Kangaroo *Cryptosporidium:* Is it a wildlife disease?

Michelle Power

Faculty of Veterinary Science
University of Sydney, NSW 2006
E-mail: mpower@vetsci.usyd.edu.au

Cryptosporidium, an apicomplexan protozoan parasite, is a causative agent of enteric disease in a broad range of hosts. The parasite has been identified in greater than 170 vertebrates, including 13 marsupial species. *Cryptosporidium* is well known to Sydney residents, who were forced to boil their drinking water during the 1998 Sydney water crisis when high levels of *Cryptosporidium* oocysts (the infective stage) were detected in the drinking water supply. During the crisis it was not ascertained if the oocysts were of a type infectious to humans, however as there was no reported increase in human cryptosporidiosis it appears unlikely that they were. Although there was much speculation the source of oocysts causing the contamination was not identified.

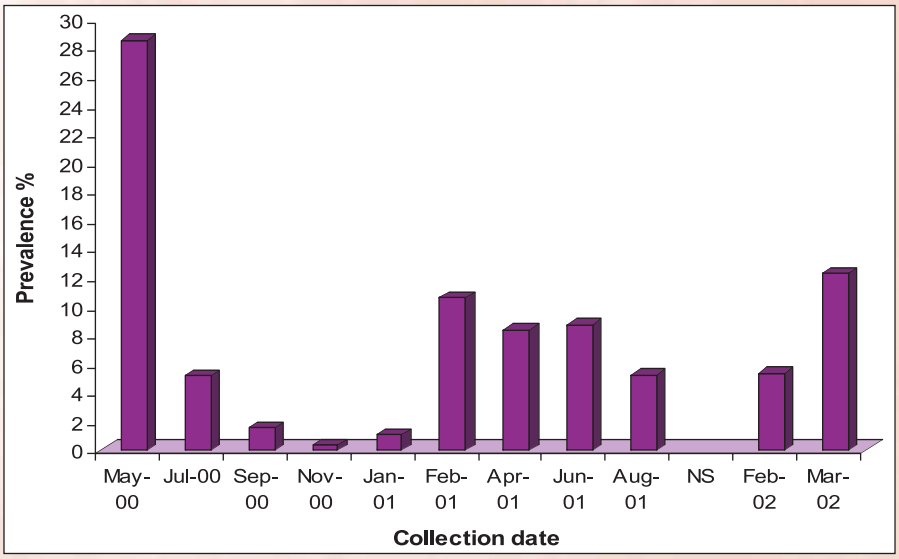
Sydney's drinking water is collected into Lake Burragorang from some 900,000 ha of land. The inner catchment area (250,000 ha) is comprised of lands directly surrounding Lake Burragorang and management strategies in this area aim to exclude humans, minimise recreational activity and reduce feral animal numbers. Native animals are not subject to population controls leaving the eastern grey kangaroo (EGK) as the most abundant large mammal inhabiting the area. Producing 1.5 kg faeces/adult /day[1] EGKs are a substantial source of faecal contamination within the catchment. Could EGKs have been the source of the *Cryptosporidium* contamination in Sydney's drinking water?

Our knowledge of Cryptosporidium in Australian marsupials is limited to information from animals in captivity or undergoing rehabilitation. Compared to wild populations, captive animals are potentially exposed to pathogens not typically present in the host's natural habitat, limited gene pools may exist in the captive host and its associated pathogens, and factors such as the stress of crowding may influence the disease process in the captive environment. Understanding disease in wild populations requires knowledge of the types of pathogens that may be present in their environments and other epidemiological details such as transmission and population susceptibility.

Over a two year period 3,557 EGK faecal samples from animals inhabiting the inner catchment were tested for *Cryptosporidium* oocysts using a combined immunomagnetic separation and flow cytometric technique. This technique has a detection limit of <20 oocysts/g faeces[2] and is significantly more sensitive than the diagnostic methods used in previous *Cryptosporidium* prevalence studies that have a detection limits >500 oocysts/ g faeces. Within host populations, parasite distribution tends to be aggregated, with many individuals having a low parasite burden and few with a high parasite load. Hence, for accurate estimates of prevalence, diagnostic methods need to be able to detect as few parasites as possible and sampling of large numbers is required.

Cryptosporidium occurrence in EGK faecal samples ranged from 0.32% to 28.5%, with peaks during the autumn months over three consecutive years (Figure 1). Generally, juveniles are more susceptible to *Cryptosporidium* infection and the autumn peaks in prevalence in the EGK population coincide with weaning. During this time, the exposure of the total host population to the infectious stage would be increased though greater environmental contamination from juvenile oocyst shedding. Kangaroos defecate several times during grazing periods[1] and the likely transmission route would be via ingestion of oocysts whilst grazing. Oocyst shedding ranged from 20 oocysts/ g faeces to as high as 10^6 oocysts/g.

Figure 1: Prevalence of *Cryptosporidium* in EGKs. Peaks occur during the autumn sampling months when high numbers of juveniles are present in the population (the NS period on the graph resulted from an inability to access the area due to bushfires).



If the formal ether concentration or direct faecal smears were used in this EGK study, 65% of positive samples would not have been detected as they contained oocyst numbers below the detection limit of these commonly used diagnostic techniques (Figure 2).

Morphologically, *Cryptosporidium* species are difficult to differentiate and genetic characterisation is most often used for parasite speciation. Molecular characterisation has led to the description of greater than 30 cryptic species (genotypes) [4] and Australian marsupials have their own cryptic species named the 'marsupial' genotype. The *Cryptosporidium* 'marsupial' genotype was first described in faeces from captive koalas [5] and has since identified in an orphan juvenile red kangaroo [4] and captive yellow footed rock wallaby faeces. Molecular characterisation of EGK *Cryptosporidium* isolates from this study revealed that this host was susceptible to three types of *Cryptosporidium* [3]. The *Cryptosporidium* 'marsupial' genotype was identified in 43% of positive EGK samples and a similar isolate in 18% of the sequenced samples. The variation between these two isolates at four loci was not considered enough to warrant the second isolate being named as a new genotype. However, the third EGK *Cryptosporidium* isolate, was genetically distinct from previously described *Cryptosporidium* species and genotypes. It is likely that this genotype will be named as a new species once further genetic characterisation, host specificity data and pathology are completed.

The three types of *Cryptosporidium* identified in EGKs, along with second novel genotype, were identified in brushtail possums inhabiting suburban areas of Northern Sydney. In an urban environment, marsupials would be exposed to *Cryptosporidium* species shed by other predominant hosts such as dogs *(C. canis)*, cats *(C. felis)* and rodents *(C. muris);* despite this exposure, possums were shedding *Cryptosporidium* types that, to date, have only been identified in marsupials. Given the recognition of new *Cryptosporidium* genotypes, it is important to determine the zoonotic potential of marsupial-derived *Cryptosporidium* and to define the role of marsupials in transmission of known *Cryptosporidium* zoonoses such as *C. parvum* which causes disease in a broad range of hosts including humans.

On a more fundamental note, understanding of *Cryptosporidium* in Australian marsupials may also provide insights into parasite evolution. Similarities between American opossum and Australian marsupial *Cryptosporidium* genotypes suggests that *Cryptosporidium* was present in marsupial ancestors prior to the separation of Gondwana [6]. Genetic characterisation of *Cryptosporidium* from monotremes could provide evidence to support this theory of *Cryptosporidium* evolution. A long association between marsupials and *Cryptosporidium* is also apparent by the lack of clinical signs observed in animals, including the wild EGKs and possums, suggesting that infection with marsupial genotypes is asymptomatic. Although host specific genotypes of *Cryptosporidium* in marsupials do not appear to cause disease, this study has provided significant information on the epidemiology of this parasite in wild marsupial populations and has provided solid data for future investigations of *Cryptosporidium* in marsupials.

References

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Figure 2: Distribution of oocyst numbers shed in 239 positive faecal samples from eastern grey kangaroos. The distribution of *Cryptosporidium* in this host is highly aggregated (K < 1), with 67% of animals shedding < 500 oocysts/ g faeces. The arrow indicates minimum detection limit formal ether concentration, a common *Cryptosporidium* diagnostic method.