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Research brief

Prevalence of *Cryptosporidium* and *Giardia* species in animals in irrigation catchments in the southwest of Australia

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Abstract

Screening of 445 animal faecal samples in irrigation catchments in Western Australia (WA) was conducted to identify the prevalence of *Cryptosporidium* and *Giardia* species. Of the samples positive for *Giardia duodenalis*, 30.7% (12/36) were the zoonotic Assemblage A, while approximately 13% (4/30) of *Cryptosporidium* positives were zoonotic. This is the first finding of *Giardia* Assemblage A in native marsupials and birds and indicates that marsupials and possibly birds may potentially be a reservoir of zoonotic *Giardia*.

Index Descriptors and Abbreviations: *Cryptosporidium*; *Giardia*; Catchments; Genotyping; Zoonotic; Marsupial; Environment

1. Introduction

Cryptosporidium and *Giardia* are the most common causes of waterborne outbreaks of gastroenteritis worldwide (Pond, 2005). *Cryptosporidium* oocysts and *Giardia* cysts have been detected in pristine surface water, ground water, filtered drinking water, lakes, swimming pools and coastal marine waters (Hlavsa et al., 2005a,b; Kistemann et al., 2002). These parasites have gained the attention of water utilities world-wide due to drinking water related outbreaks, because the oocysts and cysts produced by these parasites are extremely hardy, easily spread via water, and difficult to inactivate or remove from water intended for consumption without the use of filtration (Chen et al., 2002; Fayer, 2004; Fayer et al., 2000). Although, Australia has yet to experience an outbreak, the Sydney water crisis in 1998, where high levels of (oo)cysts were found in the Warragamba catchment (Ryan et al., 2005a), highlighted the importance of identifying sources of zoonotic species in catchments. To date no genotyping studies have been undertaken to assess the potential risk of *Cryptosporidium* sp. and *Giardia* sp. contamination in Western Australia catchments. The present study was an attempt to understand the public health significance of these parasites in animals in southwestern Australian irrigation catchments using molecular tools.

2. Materials and methods

A total of 445 animal faecal samples were collected directly from the ground from various wildlife and livestock species residing within three irrigation catchments; Waroona, a non-agricultural catchment consisting of state forest that has some recreational activities; Drakesbrook Weir which is used for recreational and agricultural use and Wokalup Pipehead dam which is used for agricultural but not recreational activities. The identity of the animal host of the faecal sample was determined in most cases by visual identification of the host whilst excreting and with the aid of a scat and tracking manual (Triggs, 2004), where defecation was not witnessed.

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Faecal samples were stored at 4 °C until processing using a QIAmp stool kit (Qiagen, Hilden, Germany). A two-step nested PCR protocol was used to amplify the 18S rDNA gene of *Cryptosporidium* as previously described (Ryan et al., 2003). For *Giardia*, a primary PCR product of 292 bp was amplified from the 18S rDNA gene as previously described (Hopkins et al., 1997). For the secondary PCR, a fragment of 130 bp was amplified using internal primers described by Read et al. (2002). PCR products were purified using a freeze–squeeze method (Ng et al., 2006) and sequenced using a Big Dye version 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Nucleotide sequences were analysed using Chromas v2.3 (http://www.technelysium.com.au) and aligned using Clustal W (Thompson et al., 1994).

Statistical analysis was performed using SPSS 11.0 (Statistical Package for the Social Sciences) for Macintosh OS X (SPSS Inc., Chicago, USA) to determine if there was any association between the prevalence of *Giardia* and *Cryptosporidium* in each catchment and factors such as faecal consistency, seasonality, rainfall and temperature. Total daily rainfall and maximum daily temperatures were obtained from remote weather monitoring stations closet to the sampling site from the Bureau of Meteorology (www.bom.gov.au) for a period of 2 weeks prior to the sampling dates from each site. From this, the average temperature and rainfall was calculated, resulting in one average temperature and rainfall measurement for each sampling date.

3. Results and discussion

Thirty *Cryptosporidium* positive isolates were detected by PCR, for a total prevalence of 6.7% (30 of 445) for the three irrigation catchments. A higher prevalence of *Cryptosporidium* (11.3%; 17 of 150) was detected at Waroona, than at the agricultural catchments (Drakesbrook Weir and Wokalup pipehead) 4.4% (13 of 295) (p-value 0.008). Sequences were obtained for 14 positives (see Table 1), the remaining 16 positives were unable to be sequenced due to insufficient template and/or mixed template. The non-zoonotic marsupial genotype I was identified in faeces from two kangaroos which supports an earlier study conducted by Power et al. (2005), on eastern grey kangaroos in a Sydney watershed. The *Cryptosporidium* cervine genotype was identified in faeces from an unknown host from Drakesbrook and the *Cryptosporidium* pig II genotype was identified in faeces from two pigs from Wokalup. Four *Cryptosporidium parvum* isolates were detected; two in faeces from beef cattle from Drakesbrook, one in faeces from a dairy cow from Wokalup and one in faeces from an unknown host from Waroona. Four *Cryptosporidium bovis* isolates were detected in faeces from three beef cattle from Drakesbrook and Wokalup and one in faeces from dairy cattle from Wokalup. Previous studies have detected *C. bovis* in cattle in the United States (Fayer et al., 2005) and sheep in Australia (Ryan et al., 2005b). This is the first report of *C. bovis* in cattle in Australia.

Cryptosporidium hominis was detected in a faecal sample from a wild bird from Waroona. This species has been previously reported in geese in Ohio and Illinois in the United States (Zhou et al., 2004) and was thought to be the result of mechanical transmission rather than an established infection and may also be the result of mechanical transmission in the present study. The presence of *C. hominis* in the present study could be attributed to the high recreational use, overcrowding and limited toilet facilities within the Waroona catchment.

The overall prevalence of *Giardia* was 8.7% (39 of 445) and was detected in significantly higher (p-value 0.004) levels (11.5%; 34 of 295) in the agricultural catchments compared to Waroona at 3.3% (5 of 150). Sequences were obtained for 36/39 positives and of these, 61% (24/36) were the non-zoonotic *G. duodenalis* Assemblage E identified in sheep and cattle, and 30.7% (12 of 36) were the zoonotic Assemblage A identified in cattle, birds, kangaroos and a fox (Table 1). This study is the first report of Assemblage A in kangaroos. A previous study identified a novel host-adapted non-zoonotic marsupial *Giardia* genotype in one marsupial, Quenda (*Isoodon obesulus*) (Adams et al., 2004). In the present study, a total of 72 kangaroo isolates were screened but only three Assemblage A isolates were identified. Another study reported a prevalence of 61.5% (16 of 26) for *Giardia* in Eastern barred bandicoots in Tasmania, but did not conduct genotyping (Bettiol et al., 1997). The same study also reported that bandicoots could be experimentally infected with *G. duodenalis* from a human source, raising concern that wildlife may play a role in zoonotic transmission of *Giardia*. The finding of Assemblage A in kangaroos in the present study provides supporting evidence that marsupials may be a reservoir of zoonotic *Giardia*. The three kangaroos that were positive for Assemblage A were from the Waroona dam, which has substantial recreational use. It is therefore possible that the kangaroos may have acquired this zoonotic genotype from vegetation, soil or by drinking irrigation water contaminated from human activity. As water samples were not tested during this study, the source of the human-infectious Assemblage A is difficult to fully determine. Assemblage A was also found in a fox (Drakesbrook) similar to the study by Hamnes et al. (2007), in which Assemblage A was found in both adult and juvenile Norwegian red foxes. The finding of Assemblage A in a wild bird (Waroona Dam) is a first, but may be due to mechanical transmission.

A preliminary assessment of environmental risk factors that may increase water shed contamination with *Cryptosporidium* and *Giardia* sp. indicated that periods of heavy rainfall (>200 mm/month) and cooler temperatures (mean daily temperature <17 °C) (p-value 0.004) appeared to be risk factors for the increased prevalence of *Giardia*. A similar pattern for *Cryptosporidium* was noted, however, it was not statistically significant ($p > 0.05$).

The present study has been the first opportunity in Western Australia to assess the potential public health signifi-