



Innovative approaches to understanding and limiting the public health risks of *Cryptosporidium* and *Giardia* in Australian catchments.

Project summary (LP130100035)



Cryptosporidium spp. and *Giardia duodenalis* are the most prevalent waterborne parasitic alveolates that can cause a gastrointestinal illness that involves watery diarrhea and can interfere with the body's natural absorption of nutrients, therefore presenting a major public health concern for water utilities in developed nations. The Oo/cyst is a hardy thick-walled stage of the life cycles of these parasites and are highly-resistant to environmental stresses and disinfection treatments (including chlorine treatment of community water supplies). Humans, wildlife and domestic livestock all potentially contribute *Cryptosporidium* and *Giardia* to surface waters, and thus, this research looks at the potential role that these host species play in the dissemination of *Cryptosporidium* and *Giardia* to drinking water sources and the associated public health risks.

In total 5,774 faecal samples were collected from 17 known host species and seven unknown bird samples, in 11 water catchment areas across three states (WA, NSW and QLD).

The most significant findings were the detection of the human-infectious *C. hominis* in cattle and kangaroo faeces, the high prevalence of the zoonotic *C. parvum* in cattle, the high prevalence of the zoonotic *C. meleagridis* in wastewater and the identification for the first time of *Giardia duodenalis* assemblage E in humans in Australia.

Where are they?

To date nine species of *Cryptosporidium* have been identified in humans in Australia; *C. hominis* and *C. parvum* (the main species infecting humans), *C. meleagridis*, *C. viatorum*, *C. cuniculus* (from rabbits), *C. fayeri* (from marsupials), *C. andersoni*, *C. bovis* (from cattle) and *C. felis* (from cats).

Oo/cyst transport to surface waters can occur by deposition of manure directly in the water (i.e. animal excretion or sewage discharge) or by rainfall runoff from the land surface.

To understand the extent of the problem 5,774 faecal samples were collected, from 17 known host species and seven unknown bird samples, in 11 water catchment areas across three states (WA, NSW and QLD) over a period of 30 months (July 2013 to December 2015).

In addition, a total of 730 raw influent samples from 25 Australian wastewater treatment plants (WWTPs) across NSW, QLD and WA were also collected between 2014 and 2015 and analysed for the diversity of *Cryptosporidium* species/genotypes present using Next Generation Sequencing (NGS) analysis of 18S amplicons (after initial screening using quantitative PCR, qPCR).

DNA was extracted and samples were screened for *Cryptosporidium* and *Giardia* by qPCR to determine oo/cyst numbers. Positives were typed by Sanger sequencing and where mixed infections were encountered by Sanger, NGS was conducted. Oocyst numbers per gram of faeces (g⁻¹) were also determined using qPCR (with droplet digital PCR, ddPCR calibrated standards), with medians varying from 6,021 - 61,064 across the three states.

Quick facts



Giardia duodenalis assemblage E detected to have infected humans for the first time in Australia



To date nine species of *Cryptosporidium* have been identified in humans in Australia



C. hominis which is normally found in human faeces was found in kangaroo and cattle faeces, suggesting possible introduction of the parasite into these populations from humans



High prevalence of *C. parvum* in mature cattle (previously considered to be dominant only in calves)

The diversity of *Cryptosporidium* species in WWTPs was analysed using NGS analysis of 730 raw influent samples and the overall *Cryptosporidium* prevalence was 11.4% (83/730): 14.3% (3/21) in NSW; 10.8% (51/470) in QLD; and 12.1% (29/239) in WA. A total of 17 *Cryptosporidium* species and six genotypes were detected by NGS.

In NSW, *C. hominis* and *Cryptosporidium* rat genotype III were the most prevalent species (9.5% each). In QLD, *C. galli*, *C. muris* and *C. parvum* were the three most prevalent species (7.7%, 5.7%, and 4.5%, respectively), while in WA, *C. meleagridis* was the most prevalent species (6.3%) (Figure 1).

The oocyst load/litre ranged from 70 to 18,055 oocysts/L (overall mean of 3,426 oocysts/L: 4,746 oocysts/L in NSW; 3,578 oocysts/L in QLD; and 3,292 oocysts/L in WA).

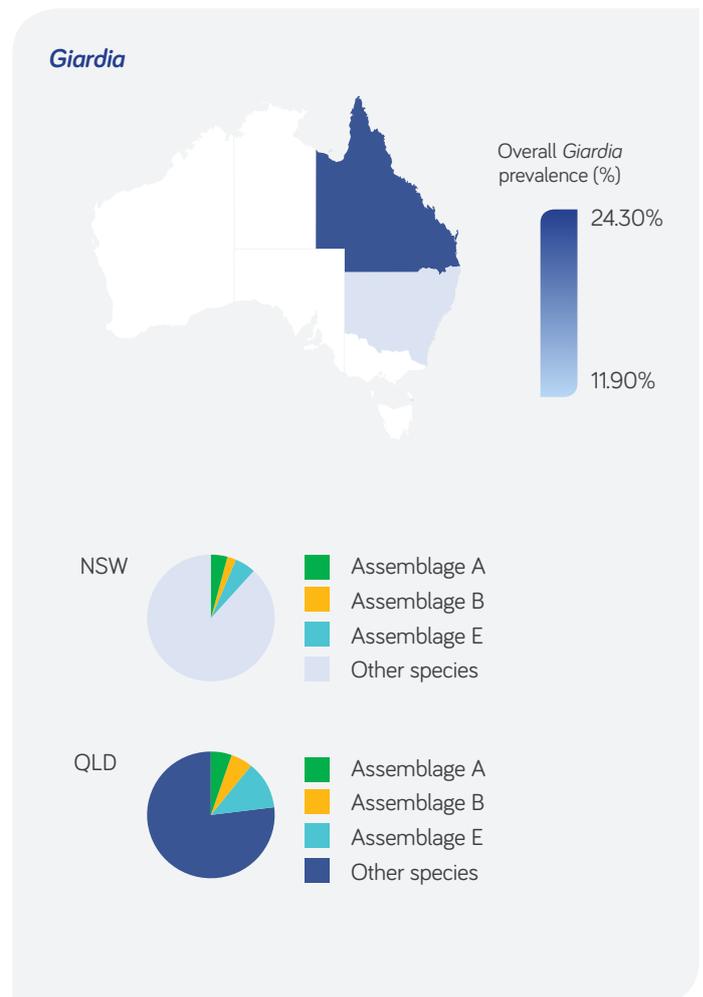
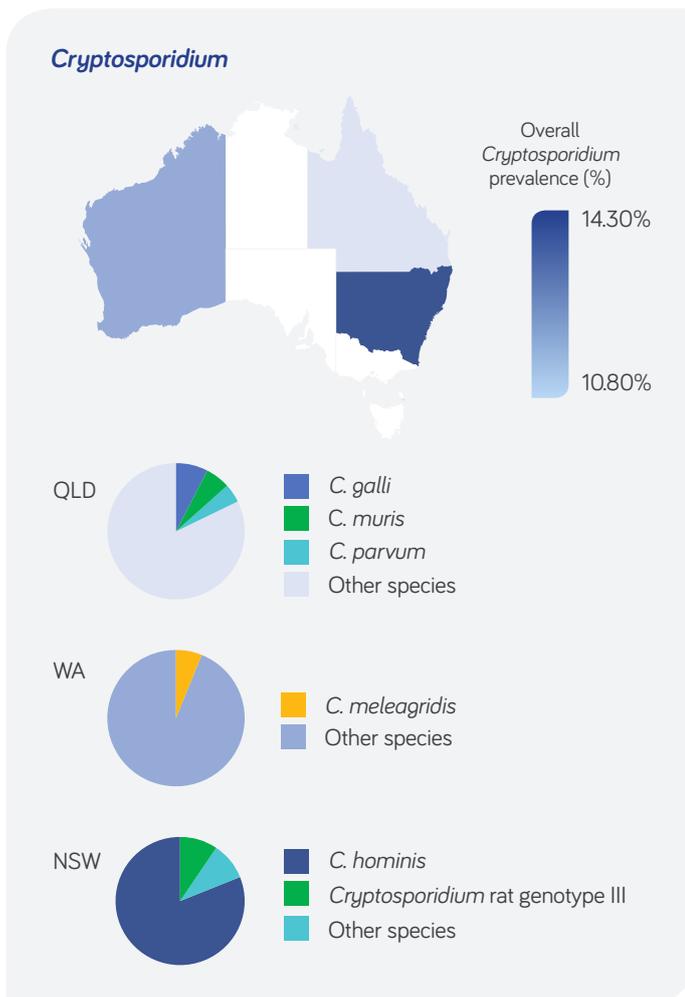
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Figure 1. *Cryptosporidium* prevalence in WA, NSW and QLD

Figure 2. *Giardia* prevalence in QLD and NSW



The overall prevalence of *Cryptosporidium* across the various hosts sampled was 18.3% (1,054/5,774; 95% CI, 17.3-19.3). Of the 17 *Cryptosporidium* species and four genotypes detected (Sanger sequencing combined with NGS), 13 are capable of infecting humans; *C. parvum*, *C. hominis*, *C. ubiquitum*, *C. cuniculus*, *C. meleagridis*, *C. canis*, *C. felis*, *C. muris*, *C. suis*, *C. scrofarum*, *C. bovis*, *C. erinacei* and *C. fayeri*.

In addition, 88 microscopically *Giardia*-positive isolates from QLD were typed and in addition to *G. duodenalis* assemblage A (50% (44/88)) and assemblage B (38.6% (34/88)), assemblage E was identified in 6.8% (6/88) of samples. This is the first report of assemblage E in humans in Australia, indicating that in certain settings, this assemblage may be zoonotic.

Due to time constraints, *Giardia* was only analysed in QLD and NSW samples. The overall *Giardia* prevalence in QLD samples was 24.3% (151/653, 95%CI; 19.9-26.6). This comprised Assemblage A 36/653 (5.5%, 95%CI; 3.9-7.6), Assemblage B 35/653 (5.4%, 95%CI; 3.8-7.4) and Assemblage E 80/653 (12.2%, 95%CI; 9.8-15). In NSW, the prevalence was 11.9% (181/1521, 95%CI; 10.3-13.6). This comprised Assemblage A 66/1521 (4.3%, 95% CI; 3.4-5.5), Assemblage B 32/1521 (2.1%, 95%CI; 1.4-3.0) and Assemblage E 83/1521 (5.5%, 95%CI; 4.4-6.7) (Figure 2).

What does Zoonotic mean?

“Zoonotic” relates to zoonosis: a disease that can be transmissible between animals and humans.

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Key findings

The most significant findings were the detection of the human-infectious *C. hominis* in cattle and kangaroo faeces, the high prevalence of the zoonotic *C. parvum* in cattle, the high prevalence of the zoonotic *C. meleagridis* in wastewater and the identification for the first time of *Giardia duodenalis* assemblage E in humans in Australia.

Cryptosporidium hominis was identified in 5.2% (43/835; 3.8–6.9) of kangaroos screened in NSW. Analysis of the kangaroo-derived *C. hominis* isolates identified only one *C. hominis* subtype in the kangaroo-derived DNA samples, suggesting a single, recent introduction of *C. hominis* into kangaroos.

 The *C. hominis* in the kangaroos may have come from spill-back from humans in the catchments, which may have also have spilled-over to infect cattle in the catchments. *Cryptosporidium hominis* was not detected in kangaroos from WA and kangaroo samples were not collected from QLD.

 *Cryptosporidium hominis* was detected in cattle faecal samples across all three states at a prevalence ranging from 4.5 to 14.1%. In cattle, *C. parvum* was the dominant species found in 39.1% to 50.7% of samples in each state. The importance of site-specific analysis for accurate Quantitative Microbial Risk Assessment (QMRA) is highlighted by the presence of *C. parvum* in adult cattle, previously thought to dominate in pre-weaned calves but that *C. bovis*, *C. ryanae* and *C. andersoni* dominate in older cattle.

 *Giardia duodenalis* assemblage E has recently been reported in humans in Egypt and Brazil, and until now, not reported in humans in Australia. Analysis of *Giardia* in sporadic human cases of giardiasis in QLD identified *G. duodenalis* assemblage E (previous thought to be specific to livestock) in six out of 88 microscopically *Giardia*-positive isolates. The humans that were positive for Assemblage E were experiencing diarrhoea, came from both rural and urban areas and shed variable levels of cysts in their faeces. All were identical to assemblage E from Australian cattle and sheep, suggesting zoonotic transmission.

 *Cryptosporidium meleagridis* is a common parasite of humans in Australia, it infects a wide range of birds suggesting both anthroponotic and zoonotic transmission and is commonly reported in wastewater worldwide. In this study, *C. meleagridis* was the most prevalent species detected in WWTP samples collected from WA and in many cases was the only species detected. Some of the *C. meleagridis* detected in WWTPs in WA could have originated from humans, however, bird contamination was deemed a likely source due to a) site of sample collection, a distribution chamber located just before the primary ponds, covered with a layer of mesh, providing easy access to bird contamination, and b) likely industrial sources of wastewater from poultry farms. *C. meleagridis* was not detected in NSW or

QLD, however, the potential role of birds in the contamination of wastewater by *Cryptosporidium* was also seen in the predominance of bird-specific *C. galli* in WWTP samples from QLD. The known contribution of poultry farms to WWTP in both WA and QLD is lacking and is an important knowledge gap.

Business benefits

The data generated can be used by utilities to:

- perform much more accurate QMRA analysis based on
 - quantitative data on oo/cyst excretion rates for the different hosts in various catchments and
 - quantitative data on the prevalence of zoonotic and non-zoonotic species in the various hosts over time and space, this analysis is ongoing
- identify relative risks from various contamination sources
- provide quantitative insights to managing control points
- develop “what if” scenarios and model alternative management scenarios to provide the ability to generate scientifically defensible, site specific criteria for improved management
- take these health impacts into account when considering recreational access to catchments and reservoirs with cattle and sheep present
- proactively develop programs of investigation and monitoring to address knowledge gaps.

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