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Eimeria spp. infecting quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia

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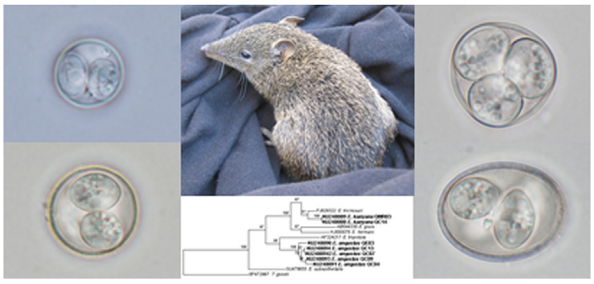
HIGHLIGHTS

- *Eimeria* sp. infections are highly prevalent in free-ranging quenda in Perth.
- Concurrent infection with multiple *Eimeria* sp. is common in Perth quenda.
- *Eimeria angustus*, a novel species of *Eimeria* parasitic in the quenda, is described.
- *Eimeria kanyana* is documented infecting quenda for the first time.
- *Eimeria angustus* and *Eimeria kanyana* are genetically characterised.

GRAPHICAL ABSTRACT



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ABSTRACT

Parasites of wildlife inhabiting urbanised and peri-urban environments are of interest regarding wildlife population health, and also veterinary public health in the case of parasites that can also infect humans and domestic animals. This study aimed to: identify, and estimate the prevalence of, species of *Eimeria* parasitic in quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia; 2) morphologically describe and genetically characterise a novel observed species of *Eimeria* as *E. angustus;* and 3) genetically characterise *E. kanyana*. *Eimeria* spp. prevalence was 76.1% (95% CI 64.9–84.5%), and four putative species of *Eimeria* were identified. *Eimeria kanyana* was identified infecting quenda for the first time, with a prevalence of 54.9% (43.4–66.0%). *Eimeria quenda* was less prevalent, at 7.0% (3.1–15.5%). The novel species *E. angustus* was present in 45.1% of sampled quenda (34.0–56.6%). A second novel morphotype of *Eimeria* was present in 2.8% of sampled quenda (0.9–9.7%). Mixed *Eimeria* spp. infections were present in 21/71 quenda (29.6%, 95% CI 20.2–41.1%). Molecular phylogenetic analyses of *E. kanyana* and *E. angustus* were conducted at the 18S rRNA and mitochondrial cytochrome oxidase loci. At both loci, two isolates identified as *E. kanyana* grouped in a phylogenetic clade with *E. trichosuri*. Five isolates identified as the novel *E. angustus* were most closely related to *E. tropidura* at the 18S locus. At the COI locus, no sequence data were available for *E. tropidura*; isolates of *E. angustus* grouped with *E. sciurorum*.

1. Introduction

Parasites of wildlife inhabiting urbanised or peri-urbanised environments are of importance regarding their potential impact

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on population health (Thompson et al., 2009). The role of parasites as a density-dependent regulator of host population size may be of particular significance in this case, as urbanisation can be associated with abnormally increased population densities of wildlife species that adapt to living in urban areas (Bradley and Altizer, 2007). Additionally, zoonotic parasites present in wildlife in urban or peri-urban areas are of public health significance (Mackenstedt et al., 2015).

Though urbanisation is associated with habitat loss for wildlife in Australia and worldwide (McKinney, 2002; Garden et al., 2006), some wildlife species are able to adapt and survive in urbanised environments. Quenda (syn. southern brown bandicoots, *Isoodon obesulus*) are a small, terrestrial peramelid marsupial, and have survived in many urbanised areas of Perth, Western Australia (Howard et al., 2014). Published documentation of parasites infecting quenda in this region are restricted to small samples of quenda tested for *Giardia* spp. (Thompson et al., 2010) and *Eimeria* spp. (Bennett and Hobbs, 2011).

Eimeria is a genus of apicomplexan parasites, species of which have been recorded in a wide range of vertebrates (including mammals, birds, reptiles and fish). Two species of *Eimeria* have been documented parasitising peramelid marsupial hosts: *E. kanyana*, parasitic in western barred bandicoots (*Perameles bougainville*) (Bennett et al., 2006), and *E. quenda*, parasitic in quenda (Bennett and Hobbs, 2011). *Eimeria* spp. have also been described, morphologically and in some cases genetically, from a range of other Australian marsupial species (Mykytowycz, 1964; Barker et al., 1988a, 1988b,1988c, 1989; O'Callaghan and O'Donoghue, 2001; Power et al., 2009; Hill et al., 2012; Austen et al., 2014). Although *Eimeria* species are typically host-specific (Joyner, 1982), there are a number of examples of infection of multiple host species within the same marsupial genus (*Macropus* spp. - Barker et al., 1988a, 1989; *Trichosurus* spp.- O'Callaghan and O'Donoghue, 2001; Power et al., 2009).

Eimeria spp. are an important cause of gastrointestinal illness in livestock (Chapman et al., 2013). However, though morbidity and mortality attributable to coccidiosis can occur in various Australian marsupial species (e.g. Winter 1959; Barker et al., 1972), no studies have specifically investigated how common morbidity or mortality are following *Eimeria* sp. infection in these hosts. Previous research has suggested that the pathogenicity of various *Eimeria* sp. in certain marsupial hosts may be mild in otherwise healthy individuals (Mykytowycz, 1964; Bennett et al., 2006).

We aimed to: 1) identify, and estimate the prevalence of, species of *Eimeria* parasitic in quenda in the greater Perth region, Western Australia; 2) morphologically describe and genetically characterise a novel observed species of *Eimeria* as *Eimeria angustus;* and 3) genetically characterise the previously described species *Eimeria kanyana*, for the purposes of distinguishing it genetically from *E. angustus*.

2. Materials and methods

The target host population was free-ranging quenda in the Statistical Division of Perth. Quenda were trapped using Sheffield (cage) traps. Trapping was undertaken on 29 bushland sites and 35 urbanised sites (7 private non-residential properties and 28 private residential properties), from March 2013 to July 2015. A subset of trapped quenda were included in this study. Inclusion was based on the availability of fresh faeces from the animal. Trapping and sampling was undertaken under a Murdoch University Animal Ethics Permit (R2530/12), and Department of Parks and Wildlife Regulation 17 (SF009640) and Regulation 4 (CE004287) permits.

2.1. Identifying species of Eimeria present in Perth quenda

Faeces were collected directly from the traps after removal of the quenda, and traps were cleaned and disinfected between animals. Faecal samples may have been passed from the animal up to approximately 10 h before collection from the trap. Faecal samples were stored in an insulated field box until processed, which was no later than 6 h after collection.

One millilitre of faeces was preserved for genetic characterisation of coccidia by mixing thoroughly into 4 mL 70% ethanol. Preserved samples were stored at 4 °C until analysis. The rest of the fresh faeces were mixed 1:4 into 2% potassium dichromate solution ($K_2Cr_2O_7$). This faecal mixture was poured into small petri dishes, and left in a dark cupboard at room temperature to facilitate sporulation of coccidian oocysts. The faecal mixture was checked intermittently for sporulated coccidian oocysts, by concentrating oocysts present in a small portion of the sample by zinc sulphate flotation, and examining the sample microscopically. Briefly, a portion of faecal mixture was centrifuged at 850 G for 2 min, with supernatant discarded, and then resuspended in distilled water and re-centrifuged twice, to remove potassium dichromate. Samples were then mixed thoroughly with zinc sulphate solution (SG 1.18) at a ratio of 1:4, and centrifuged at 850 G for 2 min. A flamed wire loop was used to transfer surface material (containing coccidian oocysts) on to a slide. Sporulated oocysts were examined at 400× to 1000× magnification, using an Olympus BX50 microscope. Photographs of sporulated oocysts were taken using bright field and Nomarski differential interference microscopy.

2.2. Prevalence estimates

Only oocysts that sporulated and could be unequivocally assigned to species based on morphology were included in estimates of prevalence. Ninety-five per cent confidence intervals were calculated using Jeffrey's method (Brown et al., 2001).

2.3. Morphological description of Eimeria angustus

One hundred and fifteen sporulated *Eimeria* sp. oocysts of a consistent, novel morphology (obtained from 21 quenda hosts) were examined and photographed at 1000× magnification, using bright field and Nomarski differential interference microscopy (Olympus BX50 microscope). Images were analysed using ImageJ software (US National Institute of Health, Bethesda, Maryland), to obtain measurements of oocyst length and width, oocyst wall thickness and sporocyst length and width. Due to the compacted nature of this species of *Eimeria,* measurements were only taken from one sporocyst per oocyst- the sporocyst that was subjectively identified as being positioned laterally. Where no sporocysts could be manipulated into lateral position within the oocyst, sporocyst length measurement was not taken.

2.4. Genetic characterisation of Eimeria kanyana and Eimeria angustus

Ethanol-preserved faecal samples from two quenda were used to characterise *E. kanyana*, and ethanol-preserved faecal samples from five quenda were used to characterise *E. angustus*, at the nuclear 18S rRNA and mitochondrial cytochrome oxidase (COI) loci. *Eimeria quenda* was not characterised as part of this study, due to logistical limitations.

DNA was extracted using the Power Soil DNA Kit (MolBio, Carlsbad, California), as described in Yang et al. (2016). A nested PCR protocol was employed to amplify a 1285 bp product of the 18s rRNA locus, using methods described in Yang et al. (2016). A nested