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Giardiasis in Native Marsupials of Tasmania

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ABSTRACT: Tasmanian native marsupials were screened for the presence of *Giardia* spp. over a 3 yr period, revealing a 21% prevalence in the 295 animals tested. A pilot study of experimentally infected eastern barred bandicoots (*Perameles gunnii*) indicated susceptibility to infection with *Giardia duodenalis* from a human source.

Key words: Tasmania, *Giardia duodenalis*, marsupials, infection, eastern barred bandicoot, *Perameles gunnii*.

In Australia, *Giardia duodenalis* is the most commonly reported intestinal parasite of human populations (Meloni et al., 1993). Transmission of human giardiasis occurs predominantly via the fecal-oral route when cysts are ingested from food or water, including chlorinated water, contaminated by infected human or animal feces (Thompson et al., 1993). Resistant infective cysts may remain viable for long periods in the environment under the right conditions (Feely et al., 1990; Thompson et al., 1993). Giardiasis has been reported world-wide in companion animals and was classified by WHO as a zoonotic disease in 1979 (Faubert, 1988). Numerous water and foodborne outbreaks have since been described, although debate continues on the importance of pets and other mammals as possible reservoirs for human infection (Bemrick and Erlandsen, 1988). Even with successful experimental infections of humans with *G. duodenalis* from an animal source (Majewska, 1994), the epidemiological implications remain unresolved.

The reported prevalence of *G. duodenalis* varies widely depending on such factors as the method of detection, the population studied and geographic locality (Horejs and Koudela, 1994). Relatively few studies have been conducted on the prevalence and epidemiology of human parasitic infections in Tasmania. Goldsmid et al. (1984) first demonstrated *G. duodenalis* as an intestinal pathogen in Tasmanian communities, with strong suggestions of familial transmission. Tasmania's natural beauty and reputation as a holiday destination for hikers is often marred by "Bushwalkers Diarrhea" for which *G. duodenalis* is often blamed, although there is little laboratory data to justify this claim. The first case of clinical giardiasis in a pet dog in Tasmania was reported by Davies et al. (1993), suggesting that an uninvestigated reservoir for human infection might exist. Subsequent investigations have revealed the presence of *Giardia* sp. cysts in fecal samples of dogs, cats and marsupials such as possums, wallabies, wombats, Tasmanian devils and bandicoots (Milstein, 1993; Milstein and Goldsmid, 1995; Davies, 1995). *Giardia duodenalis* has not been confirmed from prototherian mammals (monotremes).

Between 1993 and 1995, 295 fecal samples were collected from native animals across Tasmania. Specimens were examined for the presence of *G. duodenalis* using a combination of Trichrome staining, formalin-ethylacetate sedimentation concentration (Garcia and Bruckner, 1993) and antigen detection (*Giardia* CELISA, Cellabs Diagnostics, Pty., Ltd., Brookevale, NSW, Australia; Hopkins et al., 1993). Examination of repeat fecal samples is thought to be a reliable method of detecting intestinal parasites, but since it was not possible to obtain repeat samples from individual animals, antigen detection was used as a supplementary test. Hopkins et al. (1993) reported a lowered specificity (91 to 95%) and sensitivity (55 to 64%) when the kit was used on animal samples as opposed to specimens from humans. This being the case, it seemed that a

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screen of native animals using the *Giardia* Celisa test for antigen detection would more likely underestimate the rate of infection in these animals than give an exaggerated picture.

In 1995, four eastern barred bandicoots (*Perameles gunnii*) from the Huon valley in southern Tasmania (Grove site: 42°03'S, 147°02'W), were used in this study. Animals were caught in wire cage traps (Mascot Wire Works, Enfield, New South Wales, Australia) with a permit to take protected wildlife (Section 35 of the National Parks and Wildlife Act 1970; permit numbers 95249 and 95256). The marsupials were housed in the Central Animal House at the University of Tasmania. All animals were acclimatised to their environment and food source for over a month prior to the experiment. After this period all animals were transferred from the original holding pens to sterilized enclosures. Before experimental infection all animals were screened for *Giardia* sp. using a combination of techniques as described above, including antigen detection. Examination of daily stools over 10 days confirmed that the animals were healthy and not infected with *Giardia* sp. Care was taken to ensure that antibiotics were not administered prior to the experiment, as a precaution to unforeseen complications. Two animals were infected with cysts from a human case of *G. duodenalis* and two uninfected animal controls were housed in the same room although in separate cages throughout the experiment (University of Tasmania Ethics Committee, Animal Experimentation; Investigation number 95082). All animals were weighed before and after the experiment.

The *G. duodenalis* cysts were isolated from a human patient with chronic giardiasis. The fecal material was mixed with a small volume of Phosphate Buffered Saline and Triton X-100 (Mallinckrodt, Kentucky, USA) and filtered using parafilters. It was then centrifuged at 500 g for 5 min, 3 times. Each time the supernatant was discarded. At the end of a fourth spin the pellet was resuspended in 5 ml of distilled water and 0.5 ml Mefoxin (100 mg, equivalent to cefoxitin 2 g). The solution was left standing for 5 min, and centrifuged. This solution was again resuspended in 10 ml of sterile water.

One ml of the *G. duodenalis* cyst suspension (1,500 cysts/ml) collected from the human case of giardiasis was diluted with 2 ml of sterile distilled water. The 3 ml inoculum was administered by carefully restraining the animal and gently pouring the dose down the oesophagus with the use of a catheter.

The formalin-ethylacetate sedimentation concentration technique was implemented using the Johns Parafilter System (Biolab Scientific Pty. Ltd., Mulgrave, North Victoria, Australia) for screening fecal material for the presence of *Giardia* spp. Concentrated specimens were examined microscopically with a drop of iodine.

Diagnosis using microscopic methods is difficult and accuracy of the result is dependent on the examination of repeat specimens. Therefore, the microscopic parasitological techniques were supplemented with a method for detecting *Giardia* spp. antigen in fecal samples. Each specimen was examined for *Giardia* spp. antigens using a commercially available ELISA kit, *Giardia* CELISA (Cellabs Diagnostics Pty. Ltd.,). All specimens were prepared and tested according to the manufacturers guidelines. The results of the screening of native animals is as shown in Table 1.

Ingestion of cysts produced infection in one of the two experimentally infected bandicoots, with a prepatent period of 9 days. Cyst excretion was continuous until the 20th day post infection. No clinical symptoms were detected. Diarrhea was not detected in any of the animals used in this study and no loss of weight in the infected animal was observed, although the feces of this animal appeared to be more pale and loose on occasions. Based on both parasitological and antigen detection techniques, the two control animals remained

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TABLE 1. Prevalence of *Giardia* spp. in native animals obtained by microscopy and antigen detection on fecal specimens.

Animal	Number tested	Number positive	Prevalence
Bennetts wallaby (*Macropus rufogiseus*)	93	18	19
Possum (*Pseudocheirus peregrinus, Trichosurus vulpecula*)	89	14	16
Common wombat (*Vombatus ursinus*)	35	7	20
Dasyuridae	32	2	6
Bandicoot (*Isoodon obesulus, Perameles gunnii*)	26	16	62
Tasmanian pademelon (*Thylogale billardierii*)	13	3	23
Long-nosed potoroo (*Potorous tridactylus*)	7	2	29
Total	295	62	21

free from *G. duodenalis* and other intestinal parasites during this period as did the second animal fed cysts of *G. duodenalis*.

This preliminary study elicits many questions rather than providing many firm answers and there is enormous scope for further investigation. It is important to continue the investigation for both the eastern barred bandicoot and affected human populations. Questions which remain unanswered refer to the species of *Giardia* infecting bandicoots and other native animals, and whether they are generally susceptible to infection with *G. duodenalis*. In particular, it is necessary to culture and identify the *Giardia* spp. and assess the role of water as the mode of transmission.

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