

## A NEW *EIMERIA* SPECIES PARASITIC IN *ISOODON OBESULUS* (MARSUPIALIA: PERAMELIDAE) IN WESTERN AUSTRALIA

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**ABSTRACT:** Feces from southern brown bandicoots, *Isoodon obesulus*, inhabiting the Perth metropolitan area were examined using fecal flotation and light microscopy, and were frequently found to contain oocysts (10/24; 42%). To enable formal description of the proposed new *Eimeria* species, i.e., *Eimeria quenda* n. sp., fecal oocysts from 1 juvenile male *I. obesulus* were allowed to sporulate in 2% potassium dichromate ( $K_2Cr_2O_7$ ) at room temperature. Sporulated oocysts are spheroidal to subspheroidal  $24.5 \times 23.6$  (22.5–26.0  $\times$  22.5–24.8)  $\mu m$ , with L/W ratio of 1.04 (1.00–1.13), lack a micropyle and oocyst residuum, and are contained within a smooth trilaminar oocyst wall 1.8 (1.6–2.0)  $\mu m$  thick. Sporocysts are ovoid,  $12.6 \times 9.2$  (12.0–13.8  $\times$  8.5–10.0)  $\mu m$ , with L/W ratio of 1.37 (1.20–1.53), have a sporocyst residuum, and 2 comma-shaped sporozoites, each containing 2 spheroidal refractile bodies. Sporulation takes 1–3 days at room temperature. This is the second formal description of an *Eimeria* species parasitic in the order Peramelemorphia.

The southern brown bandicoot, *Isoodon obesulus* (Shaw, 1797), is an Australian peramelid marsupial species that usually inhabits southern mainland Australia, Tasmania, and several islands off the southern coast of Australia (Braithwaite, 2002). In Western Australia, the southern brown bandicoot is commonly known as the ‘quenda’, from the name for *I. obesulus* in the local Nyoongar language (Braithwaite, 2002).

*Eimeria kanyana* Bennett, Woolford, O’Hara, Nicholls, Warren, and Hobbs, 2006, has previously been reported from the feces of western barred bandicoots (*Perameles bougainville*) in Western Australia (Bennett et al., 2006). Two *Eimeria* species that have not yet been formally described have been noted in other peramelid species, including the eastern barred bandicoot *Perameles gunnii* (Obendorf and Munday, 1990) and the northern brown bandicoot *Isoodon macrourus* (Mackerras, 1958; Mackerras and Mackerras, 1960). No measurements or description of sporulated oocysts were given for either of these *Eimeria* species, making comparison to *Eimeria quenda* n. sp. impossible.

### MATERIALS AND METHODS

To estimate the local prevalence of coccidian oocysts in *I. obesulus* feces, 24 fecal samples were collected from wild *I. obesulus* brought to Kanyana Wildlife Rehabilitation Centre (KWRC) in late 2007 and early 2008. These individuals were admitted to KWRC for a variety of reasons, including suspected predation, suspected poisoning, habitat destruction, and motor vehicle trauma. Their feces were collected within 2 days of admission and stored at 4 C until further laboratory analysis could be conducted. Feces were mixed in a 10 ml conical tube with fecal flotation solution (saturated sodium chloride and 50% sucrose [w/v]) to produce a homogenous slurry; then further fecal flotation solution was added to achieve a total volume of 10 ml, and the mixture was centrifuged at 720 g for 5 min. Oocysts were transferred from the liquid at the meniscus of the conical tube onto glass microscope slides using a flamed wire loop and mounted using a cover slip.

Fresh feces from a juvenile male *I. obesulus* kept by a wildlife care-giver associated with KWRC were collected directly from that individual’s enclosure and placed in 2% (w/v) potassium dichromate solution ( $K_2Cr_2O_7$ ), mixed thoroughly, then poured into large Petri dishes to a depth of <1 cm and kept at room temperature in the dark to facilitate oocyst sporulation. Sporulated oocysts were concentrated by fecal flotation as described above. Slides were again produced using a flamed wire loop to transfer oocysts; however, these slides were mounted using a cover slip edged with petroleum jelly. Sporulated oocysts were observed using a  $\times 100$  oil immersion objective of an Olympus BX50 microscope and measured by Olympus Advanced digital micro-imaging DP71

technology. Bright field and Nomarski differential interference microscopy techniques were used to measure and photograph sporulated oocysts.

Sporulation time in potassium dichromate was determined in a concurrent experiment in which feces from the juvenile male *I. obesulus* were collected as described above and distributed into 6-well plates. The content of each well was concentrated and prepared as described above and then examined for the relative proportions of sporulated, unsporulated, and degenerate oocysts at 8 time points throughout the experiment, which lasted 9 days. The number of oocysts examined at each time point was between 348 and 512 (mean = 465).

Measurements of 50 sporulated oocysts are given in micrometers (mean  $\pm$  SD) with the observed range in parentheses.

### DESCRIPTION

#### *Eimeria quenda* n. sp.

(Figs. 1–4)

**Diagnosis:** Sporulated oocysts spheroidal to subspheroidal, with trilaminar oocyst wall,  $1.8 \pm 0.1$  (1.6–2.0) thick. Outermost wall smooth and yellow, encircling middle brown layer and inner black layer. Oocysts with 4 ovoid sporocysts. Oocyst length,  $24.5 \pm 0.8$  (22.5–26.0); oocyst width,  $23.6 \pm 0.6$  (22.5–24.8); oocyst L/W ratio,  $1.04 \pm 0.03$  (1.00–1.13). Micropyle and oocyst residuum absent. Sporocysts with 2 comma-shaped sporozoites. Sporocyst length,  $12.6 \pm 0.5$  (12.0–13.8); sporocyst width,  $9.2 \pm 0.4$  (8.5–10.0); sporocyst L/W ratio,  $1.37 \pm 0.06$  (1.2–1.53). Stieda, parastieda, and substieda bodies absent. Each sporozoite with 2 refractile bodies; diffuse, granular sporocyst residuum surrounding sporozoites.

### Taxonomic summary

**Type host:** *Isoodon obesulus* (Shaw, 1797), southern brown bandicoot.

**Type locality:** Forrestfield, Western Australia (31°59’S, 116°00’E).

**Prevalence:** Unknown. Ten of 24 (42%) fecal samples from *I. obesulus* contained coccidian oocysts; however, only 3 of the 10 oocyst-containing samples were sporulated. All 3 of these fecal samples contained *E. quenda* n. sp. oocysts.

**Sporulation time:** One to 3 days at 25 C in 2% (w/v) potassium dichromate (see Fig. 5).

**Site of infection:** Unknown.

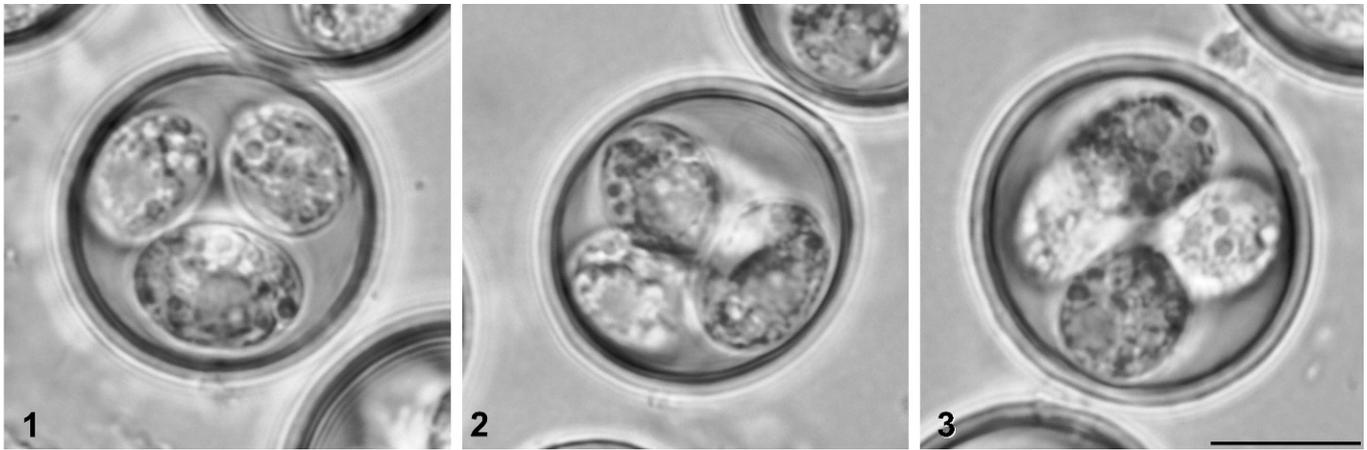
**Prepatent and patent periods:** Unknown.

**Material deposited:** Photomicrographs of sporulated oocysts have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland, USNPC No. 104679 and the Australian Registry of Wildlife Health, Taronga Zoo, Mosman, New South Wales, Australia, ARWH No. 8015.1.

**Etymology:** The specific epithet *quenda* is the Nyoongar (local Australian aboriginal) word for *I. obesulus*.

### Remarks

Compared with *Eimeria kanyana*, the only other species of *Eimeria* formally described from a peramelid host, *E. quenda* n. sp. is approximately 30% larger in its dimensions. In contrast to *E. kanyana*,



FIGURES 1–3. Nomarski interference contrast photomicrographs of *Eimeria quenda* n. sp. sporulated oocysts from *Isoodon obesulus* in Western Australia. Bar = 10  $\mu$ m.

*E. quenda* n. sp. oocysts lack a polar granule, and their sporocysts lack a Stieda body.

Nearly 50 species of *Eimeria* have been described from members of other families of marsupials in Australia, mostly from the Macropodidae (O’Donoghue and Adlard, 2000; O’Callagan and O’Donoghue, 2001). Only 3 of these species have oocysts that are spheroidal or subspheroidal in shape, and the sporocysts of all 3 have a Stieda body.

**DISCUSSION**

It is worth noting that the juvenile male *I. obesulus* whose feces were used in this formal description of *E. quenda* n. sp. had a male pouch-mate, whose feces also contained *E. quenda* n. sp. oocysts, though the concentration of oocysts in his feces was much lower.

Furthermore, their mother’s feces also contained a low concentration of *E. quenda* n. sp. oocysts.

Adult *I. obesulus* were more likely to have fecal oocysts (5/8 = 62.5%) compared with juveniles (5/16 = 31.25%). Of the 16/24 individuals for which a sex was recorded, males were slightly more likely to have fecal oocysts (5/8 = 62.5%) than females (3/8 = 37.5%). However, owing to the low number of samples in this epidemiological analysis, no clear conclusions regarding any possible age or sex predilection can be drawn.

In 1 fecal sample from an adult female *I. obesulus* victim of motor vehicle trauma, a heavy fecal oocyst burden was detected. While the majority of these unsporulated oocysts were morphologically consistent with *E. quenda* n. sp., there were also other coccidian oocysts with a distinctly oval, rather than spheroidal to subspheroidal morphology. Furthermore, there are 2 other morphologically distinct sporulated oocyst types recovered from *I. obesulus* feces (both in 1999), recorded in the archives of the Parasitology Department, Murdoch University School of Veterinary and Biomedical Sciences. These findings indicate that further coccidian species parasitic in the bandicoots of Western Australia are awaiting description.

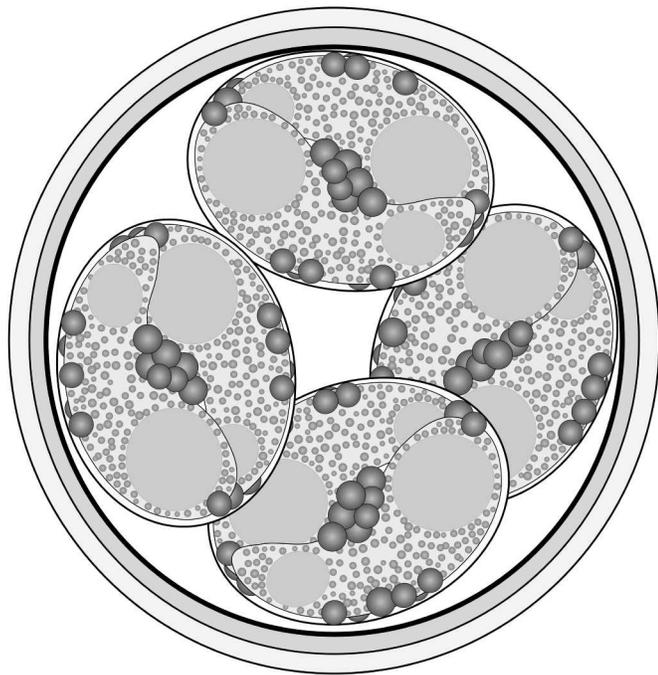


FIGURE 4. Composite line drawing of *Eimeria quenda* n. sp. sporulated oocyst. Bar = 10  $\mu$ m.

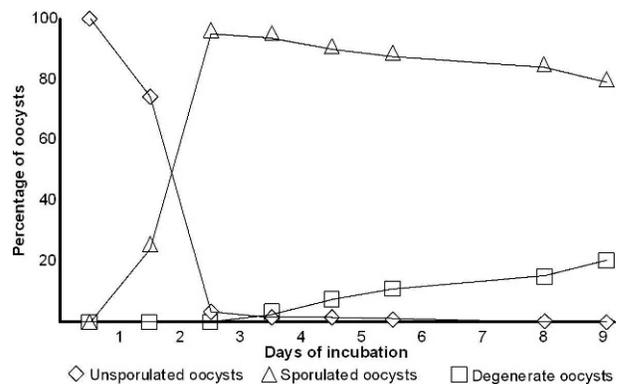


FIGURE 5. Graph illustrating the percentage of unsporulated, sporulated, and degenerate oocysts of *Eimeria quenda* n. sp. over 9 days of incubation in 2% (w/v) potassium dichromate solution at room temperature.

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