is endangered. Therefore, a preliminary serosurvey was conducted to evaluate exposure to *Toxoplasma gondii* in 57 captive eastern barred bandicoot and to estimate the possible impact of *Toxoplasma* on recovering populations. Five (9%) bandicoot were classified as seropositive using a modified agglutination test. Nineteen additional bandicoot (33%) were classified as serosuspect using a direct agglutination test. No bandicoot showed signs of clinical disease. Seropositive titers were IgG associated, suggesting that infections were chronic and latent. Serostatus was not associated with either sex or being wild-caught, although each seropositive bandicoot was wild-caught. Seropositive animals ranged from 1.25- to 2.5-yr-old. Computer simulations using Vortex 5.1, based on the proportion of seropositive and seronegative bandicoot in this study, indicate that mortalities from *T. gondii* should have little impact upon captive populations. However, the potential impact of toxoplasmosis on recovery efforts for wild, mainland bandicoot populations is not clear.

Key words: Agglutination test, Eastern barred bandicoot, *Perameles gunnii*, population viability, *Toxoplasma gondii*, toxoplasmosis, serosurvey.

INTRODUCTION

Marsupials are perceived to be particularly vulnerable to *Toxoplasma gondii*, due to clinical outbreaks with high mortality rates at several zoos (Dobos-Kovács et al., 1974; Boorman et al., 1977; Jensen et al., 1985; Patton et al., 1986; Dubey et al., 1988; Canfield et al., 1990). Similarly, wild marsupial mortalities due to toxoplasmosis have been documented (Obendorf and Munday, 1983, 1990; Johnson et al., 1988; Lenghaus et al., 1990; Obendorf et al., 1996). Although clinical signs are often acute, it is often not clear whether epizootic mortalities in captive and free-ranging marsupials due to toxoplasmosis are due to recent infections (Jensen et al., 1985; Dubey et al., 1988; Johnson et al., 1989), or chronic infections which are reactivated during times of stress (Obendorf and Munday, 1983, 1990).

The eastern barred bandicoot (*Perameles gunnii*) is a 0.5–0.9 kg marsupial whose remnant mainland population in Hamilton, Victoria, Australia number less than 100 free-ranging individuals (Robinson, 1995). Factors which may have contributed to their decline include habitat loss, predation by domestic cats and fox, and pesticides (Seebeck et al., 1990). In addition, free-ranging bandicoot with neurologic abnormalities (motor dysfunctions, apparent blindness, and atypical activity levels) have been documented to have *T. gondii* lesions (Lenghaus et al., 1990; Obendorf and Munday, 1990; Obendorf et al., 1996). This suggests that toxoplasmosis is potentially an important source of mortality in bandicoot.

The antemortem diagnosis of toxoplasmosis and distinctions between infection and active disease can be difficult. A direct agglutination test (DAT)(Fulton and Turk, 1959) was developed which measured the sum of IgG and IgM serum antibodies to *T. gondii*, and is an assay which can be applied to multiple species. However, in eutherians, non-specific IgM reactions limit the diagnostic value of the DAT. Consequently, the DAT was modified by the

addition of 2-mercaptoethanol to eliminate IgM reactivity (Desmonts and Remington, 1980). The resulting assay, the modified agglutination assay (MAT), is commonly used to measure IgG antibodies to *T. gondii*. Work with marsupials suggests that both the DAT and the MAT have diagnostic value (Johnson, 1989; Obendorf et al., 1996) although specific cutoffs for the MAT and DAT have not been universally established and accepted for all applications.

Small populations are vulnerable to extinction due to a number of deterministic and stochastic processes, including disease. Disease has limited reintroduction and captive propagation efforts of several endangered species, including Arabian oryx (Woodford and Kock, 1991), black-footed ferret (Thorne and Williams, 1988), and golden lion tamarin (Bush et al., 1993). Computer simulations have been a valuable tool for estimating the impact of various factors on endangered species populations, and have been used to guide management decisions. As toxoplasmosis may have contributed to the bandicoot decline on mainland Australia and may limit recovery efforts, this paper reports results of a preliminary serosurvey for toxoplasmosis and computer simulations which estimate the potential effects of this disease on captive populations.

METHODS

A serological survey for *T. gondii* was conducted on plasma samples from 57 bandicoot. Forty bandicoot located at the Royal Melbourne Zoological Gardens (Parkville, Victoria, Australia; 37°47′S, 144°57′E), Healesville Sanctuary (Healesville, Victoria, Australia; 37°43′S, 145°36′E), or Gellibrand Hill Park (Greenville, Victoria, Australia; 37°37′S, 144°52′E) were humanely immobilized with isoflurane (Aerrane, Anaquest, Madison, Wisconsin, USA) gas anesthesia for sampling and routine complete physical examinations during March and April 1992. Blood was collected for routine hematology, blood chemistry, and serology. Sera from 17 bandicoot which were collected at these sites on earlier dates were also analyzed. Plasma and serum samples were frozen at −70 C to establish a serum reference bank, and were stored in multiple small aliquots.

Serum and plasma samples were tested using a commercially available direct agglutination (DAT) and modified agglutination (MAT) kit (BioMerieux, 69280 Marcy-l'Etoile, France) which is not species specific and has been used for bandicoot (Obendorf et al., 1996). Directions were followed for the test kit with the exception of modifications made to conserve serum and the test kit's antigen. To conserve serum and plasma, 150 μl volumes each of sample and phosphate-buffered saline (PBS) for the DAT and MAT, and 150 μl of 0.2 M 2-mercaptoethanol (2-ME) for the MAT were used, rather than the 200 μl volumes which are recommended by the manufacturer. Preliminary trials confirmed that our modifications did not alter the results of our assays. The kit's positive and negative controls provided quality control for each plate. To conserve costly antigen, samples were initially screened from 1:4 to 1:256 using doubling dilutions for the DAT. Samples greater than 1:64 for the DAT were retitrated to 1:4,096 to determine the endpoint, and MAT's were performed on samples with DATs greater than 1:32. Plates were scored blindly with a reverse-light microscope at 40 by at least two experienced, independent observers at 4 hr.

A MAT titer of ≥ 64 was classified as positive for antibodies to *T. gondii* (Johnson et al., 1989; Obendorf et al., 1996). As serologic cutoffs for bandicoot are currently provisional, a serosuspect group was established. The cutoff for serosuspect status (DAT titer > 64, exclusive of animals with seropositive MAT titers), was based on cutoffs established for experimental *T. gondii* infections in marsupials (Johnson et al., 1989) and field work with Tasmanian bandicoot (Obendorf et al., 1996). The proportion of seropositive and seronegative bandicoot was used to provide values for computer simulations which assessed the impact of toxoplasmosis on bandicoot populations.

Animals with known or reliable estimates of age were grouped into four age classes: <0.5 yr, 0.5–1.5 yr, 1.5–2.5 yr > 2.5 yr. Sex and whether bandicoot were captive-born or wild-caught, if known, were examined with respect to DAT cutoffs of ≥ 64 and MAT ≥ 64.

To estimate the potential impact of toxoplasmosis on captive bandicoot, computer simulations were conducted using Vortex 5.1 (Lacy and Kreeger, 1992) with the assumption that all *T. gondii* infections resulted in mortality which was additive (versus compensatory) to other sources of mortality. Baseline simulations were conducted based on data from P. Myroniuk (unpubl. data) for captive populations. Two dif-

ferent levels of mortality were evaluated by conducting two simulations which were identical to baseline simulations, except for mortality rates increased by 9% (percentage of seropositive bandicoot) and 42% (combined percentage of seropositive and serosuspect bandicoot). One thousand simulations, corresponding to 25 yr (four bandicoot generations per year) were conducted for each of the above scenarios. Values generated by simulations included probability of extinction for each 5 yr interval, mean persistence times, observed heterozygosity, and number of alleles.

RESULTS

Five (9%) bandicoots were classified seropositive for *T. gondii* using MAT's, and 19 (33%) were classified as serosuspect using DAT's. All seropositive bandicoot had MAT's ≥ 512 and all seropositive MAT values were within 2 dilutions of the corresponding DAT value. All serosuspect and seronegative bandicoot had MAT's = 0. Titers appear to be stable over time, as sequential titers for three animals with 6 mo, 3 mo, and 2 wk sampling intervals were within one dilution of each other, and were therefore not significantly different. No bandicoot showed signs of illness.

Age was known for 51 bandicoot. The mean (±SD) age of captive-born bandicoot (0.81 ± 0.72 yr) was younger than the mean estimated age of wild-caught bandicoot (1.72 ± 0.84 yr). All but two of those < 1 yr were born in captivity. Fifteen bandicoot were classified as < 0.5 yr, 16 were 0.5 to 1.5 yr, 13 were 1.5–2.5 yr, and seven were ≥2.5-yr-old. All seropositive bandicoot were 1.25–2.5-yr-old and wild-caught. Identification of sites with higher apparent infection rates is not possible due to frequent intra facility transfers of bandicoot for breeding arrangements designed to retain genetic diversity.

Three dams in this study had more than one captive offspring included in the serosurvey. Each of these dams were seronegative and their offspring's MAT = 0. Five seronegative females > 2.5-yr-old were included in the serosurvey, each of which produced a range of 8 to 17 studbook listed offspring. Bandicoot records suitable for rigorously analyzing the effects of toxoplasmosis on reproduction were not available.

Increasing toxoplasmosis related mortalities by 9% and 42% had little impact upon the mean number of years to extinction in captive bandicoot simulations (Table 1). Similarly, observed heterozygosity and number of alleles were similar for each captive simulation.

DISCUSSION

This preliminary study suggests that 9% of captive bandicoot are infected with *T. gondii* and an additional 33% are suspected of being infected, based on provisional cutoffs. A 9% seroprevalence is similar to the 10% *T. gondii* infection rate reported for wild bandicoot post-mortem examinations (Lenghaus et al., 1990). No bandicoot showed clinical signs of illness. The MAT detected bandicoot with IgG antibodies to *T. gondii*. This suggests that infections were chronic and latent.

There was no significant association between sex or wild-caught status and *T. gondii* antibody status for seropositive or seronegative bandicoot. It is not clear why all seropositive bandicoot were wild-caught and 1.25- to 2.5-yr-old, and none were older. Possible explanations include (1) this cohort was exposed to toxoplasmosis at a higher rate or level than other cohorts and mounted an appropriate and vigorous immune response, (2) it is an advanced stage of infection which may lead to premature mortality, or (3) the animal is exhibiting peak antibody levels which will subsequently decline with age.

Establishing the temporal course of *T. gondii* infections in bandicoot is an important issue to resolve, as a substantial number of offspring (n = 8 to 17) were produced by three seronegative females >2.5-yr-old. Our study was unable to establish whether seropositive status was associated with increased numbers of abortions or failure to conceive. We believe it is unlikely that *T. gondii* affects bandicoot by decreasing the viability of offspring, as ob-

TABLE 1. Results from 1,000 Vortex simulations (25 yr) for captive Eastern Barred bandicoot populations with different levels of toxoplasmosis related mortality.[a]

Scenario	Probability of extinction (yr)					Mean number yr to extinction	Observed heterozygosity	Number of alleles
	5	10	15	20	25			
Base—captive	0.001	0.023	0.058	0.095	0.138	16.42 ± 5.8	0.857 ± 0.1	11.77 ± 4.3
Captive—8% more mortality	0.013	0.041	0.077	0.126	0.194	16.23 ± 6.2	0.848 ± 0.1	11.52 ± 4.4
Captive—41% more mortality	0.028	0.093	0.214	0.349	0.455	15.33 ± 5.9	0.844 ± 0.1	10.46 ± 5.1

[a] Data presented as mean of 1,000 simulations ± SD.

served in congenitally infected eutherians; the short (12 day) gestation substantially limits the period of time in which *in utero* infections could result in live, congenitally infected offspring, rather than inducing abortion. This is supported by the absence of *T. gondii* lesions in pouch young necropsied from free-ranging bandicoot road kills (Lenghaus et al., 1990) and the seronegative status of all bandicoot < 0.5-yr-old in this study ($n = 15$).

Until infection prevalence and test sensitivity and specificity for the MAT and DAT have been determined for bandicoot populations, the predictive value of these tests individually or in combination will be uncertain for individual bandicoot. However, the cutoffs used for these tests do serve as reasonable values for estimating the potential impact of *T. gondii* infections on bandicoot using computer simulations. Due to the preliminary nature of this investigation, a serosuspect group of animals provides a means of accounting for possible errors in identifying animals infected with *T. gondii*. Computer simulations assumed mortality in all animals classified as serosuspect or seropositive because of interest in the impact of an epizootic. Computer simulations do not fully account for all variables which can influence population size, and are best viewed as tools for evaluating the relative impact of each variable, rather than as predictors of absolute population size. Therefore, the trends which occurred as mortality rates were changed are the important features of our simulations. Possible effects of *T. gondii* on bandicoot reproduction were not included in computer simulations due to an absence of data on this topic.

Our simulations suggest that captive populations appear to be relatively secure from toxoplasmosis under current management protocols and at current rates of infection. This is in contrast to computer simulations estimating the impact of toxoplasmosis on wild bandicoot populations (D. S. Miller unpubl. data). We believe this is because captive populations have

higher juvenile survivorship, longer life spans, and increased fecundity, in contrast to wild populations. However, it is important that future research determine whether captive bandicoot with latent *T. gondii* infections which are released to wild populations are at a greater risk for disease, mortality, or decreased reproduction.

Conclusive evidence for the role of *T. gondii* in the decline of mainland bandicoot populations will remain elusive. However, the potential for toxoplasmosis to directly or indirectly affect mortality and fecundity in recovery efforts is a management concern. As marsupials with toxoplasmosis can have subclinical infections, the occurrence of clinical toxoplasmosis and other diseases in bandicoot could serve as indicators of poor environmental quality or suboptimal management practices.

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