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Earthworms as Paratenic Hosts of Toxoplasmosis in Eastern Barred Bandicoots in Tasmania

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ABSTRACT: An experimental feeding study was designed to assess the role of earthworms in the transmission of *Toxoplasma gondii* infection to eastern barred bandicoots (*Perameles gunnii*). Six animals with no agglutinating antibodies to *T. gondii* were fed artificially cultured earthworms that had been maintained in autoclaved nutrient-enriched soil. Two animals were given earthworms that had been maintained in soil contaminated with *T. gondii* oocysts (P89/VEG strain); two animals were fed on earthworms, which initially had been exposed to soil containing *T. gondii* oocysts then transferred through three changes of sterile soil; two control bandicoots were fed earthworms maintained in sterile soil. Both bandicoots fed earthworms maintained in *T. gondii* contaminated soil died 11 and 14 days after feeding. The necropsy findings were consistent with acute toxoplasmosis. Bandicoots fed earthworms exposed to oocysts but then transferred through changes of sterilized soil remained healthy as did control animals. All surviving animals remained seronegative over the 6 wk observation period after feeding. These findings confirm that earthworms, a major component of the natural diet of *P. gunnii,* can transmit *T. gondii* infection. It appears that oocysts present in the alimentary tracts of the worms, rather than infective stages of *T. gondii* in worm somatic tissues, are responsible for these infections.

Key words: Eastern barred bandicoot, earthworms, experimental transmission, marsupial, *Perameles gunnii, Toxoplasma gondii.*

After European settlement of Australia, introduced domestic cats began to colonize Australia's terrestrial environments. Cats also brought with them their endoparasites including the apicomplexan protozoan, *Toxoplasma gondii.* Members of the cat family (Felidae) are the only known definitive hosts for *T. gondii* (Dubey, 1994). Consequently, domestic and feral cats were responsible for transmitting this disease to a wide range of naive intermediate hosts. Australia's marsupial fauna are known to be susceptible to toxoplasmosis (Canfield et al., 1990) with free-ranging Tasmanian marsupials, in particular the eastern barred bandicoot (*Perameles gunnii*), dying from acute primary toxoplasmosis (Obendorf et al., 1996).

For omnivorous and insectivorous marsupials such as bandicoots, a possible source of toxoplasmosis could be from eating soil-associated arthropods, insects, or annelids that have come in contact with *T. gondii* oocysts. After exposure to contaminated soil, plant material or directly from cat feces such invertebrates may act as mechanical hosts by harbouring viable oocysts within their gastrointestinal tract (Frenkel et al., 1975; Ruiz and Frenkel, 1980). This investigation presents an experimental feeding study undertaken to assess the role of annelids in the transmission of toxoplasmosis to eastern barred bandicoots.

Six adult eastern barred bandicoots (4 females; 2 males, weighing between 700–790 g) were live trapped in the Huon Valley (Tasmania, Australia: 43°03′S, 147° 02′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) and transferred to holding pens in the Central Animal House at the University (Hobart, Tasmania; Animal Ethics Committee approval number 94010). The animals were acclimatised for 3 wks prior to the commencement of the experiment. Each bandicoot was held in a separate holding pen (2.5 × 1.0 m in floor area, with 0.6 m walls) made from freshly milled timber; the flooring was clean pine sawdust. Each pen contained a wooden hutch with shredded paper as nesting material; pens were covered

with shade cloth to prevent escape and reduce distress. Food and water were provided *ad libidum.*

Twenty kg of a commercial soil mix containing compressed peat moss and dolomite (Amgrow, Castlereigh, Tasmania, Australia) was sterilized by autoclaving twice at 115 C for 1 hr. Approximately 3 kg of soil mix was distributed into a number of black plastic boxes that formed part of a worm composting kit (Worm Factory, Reln Plastic, Sydney, New South Wales, Australia).

Five hundred artificially-reared *Lumbricus rubellus* and *Perionyn excavatus* earthworms (All State Worm P/L, Adelaide, South Australia, Australia) were initially prepared by passing them through two changes of the soil mix over a 2 day period. Every 24 hr the earthworms were carefully sieved and washed in 0.9% NaCl solution and transferred to fresh soil mix. The worms were then divided equally between two plastic boxes containing fresh soil. The soil was enriched with egg and milk powder to provide nutrient for the worms. A suspension of 1.75×10^6 *T. gondii* oocysts of the P89/VEG strain (kindly provided by Dr. J. P. Dubey, USDA, Beltsville, Maryland, USA) was mixed into the soil of one box. The infectivity of the oocysts was demonstrated by inoculating bandicoots orally with approximately 100 oocysts (Bettiol et al., 2000). The remaining box was not inoculated with *T. gondii* oocysts.

After 7 days, earthworms from the soil containing oocysts were carefully removed and washed in normal saline. Twenty five g aliquots of worms were added to the daily food ration of each of two bandicoots (ear tag #7 and 8) on day 8. These animals were fed another 25 g meal on day 9. Remaining washed earthworms from this box were transferred into a new box containing freshly autoclaved soil. Over a 4 to 5 day period the worms were transferred through four changes of fresh soil as described above. Once completed the worms were washed and 25 g aliquots were added to food given to each of another two bandicoots (ear tag #2 and 5) for one or two days. An additional two bandicoots (ear tag #4 and 6) were each fed 25 g of earthworms derived from the control box (i.e., containing sterilized soil only).

Blood samples were collected from all animals by lancing the lateral ear vein into a Microtainer serum separator tube with a gel interface (Becton Dickinson & Co., Rutherford, New Jersey, USA). *Toxoplasma gondii* antibodies were screened using the Direct Agglutination Test (DAT) and Modified Agglutination Test (MAT) as outlined in Bettiol et al., (2000). All animals selected for the study tested negative for *T. gondii* antibodies prior to the commencement of experimental work. At 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.

A selection of organs and tissues from animals that died was fixed in 10% buffered formol-saline; histological specimens, and immuno-histochemical staining were prepared as outlined in Bettiol et al., (2000).

The two bandicoots (#7 and #8) fed on earthworms derived from soil containing oocysts respectively lost 80 g and 140 g in body weight and died 11 and 14 days after feeding. Both animals were active during daylight, which was abnormal, and drank excessively 2 to 3 days prior to death; one animal was unable to locate its shelter box. No change in body temperature was noted in either animal. Low agglutination titres were detected in DAT (1:16; 1:64) and one animal also had a low MAT titre (1:16). Notable necropsy findings in the two dead bandicoots were: the presence of excessive blood-tinged abdominal fluid, enlarged mediastinal lymph nodes, splenomegaly, spleen congested with focal consolidation, enlarged mottled livers and petechial hemorrhages in the pancreas and intestinal mesentery.

Extensive cell necrosis associated with acute inflammation was apparent in many

tissues and organs, the most severe histopathological changes were seen in heart muscle, liver, lung, lymphoid follicles of the spleen and lymph nodes, pancreas and skeletal muscle. Immunostaining specific for *T. gondii* trophozoites, as outlined in Bettiol et al. (2000) was used to identify the organisms as individual organisms or cyst-like aggregates within several types of host cell and in close association with areas of generalized cell necrosis.

Animals that were fed on earthworms which had initially been exposed to *T. gondii* oocysts and then passed through several changes of fresh soil remained clinically normal. They did not develop antibodies during the 6 wk after the experiment began. Similarly the control animals fed on worms maintained in autoclaved soil remained healthy and seronegative over the same period. Control animals were released back into the wild.

In soil *T. gondii* oocysts have been reported to remain infective for up to 18 months (Frenkel et al., 1975). Many millions of oocysts can be shed in the period a cat excretes the organism in its faeces. It has been calculated that the number of oocysts shed in a 20 g cat stool can be in the order of 2 to 20 million and after fecal decomposition, the local soil concentration can be as high as 100,000 oocysts/g (Frenkel et al., 1975).

Soil-associated invertebrates which are coprophagic are capable of acting as mechanical transport hosts for *T. gondii* oocysts feces (Markus, 1974; Frenkel et al., 1975; Ruiz and Frenkel, 1980). As domestic cats tend to bury their feces in surface layers of soft soil, a range of invertebrates including ground beetles, isopods, molluscs and dipteran larvae may ingest infective oocysts of *T. gondii* by contact directly with feline feces. Earthworms feeding on soil contaminated with decomposing cat feces are known to take in *T. gondii* oocysts (Frenkel et al., 1975). Ingested oocysts can be carried in their intestine and dispersed in their discarded alimentary casts. Their attraction to fecal material or soil enriched with fecal compost make earthworms important disseminators of some microbial pathogens (Brown, 1995). Proximity to the surface layers of soil also make them available as food for birds or mammals.

Earthworms (Annelida) and beetles (Coleoptera) make up a significant proportion of the diet of eastern barred bandicoots (Heinsohn, 1966; Quin, 1985; Brown, 1989; Dufty, 1994; Mallick et al., 1996). In the 3 yr dietary study conducted by Mallick et al., (1996) the number of chaetae (microscopic body hairs of annelids) in fecal samples of free-ranging Tasmanian *P. gunnii* began to rise in the autumn and reached a peak in winter months. This also corresponded to the seasonal peak in availability of earthworms.

In the present investigation the two bandicoots fed earthworms exposed to soil containing oocysts displayed abnormal behaviour prior to death. Docility, apparent blindness, incoordination and a loss of the ability to seek refuge have been described in *P. gunnii* with toxoplasmosis (Obendorf and Munday, 1990). The severity of gross and histopathological findings indicates that both animals died as a result of acute generalised toxoplasmosis. The number of oocysts received by each bandicoot is not known. The pathogenicity of this strain at a dose of 100 oocysts given orally to *P. gunnii* is documented elsewhere (Bettiol et al., 2000). On average, bandicoots fed earthworms from *T. gondii*-contaminated soil died earlier (12.5 days) whereas orally inoculated bandicoots died later (16 days) (Bettiol et al., 2000). The short time interval from exposure to death suggests that these animals ingested a larger infective challenge than orally inoculated bandicoots and as a consequence limited the detection of serum antibodies to *T. gondii.*

Based on the dietary and population studies of eastern barred bandicoots in Tasmania, the findings provide additional support for the role of earthworms as mechanical vectors of infective *T. gondii* oocysts. The study indirectly demonstrates

that these earthworms can become noninfective, suggesting that such worms lose their infectivity, probably by excreting the oocysts in their intestinal casts. Although not specifically investigated the study supports the view that infective stages of *T. gondii* do not establish in the somatic tissues of earthworms. For this reason earthworms are not considered long-term carriers of infective stages of *T. gondii*.

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