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Prevalence of *Toxoplasma gondii* Antibodies in Dingoes

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ABSTRACT: Serum samples from 62 dingoes (*Canis familiaris dingo*) trapped in five areas of southeastern New South Wales, Australia were tested for antibodies to *Toxoplasma gondii*. Six (10%) of the dingoes had direct agglutination test titers for *T. gondii* of ≥1:64, and four of these animals had *T. gondii*-specific IgM, suggesting recent exposure.

Key words: Toxoplasma gondii, dingo, *Canis familiaris dingo*, serological survey, direct agglutination test, modified agglutination test, parasite-specific IgM.

Numerous studies have determined the prevalence of *T. gondii* in human and domestic animal populations (Dubey and Beattie, 1988). Over 250 surveys have determined the prevalence of *T. gondii* in dogs (Dubey, 1985). However, there are apparently no surveys for toxoplasmosis in the dingo (*Canis familiaris dingo*).

We reported antibodies to *T. gondii* in 18% of Tasmanian pademelons (*Thylogale billardierii*) and 3% of Bennett's wallabies (*Macropus rufogriseus rufogriseus*) (Johnson et al., 1988). Antibodies to *T. gondii* have been reported in many species of small Australian wild animals (reviewed in Munday, 1970), although occurrence appears to be related to climate, higher prevalence being reported in animals from cooler, moister areas (Jakob-Hoff and Dunsmore, 1983). O'Donoghue et al. (1987) similarly reported a higher prevalence of antibody to *T. gondii* in sheep from cooler areas. Dingoes prey on Australian marsupials, predominantly wallabies such as *M. rufogriseus*, although the remains of sheep, cattle, feral pigs (*Sus scrofa*), horses and rabbits (*Oryctolagus cuniculus*) have been found in the stomachs of dingoes trapped in southeastern Australia (Newsome et al., 1983). Because toxoplasmosis can be transmitted by the ingestion of cyst-laden flesh, this study was undertaken to determine the prevalence of antibodies to *T. gondii* in dingoes in southeastern New South Wales.

Dingoes were sampled in five areas, Bondo State Forest (149°01′S, 36°32′E), Shannons Flat (149°01′S, 36°08′E), Bombala (149°28′S, 37°10′E) Kosciusko National Park (148°32′S, 35°50′E) and Wombeyan Caves (150°12′S, 34°20′E) primarily as part of a hydatid eradication campaign (Jenkins and Morris, 1990). These areas and the number of animals trapped are designated in Figure 1.

All trapping areas were above 500 m in country that is hilly with peaks ranging from 650 to 1,000 m. The areas are mostly thickly forested with native hardwood forests interspersed with pine plantations. Average annual rainfall ranges from about 10 mm (Bombala State Forest) to about 25 mm (Kosciusko National Park), and all areas are covered by snow for at least part of the winter. Most areas were within dingo territorial range of sheep and cattle grazing country, and macropodids and feral pigs were common. All areas were used to some extent for recreational activities (bushwalking and pig hunting). Established human habitation consisting of farm homesteads and holiday cabins was outside the trapping areas, but within about 8 km.

Dingoes were trapped by employees of the regional Pasture Protection Boards and shot. Approximate age of the dingoes was determined by size of the animal and their dentition. Immediately after shooting, 20 ml of blood was collected into plastic vials and allowed to clot at ambient temperature. The clot was detached from the inside of the vial and allowed to contract overnight at 4 C. After centrifugation to remove free cells, serum was collected in 5 ml aliquots and frozen at −20 C.

Sera were shipped at −20 C to the Flin-

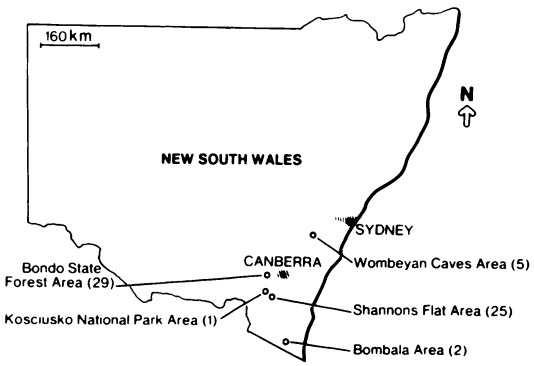

FIGURE 1. Collection areas for dingoes in New South Wales, Australia. The number of animals trapped at specific localities are in parentheses.

ders Medical Centre (South Australia 5042, Australia) for serological testing in a direct agglutination test (DAT) which measures *T. gondii*-specific total antibody (Peloux et al., 1973), and a modified agglutination test (MAT), which measures parasite-specific IgM (Dubey et al., 1985a, b; Dubey and Desmonts, 1987; Johnson et al., 1989). For the DAT, sera were serially diluted from 1:32 to 1:512 in BABS buffer (0.12 M NaCl, 0.025 N NaOH, 0.05 M boric acid, 0.4% bovine serum albumin, pH 9.0) in U-bottom microtiter plates (Disposable Products, Adelaide, South Australia). Twenty-five μl of a suspension of formalin fixed *T. gondii* tachyzoites (BioMerieux, Charbonnieres les Bains, France) was added to each 25 μl serum dilution per microtiter well. The plates were left at 22 C for 18 hr, at which time the titers of the test sera were determined by comparison with the agglutination in a serial dilution of a positive control serum (BioMerieux). We believe that a titer >1:64 indicates a positive result in the DAT based on previous studies (Johnson et al., 1987, 1989).

In order to destroy the IgM fraction of the sera giving positive DAT titers, specimens were treated with either 0.2 M 2-mercaptoethanol (2-ME) or phosphate-buffered saline, pH 7.2 (PBS), at 37 C for 1 hr, and treated as described above for the DAT. The difference in titers obtained after treatment of a serum with 2-ME or PBS in the MAT was taken to be the amount of parasite-specific IgM present in the serum (the greater the difference, the more IgM was present). Positive responses in the MAT were considered to be those where the titer after treatment with 2-ME was a four-fold or greater dilution less than that of the same serum after treatment with PBS only. We have shown previously that this MAT technique is applicable to macropodid (Johnson et al., 1989) and human serum (Johnson et al., 1987) and others have found it suitable for use with a range of species including goats (Dubey et al., 1985a), cattle (Dubey et al., 1985b), pigs (Dubey et al., 1986), horses (Dubey and Desmonts, 1987), sheep (Dubey et al., 1987a) and cats (Dubey et al., 1987b) as well as dogs (Dubey, 1985). Although we are unaware of exact figures for the specificity and sensitivity of the DAT or MAT for canid sera, Dubey (1985) reported good correlation between MAT titres and dye test titres for two dogs fed *T. gondii* oocysts and two dogs fed *T. gondii* cysts.

Overall, 10% of the dingoes had antibody to *T. gondii*. The highest prevalence (18%) was found in dingoes trapped in the Bondo State Forest, but this was not significantly different from that found in dingoes trapped in the Shannons Flat area ($0.5 < P < 0.25$, χ^2 test with Yate's correction). The number of dingoes was not large enough to perform valid statistical analyses for parameters such as approximate age or sex. The fact that four of the six dingoes with total antibody to the parasite were between 2- to 3-yr-old and possessed parasite-specific IgM (Table 1) is consistent with the hypothesis that dingoes are more often exposed to *T. gondii* in the first year or two of their life. Of course, this survey only accounts for those dingoes which were exposed to the parasite and survived the infection. Dingoes succumbing to fatal toxoplasmosis would be excluded naturally from this survey. However, we are unaware of other studies reporting prevalence of *T. gondii* in dingoes for comparison. Toxoplasmosis appears more likely to be

TABLE 1. Direct agglutination test (DAT) antibody titers and modified agglutination test (MAT) antibody titers to *Toxoplasma gondii* in dingoes (*Canis familiaris dingo*).

Animal identification	Sex	Approximate age (yr)	Area trapped	Reciprocal of DAT titer	Reciprocal of MAT titer
119	Female	2–3	Shannons Flat	64	<4
129	Female	2–3	Bondo State Forest	64	4
130	Male	3–4	Bondo State Forest	64	64
148	Female	2–3	Bondo State Forest	256	8
156	Male	2–3	Bondo State Forest	64	64
158	Female	2–3	Bondo State Forest	64	16
Remaining 56 animals	Both sexes	—	All areas	<32	not done

fatal in domestic dogs <1 yr of age, and in dogs concurrently infected with canine distemper virus (Dubey and Beattie, 1988). Antibody to *T. gondii* has been reported at prevalences of 7 and 31% in dogs in Australia (Cook and Pope, 1959; Watson et al., 1982). The dingo is at the head of a food chain which consists of a large range of native animals as well as sheep and pigs, all known to have high prevalences of antibody to *T. gondii* (reviewed by Dubey and Beattie, 1988). A random sample of the stomach contents on some of the dingoes described here gave results similar to those of Newsome et al. (1983), confirming that the dingoes trapped in these five areas would have eaten animals known to be infected with *T. gondii*. Therefore, the prevalence of 10% obtained in this survey is perhaps unexpectedly low. This is consistent with the hypothesis that some of the dingoes infected early in life may have succumbed to fatal toxoplasmosis. Alternatively, toxoplasmosis may be more commonly transmitted by the ingestion of oocysts directly from the soil to herbivores, than it is by the ingestion of cyst-laden flesh by carnivores. More studies on a larger number of dingoes in wider age ranges would be needed to attempt to confirm or refute either hypothesis.

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